PLENARYSPEAKERS

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PLENARY 1

Justin Nodwell

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PLENARY 2

Zixin Deng

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PLENARY 3

THE ECOLOGY AND EVOLUTION OF SECONDARY METABOLISM IN A MARINE ACTINOMYCETE LINEAGE

Paul Jensen

Scripps Institution of Oceanography, University of California San Diego

Actinomycetes remain one of the leading sources of microbial-derived natural products. While we have learned a great deal about the environmental distributions and diversity of these bacteria, we know little about the ecological functions of the secondary metabolites they produce and how the associated pathways are distributed among related strains and evolve to generate new structural diversity. We have addressed these questions using the marine actinomycete genus *Salinispora* as a model organism. By analyzing a large number of genome sequences, it has been possible to assess the diversity and distribution of biosynthetic pathways among the three species that currently comprise this genus. The results reveal extraordinary levels of pathway diversity, with the majority being acquired relatively recently by horizontal gene transfer. Pathways evolve by gene gain, loss, and duplication followed by divergence and are frequently exchanged within and among species. Despite the plasticity of the *Salinispora* secondary metabolome, there is clear evidence that some pathways are fixed at the species level indicating the products are of fundamental ecological importance. Furthermore, the co-occurring species *S. arenicola* and *S. tropica* can be distinguished based on competitive strategies with the former investing in defense (interference competition) and the later in growth (exploitation competition) thus providing the first evidence that these two closely related species represent distinct ecotypes. This unique genus continues to be a rich source of novel secondary metabolites and a useful model with which to address the role of secondary metabolism in the evolution of species-level units of diversity.

Keywords: Comparative genomics, *Salinispora*, Natural products, Ecology, Evolution

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PLENARY 4

IN VITRO RECONSTITUTION OF BIOSYNTHETIC MACHINERY FOR REVEROMYCIN

Hiroyuki Osada, Shunji Takahashi

Riken CSRS

Reveromycin A (RM-A) was originally isolated as an antitumor polyketide compound from *Streptomyces reveromyceticus*. RM-A exhibited anti-bone-resorption and anti-bone-metastasis activities through inhibiting the activity of isoleucyl-tRNA synthetase in osteoclasts. In order to supply a large amount of RM-A for the further therapeutic experiments *in vivo*, we investigated the molecular basis of biosynthetic machinery for RM-A

The RM-A gene cluster consists of 21 open reading frames spanning 91 kb. Polyketide synthase (PKS) gene disruption and feeding experiments revealed that the acyclic precursor (RM-A1a) is the post-PKS biosynthetic intermediate. To understand the post-PKS modification pathway, all of the genes found in the RM-A cluster were disrupted. Then, the metabolites accumulated in each mutant strain were analyzed. To clarify the mechanism of polyketide chain truncation, spiroacetal formation, and succinylation process, we performed the heterologous expression of the enzymes, which were purified for biochemical characterization. Finally we succeeded the *in vitro* reconstitution of enzymes and synthesized RM-A from RM-A1a.

Keywords: Biosynthesis, Natural products

Takahashi S, et al. and Osada H.:Reveromycin A biosynthesis uses RevG and RevJ for stereospecific spiroacetal formation.Nature Chem Biol 7: 461-468 (2011)

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PLENARY 5

DISCOVERY OF NEW TARGETS FOR VACCINATION, TREATMENT AND DIAGNOSIS OF TUBERCULOSIS

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Tuberculosis (TB) remains a formidable global health problem, causing around 1,5 million deaths and around 9 million new cases ever year. One third of the world population is considered to be infected with *Mycobacterium tuberculosis* (*Mtb*), representing a huge reservoir of infected persons that can develop reactivation disease later in life, for example during episodes of decreased immunity such as during co-infections (e.g. HIV), co-morbidity (e.g. Type 2 diabetes) and immuno-senescence.

Better vaccines against TB are urgently required to control the TB epidemic. BCG, the only TB vaccine available induces insufficient and inconsistent protection against TB, and a recent new TB vaccine trial failed to demonstrate any improved protection afforded by the new vaccine. A prerequisite for developing better vaccines is that the right antigens from the bacillus are selected for vaccination, and several recent approaches we have followed to accomplish this will be described.

In addition, the emergence of drug-resistant *Mtb* strains and the scarcity of newly approved antibiotics further aggravate the global TB problem, prompting for complementing therapeutic approaches. Intracellular pathogens such as *Mtb* manipulate host signalling networks to induce a niche for survival but knowledge of molecular interactions at the pathogen-host interface is scarce. We have developed new host directed therapy strategies, and will present new results showing how host control of Mtb can be improved significantly by genetic and chemical approaches acting through redressing perturbed intracellular host immune signaling pathways.

Besides better vaccines and therapeutics, TB biomarkers are a third, important tool that is needed to control TB. TB biomarkers would help to diagnose and stage infection, and to predict protected individuals from those at risk of developing disease. Recent findings using transcriptomic TB host biomarkers will be discussed which demonstrate highly encouraging progress in these areas.

Keywords: Tuberculosis, Immunity, Vaccines, Antibiotics, Biomarkers

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INVITED SPEAKERS

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CRITICAL EVALUATION OF PROKARYOTIC TAXONOMIC PARAMETERS USING LARGE SCALE COMPARATIVE GENOMICS

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Various taxonomic parameters, including phenotypic, chemotaxonomic and genotypic data, have been used in modern taxonomy of Bacteria and Archaea. Genome sequences is the ultimate information for taxonomy and systematics, and recent revolution of DNA sequencing technology enables us to actively apply genome sequence data in many microbiological disciplines. In this presentation, the current status of genome data for prokaryotic taxonomy will be reviewed and historically important taxonomic parameters are evaluated using comparative genomics approach on a large set of genome data available to date. These include 16S rRNA gene sequence similarity, DNA G+C contents and other widely used molecular systematics methods. Based on the comprehensive bioinformatics presented here, recommendations are suggested to facilitate routine use of genome sequence data in microbial taxonomy and ecology.

Keywords: Genomics, Taxonomy, Systematics, Average nucleotide identity

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WILL APPLICATION OF GENOMICS TO THE CLASSIFICATION OF THE GENUS *RHODOCOCCUS* HELP RESOLVE COMPLEX NOMENCLATURAL PROBLEMS?

Iain Sutcliffe

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The earliest described members of the genus *Rhodococcus* Zopf 1891 typically have complex taxonomic histories. Although relative stability was achieved through the circumscription of the genus by Goodfellow and Alderson in 1977, the genus has subsequently expanded from 10 to nearly 50 species. Moreover the subsequent advent of molecular systematics, notably 16S rRNA gene sequence analysis, has revealed both intrageneric and suprageneric relationships that are not readily discernable from classical phenotypic and chemotaxonomic approaches. In the light of these molecular analyses, it seems likely that the genus *Rhodococcus*, as currently defined, encompasses several genera. A first step towards resolving this was the proposed reclassification of *Rhodococcus equi* into a separate genus as *Prescottella equi* Jones et al. 2013. However, the formal status of the names *"Rhodococcus equi"* and "*Prescottella equi*" has yet to be resolved as both are currently deemed invalid as binomial names. This situation is further complicated by the recent conclusion that the name *Rhodococcus* Zopf 1891 is illegitimate as it is later homonym of the algal genus *Rhodococcus* Hansgirg 1884.

It is clear that the analysis of whole genome sequence data has the potential to both reform and revitalise the practice of prokaryotic systematics. To illustrate this approach, we have *a*pplied whole genome sequencing to help resolve the taxonomic status of "*R. equi*" specifically and, more broadly, the genus "*Rhodococcus*". Whole genome sequence analyses of the genus, notably yielding a phylogenetic framework from analysis 400 universal proteins with strong support from overall average nucleotide and amino acid diversities, strongly support the reclassification of '*R. equi'* into the genus "*Prescottella*". These analyses also reveal that the recently described *Rhodococcus defluvii* should be considered a second member of this taxon. Moreover, "*Rhodococcus*" itself can be resolved into at least four other robust lineages representing "*Rhodococcus"* sensu stricto and three putative novel genera. Eventually, the provision of these taxa with names that can be validated will bring nomenclatural stability and resolve the current confused situation.

The whole genome sequence data both help clarify the intrageneric structure of "*Rhodococcus*" sensu lato and provide a powerful illustration of the potential of whole genome sequence analyses to resolve complex taxonomic questions.

Keywords: Genome sequencing, *Rhodococcus*, Prescottella, Systematics

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MULTILOCUS SEQUENCE TYPING IN ACTINOBACTERIA

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Multilocus sequence analysis (MLSA) is applied as a tool using concatenated sequences of several partial protein code “housekeeping” genes with the aim to resolve the problem of phylogenetic resolution at the species level. MLSA was proposed and is still discussed as an “intermediate” method to replace the time and material consuming DNA DNA hybridization (DDH) studies. There is however, no clear recommendation of a specific set and number of genes that need to be included in a MLSA approach. In addition, no clear universally applicable similarity cut-off values are provided for species delineation and/or differentiation.

While MLSA may be very useful in identification of closely related species within some bacterial genera, MLSA analysis and its use for classification within the taxonomically quite complex genus Streptomyces can be used as a good example to illustrate some of the problems or open questions associated with MLSA. We investigated a sets of 10 to 15 Streptomyces type strains that shared more than 98.5% 16S rRNA gene sequence similarities and revealed relatively high DNA-DNA hybridization values. DNA and amino acid sequences based analysis of each of four housekeeping genes: atpD, gyrB, recA, and rpoB, showed strong differences in the phylogenetic resolution. Sequences of some species even clustered more closely with those of other species sharing much lower 16S rRNA gene sequence similarities in comparison to the investigated species. Analyses of concatenated sequences revealed again a different picture, and it is obvious from these results that clear recommendations of cut-off values in MLSA are impossible.

A large set of “core” genes will hopefully solve the problem of phylogenetic resolution of Streptomyces species with high 16S rRNA gene sequence similarities, however, also here the question of different “phylogenies” will lead to further, possibly even more complicated “phylogenies”.

Keywords: Multilocus Sequence analysis (MLSA), Actinobacteria, Streptomyces

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Klas Flärdh, Sweden

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OFF THE WALL: FROM FILAMENTOUS GROWTH TO PRIMORDIAL CELLS AND BACK AGAIN

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Institute Biology Leiden

Streptomycetes are filamentous bacteria that grow by apical tip extension. This process is orchestrated by the tropomyosin-like protein DivIVA, which is present at hyphal tips. DivIVA interacts with various proteins, among which the cellulose synthase-like protein CslA. This protein synthesizes a β-(1,4)-glycan, which is thought to protect growing apices that are continuously being remodeled. To obtain further insight in the role of DivIVA and CslA in polar growth and morphogenesis, we have recently generated so-called *Streptomyces* L-forms that can grow without peptidoglycan. As a consequence, such cells are round and lack any obvious form of polarity. L-form cells have recently been suggested to resemble primordial cell, based on the observation that their growth and proliferation do not require the canonical cytoskeletal or cell division proteins. Instead, their proliferation can merely be explained by physical processes. However, our work on *Streptomyces* L-forms suggests that these cells require glycans, such as those formed by CslA, for their growth. Such glycans might have served for protection of early life forms, before the modern cell wall was invented. We have recently isolated an L-form mutant strain, which readily switches back and forth between mycelial and L-form growth. This mutant with the capability to re-synthesize peptidoglycan is crucial to understand which genes play an essential role in proliferation of L-forms, but also to unravel the mechanism underlying filamentous growth.

Keywords:*Streptomyces*, L-Forms, Primordial Cells, DivIVA

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Gabriella Kelemen, UK

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THE PHYSIOLOGY, MOLECULAR BIOLOGY AND GENOMICS OF *FRANKIA* SP. AND ITS SYMBIOTICALLY-ASSOCIATED PLANTS

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Frankia is a well-defined group within the actinobacteria. The host range of species infected by *Frankia* is broad but comprises mainly woody species from eight different families belonging to three different orders (Fagales, Rosales and Cucurbitales). Phylogenetically, *Frankia* strains group in three different clades (Normand et al., 1996) that are associated with certain host families; eg. Clade I strains nodulate actinorhizal Fagales with the exception of Gymnostoma species, which are nodulated be clade III strains. In addition, a fourth clade has been identified (Ghodhbane-Gtari et al., 2010) that includes atypical and non-infective strains.

Up to date eighteen *Frankia* genomes have been sequenced, with sizes ranging from 5.3 to 9.9 Mbp (Tisa et al., 2013). Based on the first three *Frankia* genome sequences, a hypothesis was presented that genome size was related to host specificity and biogeography ranges (Normand et al., 2007). Continued genome sequencing confirms that hypothesis. Analysis of these genomes has revealed new potential in respect to metabolic diversity, natural product biosynthesis, and stress tolerance, which may help aid the cosmopolitan nature of the actinorhizal symbiosis (Udwardi et al., 2011).

The physiological responses of the plant and bacterium have been studied through transcriptomics. This showed that the plant upregulates known nodulins and defensins (Hocher et al., 2011; Demina et al., 2013) while the microbe upregulates *nif, hup, suf* and genes involved in the synthesis of the hopanoid lipids that constitute a barrier against oxygen (Alloisio et al., 2010). Not only are*Frankia* sp. well-known for their capacity to fix nitrogen in symbiosis with actinorhizal plants but also in free-living conditions. During nitrogen fixation there is also a production of hydrogen gas that can be considered as a loss of energy. However, all *Frankia* strains investigated but one have the capacity to use the hydrogen as an energy source by use of an enzyme called hydrogenase (Leul et al., 2009).

Keywords: Actinobacteria, Frankia, Genomes, Host plant, Physiology

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THE ROLE OF DEFENSE PEPTIDES PRODUCED BY ALNUS GLUTINOSA IN THE ACTINORHIZAL SYMBIOSIS

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Two symbiotic systems exist where nitrogen fixation done by microorganisms permits growth of plants in soils poor in nitrogen, the well-known *Rhizobium*-legumes symbiosis and the actinorhizal symbiosis between the actinobacterium *Frankia* and plants collectively designated actinorhizal. Understanding the mechanisms permitting the establishment and the trophic exchanges between partners is our purpose.

The first days after contact between the plant and the microorganism are the most active in terms of communication between the two partners, which is why we studied the interaction using micro-arrays for both partners. Among the genes upregulated, besides well-known nodulins, we have identified defense peptides. We have checked this upregulation through qRT-PCR. Then we have analyzed the effect of selected peptides *in vitro* on *Frankia* and other bacteria. We determined using propidium iodide that the membrane porosity was altered. We then analyzed the amount of some metabolites in the supernatant and measured the attachment du peptide par spectrophotometry. To determine its position *in situ,* we used a specific antibody and immunolocalization revealed it was bound to nitrogen-fixing vesicles in the nitrogen-fixing zone.

These peptides thus appear to have an important function in the symbiotic process.

Keyword: Frankia, Alnus, Defense peptides, Symbiosis

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Hocher *et al*. (2011) Transcriptomics of actinorhizal symbioses reveals homologs of the whole common symbiotic signaling cascade. Plant Physiol. 156: 1-12.

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THE SYMBIOSES OF THE UNCULTURED FRANKIA CLADE

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Phylogenetically, symbiotic *Frankia* strains group in three different clades (Normand et al., 1996), and the strains of basal clade II could never been cultured so far. *Frankia* clade II strains nodulate actinorhizal plants from the order Cucurbitales (Datiscaceae and Coriariaceae) and from the order Rosales (all actinorhizal Rosaceae and the rhamnaceous genus *Ceanothus*). The mechanisms by which *Frankia* hyphae enter the plant root differ dependent on host plant order. Actinorhizal nodules from different host plant genera show great diversity regarding anatomy, *Frankia* differentiation in infected cells and nodule metabolism. In particular, nodules from actinorhizal Cucurbitales show striking differences from nodules of Fagales and Rosales with regard to the positioning of infected cells in the nodule cortex (Pawlowski and Demchenko, 2012).

We have sequenced the genome of a member of clade II, *Candidatus* Frankia datiscae Dg1 (Persson et al., 2011) and are now comparing the plant and bacterial transcriptomes of two host plants nodulated by *Frankia* strains from the uncultured clade, *Datisca glomerata* (Datiscaceae, Cucurbitales; Demina et al., 2013) and *Ceanothus thyrsiflorus* (Rhamnaceae, Rosales).

Keywords: .*Frankia*, Actinorhizal Plants, Nodules, Nitrogen-Fixation, Datisca, Ceanothus

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Demina IV, Persson T, Santos P, Plaszczyca M & Pawlowski K. 2013. Comparison of the nodule *vs.* root transcriptome of the actinorhizal plant *Datisca glomerata:* actinorhizal nodules contain a specific class of defensins. PLoS ONE 8: e72442.

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FRANKIA GENOMICS AND GENOME-GUIDED APPROACHES TOWARD UNDERSTANDING THE ACTINORHIZAL SYMBIOSIS

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Frankia forms nitrogen-fixing symbioses with 8 different Angiosperm families, commonly known as actinorhizal plants. The actinorhizal symbiosis is a strong contributor to global biological nitrogen fixation process. *Frankia* has the ability to bind and sequester several toxic heavy metals and it has potential bioremediation and phytoremediation applications especially on heavy-metal-contaminated land. Actinorhizal plants have been successfully used to re-colonize and reclaim land that has been industrial wasteland including lands contaminated by metaliferous mine spoils and smelter waste. *Frankia* sp. strains are generally classified into one of four major phylogenetic groups that have distinctive plant host ranges. Symbiotic interactions between *Frankia* and the host plant are not well understood and very little is known about the initial molecular interactions in the rhizosphere. The nature of the chemical signals exchanged between the two partners of actinorhizal symbioses is still unknown. Due to the absence of genetic tools for *Frankia*, we have also pursued new genomic approaches toward studying these bacteria. Eighteen *Frankia* genomes have sequenced providing opportunities to use bioinformatics approaches and other new technologies. A correlation between genome size and plant host range was suggested from these data. Larger genome had broader host ranges. The absence of obvious nodulation genes similar to those found in *Rhizobia* genomes suggests that the actinorhizal symbiosis uses novel signal compounds during the infection process. Analysis of the *Frankia* genomes also demonstrated the presence of unexpected numbers of secondary metabolite gene clusters and potential novel natural products as candidates. Comparative genomic analysis will be discussed in terms of metal resistance mechanisms and the identification of potential genes involved in host recognition and other common traits. RNASeq results for three broad-host-range *Frankia* strains (EAN1pec, EuI1c and EUN1f) grown under nitrogen-replete (NH_4) and nitrogen-deficient (N_2) conditions will be presented. Besides comparative genomics approaches to identify key genes, we have used other genome-guided approaches to search for marker genes of symbiotic interaction to identify symbiotic signals emitted by actinorhizal plant roots. A molecule present in root exudates from *Casuarina glauc*a plants induced molecular and physiological changes in *Frankia* including the ability to establish root nodules on the host plant significantly earlier than untreated cells. The presence of an extracellular signaling molecule(s) produced by the exudates-treated *Frankia* was identified by the use of a bioassay with transgenic *C. glauca* plants and specific genetic markers in the nodulation pathway. These results provide support and insight on the hypothesis of chemical signaling between actinorhizal host plant and *Frankia.*

Keywords: Actinorhizal symbiosis, Genomics, Natural products, Host-Microbe Interaction

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A WHOLE NEW WORLD OF AQUATIC ACTINOBACTERIAL GENOMES

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Actinobacteria have been classically considered soil microbes with large genomes of high GC content. However, recent studies of molecular ecology and even more recent of metagenomics have shown that large clades of Actinobacteria are aquatic and many of them are planktonic, and have very small cells and small genomes with low GC contents. We have found two major groups of marine planktonic Actinobacteria. One of them forms a new subclass within the phylum and has been named Actinomarinales. They have been hypothesized to have the smallest cells and genomes yet described for free living bacteria. They constitute a substantial fraction (up to 5%) of the community in the deep chlorophyll maximum (among the largest marine habitats on Earth). The other group belongs to the order Acidimicrobiales and seems to be less prevalent. They too have small, very streamlined genomes. They are both photoheterotrophs and genuinely planktonic microbes subsisting in one of the most oligotrophic habitats. Even more remarkable is the situation of freshwater habitats such as rivers and lakes in which under oligotrophic conditions, unicellular and small planktonic Actinobacteria may constitute the vast majority of free-living bacteria (up to 40%). These largely belong to three different clades: Micrococcineae, Acidimicrobiales and a new subclass yet unnamed within the order Actinomycetales. Like their marine counterparts they are photoheterotrophs with remarkable degradative capacities for transforming plant detrital material including the recalcitrant heteropolymer lignin, persistent herbicides etc. Their presence is negatively correlated with that of harmful cyanobacterial blooms what makes them sentinel microbes for impending ecological catastrophes.

Keywords: Aquatic Actinobacteria, Metagenomics, Marine, Fresh-Water, Low GC

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THE ROLE OF DYNAMIN-LIKE PROTEINS IN THE DEVELOPMENTAL CONTROL OF CELL DIVISION IN STREPTOMYCES

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The Streptomyces developmental program involves mycelial growth and differentiation into dormant uni-genomic spores. During the reproductive phase, a massive and synchronous cell division event leads to the deposition of 50 or more regularly spaced FtsZ-rings along the sporogenic hyphae within a short time. Among the regulatory genes identified to be central to developmentally-controlled cell division is the transcription factor whiH. Exploiting the ability of S. venezuelae to sporulate in liquid culture, we have characterized the WhiH regulon. Through this route, a WhiH target promoter was identified that controls an operon of two genes encoding dynamin- like proteins (DynAB).

Dynamin-like proteins are large GTPases that play critical roles in diverse cellular processes in eukaryotes that require membrane fusion or fission. Although bacterial dynamin-like proteins have been partially characterized, their precise function in bacteria has remained poorly understood. Interestingly, in Streptomyces, the disruption of DynAB leads to the creation of long compartments containing more than one copy of the chromosome, indicating that the dynamins are required for normal sporulation septation, and for the regular assembly of cytokinetic FtsZ-rings. The dynamin mutant phenotype very closely mimics the whiH phenotype, suggesting that the dynamins largely mediate the effect of WhiH on developmentally-controlled cell division.

Keywords: Sporulation, cell division, dynamin-like proteins

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MICROBIAL DEGRADATION OF POLYISOPRENOIDES

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The two major naturally occurring polyisoprenoides (i) poly(*cis*-1,4-isoprene), the main constituent of natural rubber (NR), and (ii) poly(*trans*-1,4-isoprene), the main constituent of GuttaPercha (GP), are both biodegradable. Both are mainly degraded by Gram-positive bacteria which use these polymers as sole carbon and energy source for growth. Whereas NR-degrading bacteria are widespread in nature like *Gordoniapolyisoprenivorans*[3], axenic cultures of GP-degrading like *Nocardia nova* SH22a were only recently obtained [7].Interestingly, none of the bacteria isolated as NR-degrading strain could also degrade GP, whereas all bacteria isolated as GP-degrading bacteria were also capable of degrading NR [4, 7]. All Gram-positive NR- or GP-degrading bacteria possess a gene coding for an extracellular latex clearing protein (LCP), which is a dioxygenase that cleaves the polyisoprene molecules by addition to oxygen to the double bond [1,2].RoxA, which is an extracellular heme-dependent dioxygenase responsible for rubber cleavage in the only two so far isolated Gram-negative bacteria, exhibits no similarity to Lcp [6]. In this lecture the current knowledge on the degradation pathways for NR and GP in *G. polyisoprenivorans* VH2 and *N. nova* SH22a and their differences will be summarized [5]. This knowledge is based on an extensive analysis of numerous mutants and their biochemical as well physiological characterization as well as *in silico* analyses of the complete genomes of both bacteria [1,5]. In addition, biochemical characteristics of Lcp from *G. polyisoprenivorans* strain VH2 with regard to the kinetics, substrate specificity and cofactor dependence will be shown [2]. A better understanding of rubber degradation may allow the use of this knowledge for various biotechnological applications.

Keywords: Natural rubber degradation, Gutta percha degradation, *Gordoniapolyisoprenivorans*, *Nocardianova*, polyisoprenoides

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Manuel Hernandez, Spain

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STRATEGIES OF *STREPTOMYCES* TO SUPPRESS VERTICILLIUM DAHLIAE

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The ascomycete *Verticillium dahliae* causes worldwide vascular wilt of many field and horticultural plants. The fungus forms conidia and melanized resting structures, so-called microsclerotia, which survive for many years in soils and continuously re-infect plants. Due to the absence of known fungicides, *Verticillium* wilt causes immense crop losses. We discovered that *Streptomyces* has several strategies to suppress the plant pathogen; these include the concerted action of newly detected proteins that mediate close contact among hyphae as prerequisite to target the fungus alone or proliferating at plant seeds or roots. Consequently, a range of enzymes and metabolites support the suppression of vegetative fungal hyphae. Interestingly, one class of identified metabolites prevent the formation of the fungal microsclerotia. We discovered that several proteins and metabolites reside in newly discovered vesicles, which we characterised by several biochemical and microscopic tools. Additional studies led to elucidate the biogenesis of the vesicles and their role during interactions with the fungus. The investigations revealed the sophisticated *Streptomyces* repertoire to suppress the fungal plant pathogen.

Keywords:*Streptomyces*, Fungus Interactions and Inhibition

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Charles Thompson, Canada

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Marie Elliot, Canada

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CYCLIC DI-GMP SIGNALLING CONTROLS *STREPTOMYCES* DEVELOPMENT

Natalia Tschowri[1], Maria A. Schumacher[2], Susan Schlimpert[1], Naga babu Chinnam[2], Kim C. Findlay[1], Richard G. Brennan[2] and Mark J. Buttner[1]

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In recent years, the signalling molecule cyclic di-GMP has emerged as one of the most important and widespread second messengers in the bacterial world, with a key role in controlling the switch between planktonic, motile growth and a surface-associated, biofilm lifestyle in Gram-negative bacteria ("stick or swim") [1].

The model *Streptomyces* species *Streptomyces venezuelae* has 10 genes encoding enzymes that synthesize and/or degrade c-di-GMP, and recent experiments have shown that several of these genes are directly under the control of the developmental regulatory cascade [2-4]. Aiming to gain further insight into the regulation of development in *S. venezuelae*, we found that engineering elevated levels of c-di-GMP blocked development and that, conversely, engineering depletion of c-di-GMP greatly accelerated sporulation. Using the c-di-GMP Capture Compound (Caprotec), we identified the c-di-GMP-binding effector protein responsible for mediating the effects of c-di-GMP on the developmental cascade, and characterised this novel signalling mechanism physiologically, biochemically and structurally [4]. Thus, c-di-GMP still functions as a regulator of life-style changes in *Streptomyces*, but enters a new physiological arena in controlling the switch between vegetative growth and sporulation.

Keywords: c-di-GMP signaling, Second messenger, *Streptomyces*, Development, Sporulation

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Andreas Burkovski, Germany

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Matt Hutchings, UK

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CHARACTERIZATION OF THE VIRULENCE-ASSOCIATED COR-LIKE PHYTOTOXIN PRODUCED BY THE POTATO SCAB PATHOGEN *STREPTOMYCES SCABIES*

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Streptomyces scabies is an important causative agent of common scab disease on root and tuber crops such as potato, carrot, radish and beet. The disease negatively impacts the quality and marketability of the crop due to the formation of superficial, raised and/or pitted lesions on the root/tuber surface. The phytotoxic secondary metabolite thaxtomin A is known to be essential for scab disease development by *S. scabies* and other scab-causing pathogens. In addition, *S. scabies* is capable of synthesizing a secondary metabolite that has been predicted to resemble the coronatine (COR) phytotoxin produced by several pathovars of the Gram-negative plant pathogen *Pseudomonas syringae.*COR contributes to the virulence phenotype of *P. syringae* by facilitating invasion and proliferation of the pathogen in the plant host, by contributing to disease symptom development, and by suppressing plant defense responses. Analysis of *S. scabies* culture extracts using high performance liquid chromatography confirmed that the organism can produce multiple coronafacoyl compounds that are related to COR. The primary COR-like metabolite produced by *S. scabies* was purified, and it was shown to exhibit similar phytotoxic effects as COR in plant bioassays, though it is not as active as COR. Gene deletion studies were also conducted in *S. scabies* in order to identify genes that are required for COR-like metabolite biosynthesis. The results of this work provide important insight into the production and bioactivity of the *S. scabies* COR-like phytotoxin, which contributes to the plant pathogenic phenotype of this organism.

Keywords:*Streptomycesscabies*, Common scab, Phytotoxin, COR-like metabolite

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AN INTRIGUING SMALL ORF MODULATES TRYPTOPHAN METABOLISM IN STREPTOMYCES COELICOLOR

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In streptomycetes, amino acid biosynthesis is growth–stage dependent and, differently from other bacteria, not subjected to end-product feedback repression. In addition, amino acid metabolisms is an important clue of the physiological differentiation program. In amino acid biosynthetic gene clusters, such as tryptophan, histidine and proline, small orfs (about 100-300 nucleotides) were identified. These orfs, such as trpX, encode proteins whose cellular role could be crucial to shed light on the molecular mechanisms controlling amino acid synthesis and differentiation in streptomycetes. A *Streptomyces coelicolor* trpX mutant shows a very slow growth kinetics in minimal medium compared to wt and the addition of tryptophan (Trp) restores the growth rate. A proteomic-based multi-strategy approach allowed to investigate the biological role of TrpX and the regulatory mechanism of tryptophan metabolism. In particular, differential proteomic analysis based on 2D-differential gel electrophoresis (2D-DIGE), pull-down assay using His- tagged TrpX and mass spectrometry procedures were applied for the identification of molecular processes and metabolic pathways Trp-and /or TrpX dependent during *S.coelicolor* growth.

The results obtained from these analyses reveal that Trp controls the expression of either metabolic and regulatory proteins and that TrpX plays a modulating role in the tryptophan metabolism of *S. coelicolor*.

Keywords: small orf, tryptophan metabolism, proteomics

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Gülay Özcengiz, Turkey

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COMPARATIVE PROTEOMIC ANALYSIS OF STREPTOMYCES LIVIDANS WILD-TYPE AND PPK MUTANT STRAINS REVEALS THE IMPORTANCE OF STORAGE LIPIDS FOR ANTIBIOTIC BIOSYNTHESIS

Pierre Le Maréchal, Paulette Decottignies, Christophe H. Marchand, Jeril Degrouard, Danièle Jaillard,Thierry Dulermo, Marine Froissard, Aleksey Smirnov, Violaine Chapuis and Marie-Joelle Virolle

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Streptomyces lividans TK24 is a strain that naturally produces antibiotics at low levels, but dramatic overproduction of antibiotics occurs upon interruption of the *ppk* gene. However, the role of the Ppk enzyme in relation to the regulation of antibiotic biosynthesis remains poorly understood. In order to gain a better understanding of the phenotype of the *ppk* mutant, the proteomes of the wild-type (WT) and *ppk* mutant strains, grown for 96 h on R2YE medium limited in phosphate, were analyzed. Intracellular proteins were separated on two-dimensional (2D) gels, spots were quantified, and those showing a 3-fold variation or more were identified by mass spectrometry. The expression of 12 proteins increased and that of 29 decreased in the *ppk* mutant strain. Our results suggested that storage lipid degradation rather than hexose catabolism was taking place in the mutant. In order to validate this hypothesis, the triacylglycerol contents of the WT and *ppk* mutant strains of *S. lividans* as well as that of *Streptomyces coelicolor* M145, a strain that produces antibiotics at high levels and is closely related to *S. lividans*, were assessed using electron microscopy and thin-layer chromatography. These studies highlighted the large difference in triacylglycerol contents of the three strains and confirmed the hypothetical link between storage lipid metabolism and antibiotic biosynthesis in *Streptomyces*.

Keywords:*Streptomyces*, Triacylglycerol, Antibiotics.

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Si-Liang Zhang, China

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ROLE OF IRON IN ACTINOMYCETE INTERACTIONS AND DEVELOPMENT

Matthew Traxler[1], Stéphany Lambert[2], Matthias Craig[2], Marta Maciejewska[2], Marc Ongena[3], Gilles Van Wezel[4], Roberto Kolter[1], Sébastien Rigali[2]

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Many actinomycetes, including the model bacterium *Streptomyces coelicolor,* have evolved developmental cycles that culminate in the formation of spores on aerial hyphae that allow efficient dispersal. Analysis of co-cultures of different actinomycetes grown on solid media revealed an interspecies interaction in which one actinomycete, *Amycolatopsis sp.* AA4, inhibited aerial hyphae formation by adjacent *S. coelicolor* colonies. An unusual siderophore, amychelin, was responsible for this developmental arrest, suggesting that iron availability regulates development in *S. coelicolor*. Testing this hypothesis with direct quantification of transcripts using NanoString technology showed that elevated iron levels enhanced developmental gene expression, while iron scarcity down-regulated expression of developmental genes. This observation prompted us to examine siderophore biosynthesis in bald mutants of *S. coelicolor*. All *bld* mutants tested were affected in desferrioxamine (DFO) biosynthesis, with *bldA*, *bldJ*, and *ptsH* mutants severely impaired in DFO production, while *bldF*, *bldK*, *crr* and *ptsI* mutants overproduced DFO. Transcript analysis showed that the *bldJ* mutant had impaired expression of genes involved in the uptake of FO, whereas transcription of genes involved in both DFO biosynthesis and FO uptake is increased in *bldK* mutants. Both the *bldK* and *bldJ* mutants showed decreased transcription of *bldN* and *ram* genes, pointing to a discrete checkpoint for iron in regulating the developmental regulatory cascade.

Keywords: coelicolor iron interspecies interaction aerial hyphae

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Takeaki Tezuka, Japan

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PREDICTION OF REGULATORY NETWORKS AND CONTROL OF SECONDARY METABOLITE BIOSYNTHESIS

Sébastien Rigali

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In the post-genomic area, the prediction of the regulon of a transcription factor by position weight matrix-based programmes is an efficient bioinformatic method to decipher biological pathways and to unveil regulatory networks in bacteria. This *in silico* approach had been successfully used to discover molecules that activate or prevent the triggering of secondary metabolite biosynthesis in actinomycetes. The main difficulty once a regulon prediction had been performed is to estimate its reliability prior to start expensive experimental validations. Trying to find a way how to identify true positive hits from an endless list of potential target genes of a regulatory protein is key to minimize the costs associates with *in vitro* and *in vivo* experiments. However, the setting up of too stringent parameters in order to ensure the identification of only reliable novel hits can severely prevent the discovering of more subtle regulatory connections that really take place in vivo. The aim of this talk is to illustrate how to perform a reliable computational prediction of a regulon and how powerful is this approach to anticipate the environmental signals that control secondary metabolite production in actinomycetes.

Keywords: Regulon prediction, Secondary metabolism, Development

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PATHWAY TO VIRULENCE IN THE PLANT PATHOGEN *STREPTOMYCESSCABIES*

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Scab lesions on economically important root and tuber crops are caused by plant pathogenic streptomycetes. *Streptomyces scabies*, among other pathogenic species, evolved from a purely saprophytic life style by acquiring pathogenicity genes through horizontal gene transfer, while retaining their saprophytic capacity. Production of the phytotoxin thaxtomin A is the main pathogenicity determinant and required for scab disease development among these pathogens. This toxin is the predominant form of a family of nitrated dipeptides synthesized by non-ribosomal peptide synthases; thaxtomin A targets the cellulose synthase complex that is active dividing and expanding plant cell tissue. Although many genetic loci involvedin plant virulence have been identified in *S. scabies,* the molecular mechanisms by which this actinobacterium integrates environmental signalsto trigger the pathogenic phenotypeis still poorly understood. We present here a regulatory network involved in sensing and transporting plant signals that induces virulence in *Streptomyces* pathogens. Our work highlights a remarkable example of how the specific distribution of *cis*-acting elements inpathogenicity related loci is sufficient to evolve a *trans*-acting factor thatis connected to primary metabolism functions in non-pathogens, into a master switch which controls the access to virulence in the pathogenic counterparts.

Keywords: Regulatory Network, Virulence, *Streptomyces*

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COMPARATIVE GENOMIC ANALYSES REVEAL UNIQUE EVOLUTIONARY HISTORIES AND A LACK OF GENETIC SUPPORT FOR BIOCHEMICAL SEPARATION OF *CORYNEBACTERIUMDIPHTHERIAE* INTO BIOVARS

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Corynebacterium diphtheriae causes diphtheria, a toxin-mediated disease of the upper respiratory tract in humans that remains a significant cause of global morbidity and mortality. The diphtheria vaccine targets the toxin which is encoded by the *tox* gene within a lysogenised β -corynephage. As a potential result of strong selection due to vaccination, non-toxigenic strains are emerging around the globe, suggesting a major change to the life style of this pathogen. The primary niche of *C. diphtheriae*, the upper respiratory tract, is a hot-bed of horizontal gene transfer which may be responsible for the variation in cell adhesion and virulence properties observed between different strains.

C. diphtheriae has traditionally been biochemically characterized into four biovars: gravis, intermedius, mitis and belfanti. However, this differentiation of strains is quite complex and the only clear phenotypic variations are that intermedius is lipophilic and non-haemolytic and belfanti cannot reduce nitrate or use glycogen and starch as carbon sources.

To understand the evolutionary dynamics and the genetic basis of biovar differentiation, comparative analyses were performed on the whole genome sequences of a collection of *C. diphtheriae* strains. We also analysed the diversity at CRISPR-Cas (Clustered regularly interspaced short palindromic repeats-CRISPR associated proteins) loci that are the major barriers to recombination in bacteria.

All the strains were phylogenetically distinct to each other based on analysis of the variation in the core genome, as well as 400 universal proteins. The majority of the genetic variation was contributed by genomic islands that might be associated with differences in the adhesive and virulence characteristics. The variation in the gene content, and gain or loss of the gene functions within the functional categories that are potentially involved in biovar separation, did not correlate with the biochemical differences. However, several genes involved in carbohydrate metabolism were absent in biovars intermedius and a two base-pair insertion inactivated *narJ* gene in biovars belfanti, compromising assimilatory nitrate reduction.

Three novel CRISPR-Cas systems, variants of Type II-C or Type I-E system, were observed in *C. diphtheriae*. Most common was the Type II-C system which lacked *csn2* or *cas4* genes that are involved in spacer acquisition. The Type II-C system was replaced by a variant of Type I-E (I-E-a) in some strains, where the repeat arrays are inserted between the *cas3* and *cse1* genes. Three isolates possessed an additional variant of Type I-E (I-E-b) with a novel divergent *cas* operon gene organization. The phylogenetic incongruence of the *cas1* gene and palindromic repeats with the core genome, variation in the G+C content and the presence of these systems on genomic islands, suggest three independent horizontal acquisitions of CRISPR-Cas systems by *C. diphtheriae*. Most of spacers lack identity with known phage or plasmid sequences, indicating an unexplored reservoir of corynebacteriophages and plasmids.

In conclusion, phenotypic separation of *C. diphtheriae* into biovars is not suitable for strain classification and robust sequence based approaches should be adopted. Core genome phylogeny in conjunction with the CRISPR diversity reveals unique evolutionary histories of *C. diphtheriae* strains.

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Keywords:*Corynebacterium diphtheriae*, Biovar, Genomic comparison, Gene Contents, snps, CRISPR-cas

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CELL WALL LIPIDS OF MYCOBACTERIUM TUBERCULOSIS: ARSENAL AND ACHILLES HEEL

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Complex lipids play an important role in the biology of the tubercle bacillus *Mycobacterium tuberculosis,* as a large proportion of its genome contains genes proposed to be involved in lipid biosynthesis. Many mycobacterial lipids display immunomodulatory activities and are essential for virulence when tested in laboratory models of infection. However, some are also essential for bacterial viability and thus their biosynthetic pathways are potential drug targets. The talk will focus on one particular class of mycobacterial lipids, mycolic acids. We have taken a genetic approach to decipher how mycolic acids are synthesised in mycobacteria and the talk will cover how key enzymes involved in mycolic acid biosynthesis were identified. Additionally, work on the identification of a mycolic acid transporter will also be presented.

Keywords: Tuberculosis, mycobacteria, mycolic acids, cell wall

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DEVELOPMENT AND APPLICATION OF A SIMPLE METHOD FOR TYPING *MYCOBACTERIUM AVIUM* SUBSPECIES PARATUBERCULOSIS ISOLATES

Kapil Tahlan

Memorial University of Newfoundland

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD) in ruminants, which is of great concern to the agricultural industry. In addition, there have been some controversies regarding the association or MAP with Crohn's disease in humans. MAP displays characteristics reminiscent of other pathogenic mycobacteria as it can evade the host immune system and can undergo dormancy. One of the MAP related projects being pursued in our laboratory involve the development and application of strain typing methods for source tracking and epidemiological surveillance purposes. Previous studies have shown that Multilocus Short Sequence Repeat (MLSSR) sequencing using four genomic loci provides enough resolution to discriminate between different isolates. These loci contain highly repetitive GC rich repeat sequences and are challenging to work with using conventional DNA sequencing strategies. We have devised a simple non-sequencing dependent approach to analyze the four loci and have successfully tested the method using MAP isolates from infected animals. The methodology developed is currently being used in epidemiological studies to determine if certain MAP isolates are more effective in causing clinical versus subclinical infections for more detailed virulence studies in the future.

Keywords:*Mycobacteriumavium* Subspecies *paratuberculosis*, Johne's disease

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Haruo Tanaka, Japan

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NEW STRATEGIES FOR DRUG DISCOVERY: ACTIVATION OF SILENT OR WEAKLY EXPRESSED MICROBIAL GENE CLUSTERS

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Genome sequencing of Streptomyces and fungi showed that, although each strain contains genes that encode a plethora of potential secondary metabolites, only a fraction are expressed during fermentation. Interest has therefore grown in the activation of these cryptic pathways. The key issue for the success of this approach is to find ways to induce or enhance the expression of cryptic or poorly expressed pathways to provide material for structure elucidation and biological testing. We review current progress on this topic, describing concepts for activating silent genes as follows.

1) Broad applicability of the rpoB mutations for activation of silent genes.

2) Utilization of lincomycin to activate silent genes.

3) Applicability of “Metabolism-Remodeling” to nonribosomal-peptide antibiotics, in addition to polyketide antibiotics.

4) Mutagenic modulation of intracellular SAM level to activate bacterial silent genes.

The broad applicability of the rpoB mutation method to the expression of cryptic secondary metabolite-biosynthetic gene clusters was demonstrated recently. A subset of rifampicin resistance (rpoB) mutations result in the overproduction of antibiotics in various actinomycetes, including Streptomyces, Saccharopolyspora, and Amycolatopsis, with H437Y and H437R rpoB mutations effective most frequently. Moreover, the rpoB mutations markedly activate (up to 70-fold at the transcriptional level) the silent secondary metabolite-biosynthetic gene clusters of these actinomycetes. Analysis of the metabolite profile demonstrated that the rpoB mutants produced many metabolites, which were not detected in the wild-type strains. This approach utilizing rifampicin resistance mutations is characterized by its feasibility and potential scalability to high-throughput studies.

We found recently that lincomycin, which inhibits protein synthesis by binding to the ribosomes, is effective in activating the expression of genes involved in secondary metabolism in Streptomyces when added to the medium at subinhibitory concentrations. S. lividans grown with lincomycin at the subinhibitory concentration (one-twelfth or one-third of MIC) produced abundant antibacterial compounds that were not detected in cells grown on lincomycin-free medium. These findings indicate that lincomycin at subinhibitory concentrations potentiates the ability to produce secondary metabolites in Streptomyces.

The “Metabolism-Remodeling” is a notion developed by Nodwell group. We recently demonstrated that this notion is applicable even to nonribosomal-peptide antibiotics, in addition to polyketide antibiotics, by using certain protein synthesis inhibitors. Particular attention should be paid to combined approaches that include the transcription-activation of key genes and metabolism-remodeling. Reinforcement of biosynthetic processes by efficient substrate may be synergistic with transcription-activation, eventually leading to the efficient discovery of new secondary metabolites.

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We also talk about significant involvement of S-adenosylmethionine (SAM) in activating silent genes, referring to rsmG and mthA mutations.

These approaches may solve the early stage discovery problems of: (a) inducing some level of expression of cryptic biosynthetic gen clusters [waking the sleeping genes] and (b) rapidly increasing product yields to obtain enough material to characterize chemically and biologically [early stage yield enhancement].

Keywords: silent genes, rpoB mutation, metabolism-remodeling

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SEEING THE INVISIBLE: COMPREHENSIVE NMR FINGERPRINT OF THE DRUG-LIKE NATURAL PRODUCT METABOLOME

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An approach that uses nuclear magnetic resonance (NMR) fingerprinting to identify minor novel metabolites will be presented. In most cases de-replication requires separation of constituents before analysis and comparison with databases of known compounds. It may be more rewarding to directly identify novel compounds and then concentrate on isolating the new compounds.

Nuclear magnetic resonance is a universal detector (for all molecule that contain protons) and is also quantitative. Our motivation also lies in examining fractions to identify components that are not visible within crude extracts.

Examples applied to micro-organisms include:

- Isolation of bioactive constituents
- Induction of metabolites
- Co-culture
- New and novel metabolites

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Keywords: NMR fingerprints; metabolome

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Jean-Luc Pernodet, France

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BIOSYNTHESIS OF ARSENO-ORGANIC MOLECULES IN ACTINOBACTERIA

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Here we report the first example of a biosynthetic pathway for arsenorganic metabolites, identified by means of using an evolution-inspired genome mining approach, termed EvoMining. A biosynthetic gene cluster in the model strains *Streptomyces coelicolor* (Sco6837 – Sco6808) and *S. lividans* 66/1326 (Sli1077 – Sli1103) were predicted, and experimentally confirmed, to be involved in the biosynthesis of an arsino metabolite. Using a synthetic biology approach, the incorporation of arsenic into the key arsonopyruvate precursor via a homologous enzyme of the central metabolic AroA or 5-enolpiruvylshikimate-3-phosphate synthase (now termed as arsonoenolpyruvate synthase, Sli1096) was demonstrated using mass spectrometry analysis. Comparative metabolite profiling using HPLC and ICP-MS of selected mutants constructed in *S. lividans* and *S. coelicolor* allowed us to identify unprecedented arsenolipid metabolites. RT-PCR expression analysis of key genes of this biosynthetic gene cluster, including Sli1096, showed a positive correlation between the presence of Arsenic, phosphate starvation and synthesis of arseoniolipids. This observation suggests that arsenolipids may act as part of a contingency mechanism for restoring membrane integrity during low phosphate conditions. Moreover, we mined bacterial genomes for the occurrence of other arseno-organic metabolite biosynthetic gene clusters, finding at least 13 other *Streptomyces, Nocardiopsis*, *Kutzneria* and *Saccharomonospora* species with the potential to synthesize arsenorganic compounds beyond arsenolipids. To date, however, we have been unable to find evidence of similar biosystems outside mycelliated actinomycetes.

Keywords: Biosynthesis of arsenolipids, Genome mining, Evolution

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THE ANTIBIOTIC ROSEOFLAVIN FROM *STREPTOMYCES DAVAWENSIS*: MECHANISM OF ACTION, RESISTANCE AND BIOSYNTHESIS

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Roseoflavin (RoF) produced by *Streptomyces davawensis* and *Streptomyces cinnabarinus* is the only known natural riboflavin (RF) analog with antibiotic function and is studied as a model compound in our laboratory.

Keywords:*Streptomycesdavawensis*, Roseoflavin, Riboflavin, Vitamin analogs

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ANGUCYCLINES AS SIGNALS MODELATE THE BEHAVIORS OF *STREPTOMYCES COELICOLOR*

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Antibiotics have been reported to show signaling roles in interspecies interactions among actinomycetes, but in most cases, the signaling mechanisms are not well understood. Here we report the discovery of angucyclines as a new class of antibiotic signals in streptomycetes. These signals and their receptor, ScbR2, orchestrate the complex survival responses to the angucycline antibiotics in Streptomyces coelicolor. The signaling circuit downstream of ScbR2 receptor was characterized to reveal a novel regulatory mechanism triggered by antibiotic concentrations to generate distinct responses. Since angucyclines are the largest group of polycyclic aromatic polyketide antibiotics produced by Actinomycetes, their signaling roles and the signal response mechanism should be wide spread among these species. Our findings represent a significant advance in the understanding of antibiotic mediated signaling mechanism and the ecological roles antibiotics play in interspecies interaction.

Keywords: Antibiotics, Signals, Regulatory loop, Responses

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REGULATORY NETWORKS AND ECOLOGICAL CUES THAT CONTROL ANTIBIOTIC PRODUCTION BY ACTINOMYCETES

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Actinomycetes are prolific producers of natural products, including anticancer compounds, antifungals and antibiotics. The treasures that lie hidden in the actinomycete genomes may well be our final resource in the battle against the rapidly emerging infectious diseases associated with multi-drug resistance. We aim to understand the triggers and cues that elicit antibiotic production in the soil as well as in the laboratory. For this we use a combination of ecological insights, systems biology and genome mining to uncover the regulatory networks that control antibiotic production. One major control system revolves around the nutrient sensory protein DasR, which pleiotropically controls antibiotic production. It provides an excellent example of how fundamental understanding of the correlation between primary and secondary metabolism can be applied to activate antibiotic biosynthetic pathways. It is also clear that this is just one layer of control, and many other control systems are in place to allow actinomycetes to respond appropriately to challenges by competitors and fluctuations in nutrient composition in the habitat. Furthermore, once a bioactivity is elicited under specific growth conditions, approaches are needed to rapidly link the bioactivity to a specific gene cluster to expedite its identifcation. Application of new molecular and ecological insights to elicit antibiotic production, and approaches to identify these antibiotics, will be discussed.

Keywords: Antibiotic, Global Regulatory Networks, Chemical Ecology, Genomics

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REGULATORY MECHANISMS IN ANTIBIOTIC PRODUCING STREPTOMYCETES

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Streptomycetes are complex microorganisms in terms of morphological development and *secondary* metabolic pathways. Regulation is the node of connection that monitors environmental signals providing information on the physiological status of the cell, development, population density, and environmental living conditions, which in turn determines the initiation an amount of antibiotic production. Here, we give two examples of complex regulatory mechanisms that control physiological events in antibiotic producing streptomycetes.

1. Pristinamycin – product-specific transcriptional regulation of antibiotic biosynthesis

Pristinamycin is a streptogramin antibiotic, produced by *Streptomyces pristinaespiralis,* which consists of two types of chemically unrelated compounds: pristinamycin I (PI) and pristinamycin II (PII). Each components alone inhibits the 50S subunit of the bacterial ribosomes and acts bacteriostatically, whereas the naturally produced 30:70 (PI: PII) combination results in a synergistic effect that leads to the bactericidal activity of pristinamycin[1]. The genes encoding pristinamycin biosynthesis, regulation and resistance are organized in a single large gene region with a size of 210 kb, which represents the largest antibiotic 'supercluster' known so far[2]. Six regulatory genes were identified within this cluster: *spbR, papR1, papR2, papR3, papR4* and *papR5*, which are all involved in regulation of pristinamycin biosynthesis. SpbR is a receptor protein specific for γ-butyrolactones. *papR1, papR2* and *papR4* encode proteins that are homologous to SARPs, which are pathway-specific transcriptional activator proteins, whereas *papR3* and *papR5* encode both proteins belonging to the family of TetR repressors. The results obtained from HPLC analyses of the regulatory mutants and overexpression strains, as well as from RT-PCR- and gel shift assays revealed a complex signalling cascade, which is responsible for the fine-tuned regulation of pristinamycin production in *S. pristinaespiralis*.

2. Aconitase – pleiotropic post-transcriptional regulation of genes involved in oxidative stress response

The aconitase AcnA from the phosphinothricin tripeptide (PTT) producer strain *Streptomyces viridochromogenes* Tü494 is a bifunctional protein: under iron-sufficiency conditions AcnA functions as an enzyme of the tricarboxylic acid cycle, whereas under iron depletion or oxidative stress situations it is regulatory active. As a regulator AcnA binds to iron responsive elements (IREs) located on the untranslated region (UTR) of certain mRNAs and post-transcriptionally controls the expression of the respective target genes. *In silico* analysis of the *S. viridochromogenes* genome revealed several IRE-like structures, which were mainly located on the UTRs of genes involved in oxidative stress response. The functionality of different IRE structures was proven with RNA gel shift assays and specific IRE consensus sequences were defined[3]. Comparative proteomic studies with the wild-type and the aconitase mutant (MacnA) provided *in vivo* evidence for a direct AcnA-mediated regulation upon oxidative stress[4].

Keywords: Regulation, Secondary metabolites, Pristinamycin, Aconitase

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CURRENT MOLECULAR AND WEB TOOLS TO TRACK PREDOMINANT MYCOBACTERIUM TUBERCULOSIS CLONES WORLDWIDE

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Until not so long ago, all human-adapted strains of *Mycobacterium tuberculosis* complex (MTBC) were considered to be essentially identical, hence the question of individual genetic variation within MTBC gained little attention previously. However, the advent of molecular methods, databases and web tools over the last fifteen years and their widespread use in molecular epidemiology of tuberculosis (TB) introduced both new conceptual and technological developments. In this paper we will briefly review various MTBC genotyping databases developed at the Institut Pasteur de la Guadeloupe, which represent a great concerted initiative and effort to control the tuberculosis epidemic. Starting from the initial excel-based version in 1999 (SpolDB1; n=610 clinical isolates) to the fourth MySQL-based version in 2006 (SpolDB4; n=39,295 clinical isolates), these databases permitted to have a first phylogeographical snapshot of circulating MTBC genotypic lineages based on spacer oligonucleotide typing (spoligotyping) which allows to study the polymorphism of the Direct Repeat (DR) locus. The two most recent MySQL-based multimarker versions concern: (i) the 5th version SITVITWEB released in 2012 (n=62,582 clinical isolates) with both spoligotyping and 12-loci Mycobacterial Interspersed Repetitive Units - Variable-Number of Tandem Repeats (MIRU-VNTRs); and (ii) the 6th version SITVIT2 (n= 111,635 clinical isolates; release projected in 2014) with spoligotyping and 12-, 15- or 24-loci MIRU-VNTR data. In these recent versions, a web-based interface allows the user to search for strains through the database by criteria, such as the year, the isolation country, the country of origin, the investigator's name. It further facilitates to perform combined searches in SITVIT2, making it possible to get the genotyping data on selected strains in conjunction with their geographical distribution, as well as available data on drug-resistance, demographic and epidemiologic characteristics. Our research is focused to improve in-depth phylogenetic characterization of MTBC lineages in conjunction with epidemiological analysis of circulating clones to generate evidence-based geographical mapping of predominant clinical isolates of tubercle bacilli causing the bulk of the disease both at country and regional level. Further superimposition of these maps with socio-political, economical, and demographical characteristics available through Geographic Information Systems (GIS) allows to have a precise view of prevailing disparities as seen at the level of United Nation's sub-regional stratification. An in-depth comprehension of these disparities and drawbacks is important to take appropriate actions by decision-makers and public health authorities alike, in order to better monitor, understand and control the tuberculosis epidemic worldwide.

Keywords: Mycobacterium tuberculosis, Tuberculosis, Genotyping, Databases, Spoligotyping, MIRU-VNTR, Epidemiology, Phylogeny, Drug-resistance

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PROPOSAL OF A CONSENSUS SET OF HYPERVARIABLE MYCOBACTERIAL INTERSPERSED REPETITIVE-UNIT–VARIABLE-NUMBER TANDEM-REPEAT LOCI FOR SUBTYPING OF *MYCOBACTERIUM TUBERCULOSIS* BEIJING ISOLATES

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Mycobacterium tuberculosis Beijing strains represent targets of special importance for molecular surveillance of tuberculosis (TB), especially because they are associated with spread of multidrug resistance in some world regions. Standardized mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) typing based on 15 to 24 loci is now internationally used for *M. tuberculosis* complex genotyping. However, this format has insufficient resolution power for discriminating closely related strains of the *M. tuberculosis* Beijing lineage. So-called hypervariable MIRU-VNTR loci, because of their higher variability compared to the 24 standardised loci, might be good candidates to be used as secondary markers in order to improve this discriminatory power. Therefore, a set of 7 additional hypervariable MIRU-VNTR loci were assessed for better resolution and tracing of such strains in a multi centric study on a global sample of 535 *M. tuberculosis* Beijing isolates, from six different world regions where this lineage is prevalent. The typeability and interlaboratory reproducibility of these hypervariable loci were lower than those of the 24 standard loci. Three loci (2163a, 3155, and 3336) were excluded because of their redundant variability and/or more frequent non-interpretable results compared to the 4 other markers. The use of the remaining 4-locus set (1982, 3232, 3820, and 4120) increased the number of types by 52% (from 223 to 340) and reduced the clustering rate from 58.3 to 36.6%, when combined with the use of the standard 24-locus set. Known major clonal complexes/24-locus-based clusters were all subdivided, although the degree of subdivision varied

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depending on the complex. In addition, we evaluated the clonal stability of the markers using 92 isolates from a unique geographically restricted epidemic representing 15 years of clonal spread of a Beijing strain, and only five single-locus variations were detected among the hypervariable loci. On this calibrated basis, this 4-locus set is proposed as a consensus for subtyping Beijing clonal complexes and clusters, after standard typing.

Keywords:*Mycobacterium tuberculosis*, Beijing, Genotyping, MIRU-VNTR, Hypervariable

NEXT GENERATION TOOLS FOR TUBERCULOSIS EPIDEMIOLOGY AND PHYLOGENY

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Molecular epidemiology meaning the combination of molecular strain typing (genotyping) of Mycobacterium tuberculosis complex strains (MTBC, causative agents of tuberculosis, TB) with classical epidemiological analysis has become a crucial tool for detailed analysis of actual dynamics of tuberculosis (TB) disease. Recent studies, pioneering the application of whole genome sequencing (WGS) for MTBC outbreak analysis, indicate that classical genotyping e.g. based on IS6110 DNA fingerprint or 24 loci MIRU-VNTR (mycobacterial interspersed repetitive units - variable number of tandem repeats) typing is lacking discriminatory power to accurately determine transmission chains although these methods target highly variable regions in the genome. WGS based genotyping appears to offer an optimal resolution of MTBC isolates in molecular epidemiological studies with the advantage that additional information, e.g. on drug resistance, phylogenetic classification and virulence traits can be simultaneously gathered from the genome wide sequence data generated on rapid next generation sequencing (NGS) platform.

So far, real time prospective WGS based analysis of clinical isolates was hampered by the fact that specialized sequencing centres have mainly performed it in a retrospective manner on large expensive NGS platforms. However, recently, so-called bench top NGS systems, which can be integrated into a normal laboratory workflow, have become available to sequence bacterial genomes in few days. We established rapid genome analysis of clinical MTBC isolates using the Illumina MiSeq system and software pipelines usable in a small laboratory settings. The overall workflow from library preparation to final NGS data analysis could be optimized to last less than three working days. First evaluations of the performance indicate that MiSeq based genome analysis is excellently suited for real time investigation of MTBC outbreaks and diagnostics. A further problem is the difficulty to standardize WGS analysis to allow inter-laboratory prospective surveillance. To address this question, we developed a core genome multi locus sequence typing (cgMLST) scheme for clinical MTBC isolates that transfers the genome wide single nucleotide polymorphism (SNP) diversity into an allele numbering system that is standardized, portable and not computational intensive.

Taken together, the studies performed so far demonstrate the potential of WGS for improved molecular-guided TB surveillance and control. First evaluations of the allele based cgMLST approach resulted in a resolution comparable to SNP based typing, and tree topology allowing for a meaningful epidemiological interpretation of the WGS genotyping data. This enables standardization of WGS genotyping in longitudinal epidemiological investigations, e.g. on the regional public health office level, and the creation of web accessible databases for global TB surveillance with an integrated early warning system.

Keywords: tuberculosis, whole genome sequencing, transmission, phylogeny

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CHARACTERIZATION AND ENGINEERING OF THE BIOSYNTHETIC PATHWAYS OF AMINOGLYCOSIDE ANTIBIOTICS

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Aminoglycosides antibiotics have been widely used for the treatment of severe bacterial infections for decades, beginning with the use of the first effective antituberculosis agent streptomycin. Aminoglycosides are defined by two structural classes: kanamycins isolated from Streptomyces kanamyceticus, are representative 4,6-disubstituted 2-deoxystreptamine (2-DOS)-containing aminoglycoside antibiotics along with the gentamicins and tobramycin, whereas the neomycins and butirosins exemplify the 4,5-disubstituted aminoglycosides. As with other antibiotics, the emergence of aminoglycoside-resistant pathogens raises serious problems. This resistance issue has been addressed by the semi-synthetic modification of natural aminoglycosides such as amikacin, dibekacin, and arbekacin. A detailed understanding of the biosynthetic pathway of aminoglycosides is a prerequisite not only for the generation of more robust antibiotic agents, but also for the direct fermentative production of clinically important antibiotics. However, although the biochemistry and genetics underlying the biosynthesis of 4,5-disubstituted aminoglycosides have been relatively well characterized, those of 4,6-disubstituted aminoglycosides have still remained unclear mainly due to difficulties in genetically manipulating the producing actinomycetes and obtaining soluble functional enzymes. We recently characterized the partial biosynthetic pathway of gentamicin and the entire biosynthetic pathway of kanamycin through the heterologous expression of combinations of putative biosynthetic genes from the natural producing strains in the non–aminoglycoside-producing Streptomyces venezuelae. Furthermore, we engineered this pathway to biosynthesize 3'-deoxykanamycins including tobramycin and 1-*N*-[S-4-amino-2-hydroxybutyric acid] (AHBA)-conjugated kanamycins such as amikacin and the novel 1-N-AHBA-kanamycin X, which exhibits greater antibacterial activity than amikacin.

Keywords: aminoglycoside, biosyntheis, pathway engineering

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TARGET DRIVEN DRUG DISCOVERY FROM MANGROVE ACTINOMYCETES

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Drug discovery from marine actinomycete resources include multi-steps of screening, isolation and identification, like “look for a needle in ocean”. The efficiency is affected by where (environments), what (taxa), and how (detection methods) to look for those lead compounds. A target driven strategy has improved the quality of the microbial resource stock, and increased the new bioactive compounds discovery. This strategy was designed based on the knowledge of the actinomycetes secondary metabolites biosynthesis pattern figured by the industrial and genomic data analysis. Chemical level detection could be designed directly for target lead compounds screening from crude extracts by their HPLC spectra characteristics. Special environment such as mangrove is worth notice for medicinal microbial resources exploring.

Keywords: Drug discovery, Mangrove actinomycetes, Strategy

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ISOLATION OF RARE ACTINOMYCETES AND THEIR POTENTIAL AS UNTAPPED RESOURCE

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For discovery of novel natural products, we have isolated many actinomycete strains as new microbial resources fromdiversesource samples by various methods. We introduce our recent progress.

The first trial is the isolation of endophyticactinomycetes in plant roots. We have isolated more than 1,000 endophyticactinomycete strains, which non-*Streptomyces* strains accounted for approximately 70%. We found two new genera belonging to family *Micromonosporaceae*, *Phytohabitans* and *Rhizocola,* in their isolates. *Rhizocolahelliborii*K12-0602^T was found from the root of a *Helleborusorientalis* in Japan. Aerial spore chains were produced on only ISP medium 7, and motility was not observed. The 16S rRNA gene sequence of the strain showed the highest similarities with the members of the family *Micromonosporaceae* but the values were below 96.2%.Furthermore,the phylogenetic analysis indicated the strain is new genus.Although the family *Micromonosporaceae* is composed of 28 genera, the differences of chemotaxonomic characteristics are few. We analyzedcell-wall peptidoglycan of the genus *Rhizocola*with LC/MS and NMR. Consequently, it became clear that amino acids of the cell-wall peptidoglycan contained a unique diamino acid; 3,4-dihydroxydiaminopimelic acid, which has never been reported, to our knowledge, in other bacteria.[1)]

The second trial is the isolation of rare actinobacteria whichplace in the position of deeply branching lineage within the class *Actinobacteria*in the phylogenetic tree. Excepted order *Actinomycetales* from class *Actinobacteria*, it consists of 9 orders, 34 genera.However, almost genera consist of only the sole or a few species. We tried to investigate the distribution of the strains related to the order *Solirubrobacterales* and to isolate after selection of isolating samples by PCR using the specific gene sequences. Finally, we were successful to isolate a new species, *Conexibacterarvalis* KV-962^T, belonging to the order *Solirubrobacterales*.[2)]

Three new bioactive compounds, actinoallolides, spoxazomicins and trehangelins, were found from metabolites of endophyticactinomycetes.Plant roots are also a repository of new actinomycetes and new bioactive compounds.There are still a large number of as-yet-uncultured bacteria in the environment. For obtainingtheir untapped resource, it is important to isolate with a broad view.

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STUDIES ON THE ISOLATION, DIVERSITY AND TAXONOMY OF NON-FILAMENTOUS ACTINOBACTERIA FROM SEASHORE ENVIRONMENTS

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Non-filamentous actinobacteria (hereinafter called actinobacteria) are known as taxa which contain many industrially useful strains such as enzyme-, amino acid- and vitamin-producing or persistent chemical-degrading bacteria. Therefore, the discovery of novel actinobacteria from natural environments is important because it contributes toward an understanding potential as biological resources for industrial applications. However, studies on the selective isolation for actinobacteria are very rare, and discrimination of actinobacteria based on their colony and cell morphologies is difficult. Therefore, it is difficult to effectively obtain many actinobacterial isolates. Recently, marine environments attract attention as isolation sources of novel microorganisms including actinobacteria. The objectives of this study are to develop the isolation medium for actinobacteria from seashore environment samples and to clarify the taxonomic positions of candidates of novel species belonging to the family Demequinaceae within the suborder Micrococcineae.

As isolation sources, we used sea-sediment, sea-sand and various mangrove forest sediment samples collected in Japan and Indonesia. For isolation of actinobacteria, an isolation medium, named ‘SPPY agar’ (sodium chloride 5.0 %, polypeptone 0.2 %, yeast extract 0.04 %, MgSO4·7H2O 0.02 %, cycloheximide 0.005 %, nalidixic acid 0.002 % and agar 1.5 %) and a dilution plate technique were used. For the identification of isolates, the 16S rRNA gene sequencing approach was applied. In addition, among the candidates of new taxa, the taxonomic positions of some isolates related to the family Demequinaceae were investigated by using a polyphasic approach.

Using the ‘SPPY agar’, over 600 strains were isolated from 65 samples. Approximately 80 % of the isolates were actinobacteria and most of them were assigned to the suborder Micrococcineae. The genera Isoptericola and Microbacterium were most abundant and occurred in almost of all samples. Meanwhile, the genera Lysinimicrobium and Paraoerskovia were occurred only from mangrove-forest sediments and sea sediments, respectively, although many isolates were obtained. In addition, several novel species belonging to the suborder Micrococcineae could be detected in this study. In this presentation, we introduce the diversity of non-filamentous actinobacteria isolated from seashore environments using the ‘SPPY agar’ and also report the results of taxonomic studies of the isolates belonging to the family Demequinaceae, along with the result of whole genome sequencing.

Keywords: actinobacteria, isolation, taxonomy, Micrococcineae, Demquinaceae

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PHAGE-GUIDED SELECTIVE RECOVERY OF THE NOCARDIACEAE AND PSEUDONOCARDIACEAE ASSOCIATED WITH THE FOAMING OF COASTAL WATERS OF THE SUNSHINE COAST REGION OF AUSTRALIA

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Foaming is a worldwide recognized problem caused by proliferations of filamentous actinomycetes at activated sludge plants resulting in thick, stable scum on aeration basins and clarifiers, subsequently resulting in operational problems. Microbially-mediated foaming events have also often been correlated with warmer temperatures and over existence of fats, oils and nutrients in the plants. Due to increasing seasonal temperatures and urban and industrial activities resulting in environmental pollution, similar foaming events have become prominent on the shores of Sunshine Coast region of Queensland, Australia. In particular, during summer seasons following heavy rains, storms and flooding events when local beaches have been reported to be exposed to pollutants, significant foam formation was recorded. At the activated sludge plants bacteria commonly associated with foaming events reported to belong Nocardiaceae and Pseudonocardiaceae families of actinomycetes. Some of these bacteria were also reported to belong the pathogenic or opportunistic pathogenic actinomycete clusters, and if disseminated via generated bio-aerosols during foaming events they can constitute public risk.

An overview of the foaming events (2003-2013) which has taken place in the region as well as phage-guided selective recovery of the species of Nocardiaceae and Pseudonocardiaceae from these foams and shores will be communicated.

Keywords: Phage-Pseudonocardiaceae-Nocardiaceae

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MICROMONOSPORACEAE STRAINS ISOLATED FROM MANGROVE FOREST AT IRIOMOTE ISLAND JAPAN

Tomohiko Tamura

National Institute for Technology and Evaluation, Biological Resource Center (NBRC)

Mangrove forest forms significant ecosystem with wide biodiversity in tropical and subtropical areas. Major Mangrove trees belong to the family *Rhizophoraceae, Verbenaceae* and *Sonneratiaceae.* The species of mangrove trees are vary considerably from one region to another. Mangrove forest is habitat of wide diversity of bacteria. This decade, many novel bacteria isolated from mangrove environment have been reported. Half of them belong to the class *Actinobacteria*, especially many Micromonosporaceae strains (22.6%) have been isolated.

Five families, 7 species of mangrove grow in Japan, and all species grow in Iriomote Island only. We have surveyed on inhabitants actinomycetes in mangrove forest with a focus on Iriomote Island. Two actinomycetes, designated TS60-4A and TS60-4C were isolated by SDS-YE (Hayakawa & Nonomura, 1989) on HV agar (Hayakawa & Nonomura, 1987) from mangrove rhizosphere soil at Iriomote Island, Okinawa, Japan. Sequence comparisons were carried out using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Strain TS60-4A shared highest sequence similarities with *Planosporangium mesophilum* YIM 48875T GU126491 (99.00%), *Planosporangium flavigriseum* YIM 46034T AM232832 (98.77%) and *Planosporangium thailandense* HSS8-18T AB560970 (97.94%). These results demonstrate that strain TS60-4A is considered as a member of the genus *Planosporangium*. The genus Planosporangium were reported to develops two type species which are motile sporangiospore in short and narrow sporangia and globose spore on the tip of short sporophore. Strain TS60-4A showed similar chemotaxonomic characteristics but 3 to 11% of DNA-DNA relatedness with Planosporangium flavigriseum NBRC 105377T and Planosporangium mesophilum NBRC 109066T. This result shows that strain TS60-4A is a candidate of novel species of the genus Planosporangium.

Strain TS60-4C shared highest 16S rRNA gene sequence similarity with Salinispora arenicola CNH-643T AY040619 (98.28%), *Micromonospora* pattaloongensis TJ2-2T AB275607 (98.08%), Salinispora pacifica CNR-114T DQ224161 (98.07%), *Salinispora* tropica CNB-440T CP000667 (98.07%), *Xiangella phaseoli* NEAU-J5T JQ073732 (98.01%), Jishengella endophytica 202201T EU560726 (98.00%). *The genus Salinispora* were reported to need seawater or NaCl for their growth, but strain TS60-4C did not required seawater or NaCl for their growth. Strain TS60-4C showed similar chemotaxonomic characteristics but 2 to 25% of DNA-DNA relatedness with *Salinispora arenicola* NBRC 105043T, *Salinispora tropica* NBRC 105044T and Salinispora pacifica NBRC 109968T. This result shows that strain TS60-4C is also a candidate of novel species.

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Keywords: Micromonosporaceae Mangrove

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LANTIBIOTICS FROM ACTINOMYCETES – STRUCTURE, BIOSYNTHESIS AND ENGINEERING

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Peptides are of considerable importance for drug use, e.g. as antibacterials, antivirals and immunosuppressive drugs. Because of their remarkable properties they are currently of interest for the pharmaceutical industry. To accomplish peptide synthesis Nature provides two fundamental biosynthesis principles for the synthesis of these peptides: the ribosomal synthesis together with posttranslational modification and processing and the non-ribosomal peptide synthesis. Important representatives of ribosomally synthesised and posttranslationally modified peptides (RiPPs)[1] are lantibiotics (nisin, epidermin, labyrinthopeptin).

Over the past years a deepened understanding of the biosynthesis pathways of RiPPs and NRPS peptides has been achieved. This sets the stage for largely expanding the structural space by engineering biosynthetic pathways and biosynthetic enzymes involved, aiming at new pharmacological properties, e.g. antiallodynic effects.[2,3] More recent discoveries[2] from our group on biosynthesis principles and successes on increasing the structural space by pathway engineering,[3] combinatorial biosynthesis and Synthetic Biology[4] will be presented. On the basis of these results chances and limits of engineering approaches for drug development will be discussed.

Keywords: lantibiotics, Ribosomal biosynthesis, Secondary metabolites, Synthetic biology

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Linquan Bai, China

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TWO CLUSTERS AND THREE MOLECULES: BIOSYNTHESIS OF PYRROLAMIDES IN STREPTOMYCES NETROPSIS

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Pyrrolamides constitute a family of natural products produced by *Streptomyces* and related actinobacteria. Their ability to bind DNA in the minor groove confers on them a variety of biological activities, such as antimicrobial and antitumoral activities. In 2009, we isolated and characterised the first gene cluster directing the biosynthesis of a pyrrolamide, the congocidine gene cluster of *S. ambofaciens*. Using a combination of molecular genetics and analytical chemistry, we identified the precursors of congocidine, guanidinoacetate, 3-aminopropionamidine and 4-acetamidopyrrole-2- carboxylic acid. We proposed the biosynthetic routes to these precursors, all unprecedented in the literature. The pathway to 4-acetamidopyrrole-2-carboxylic acid is particularly original, starting from N-acetylglucosamine and involving carbohydrate-processing enzymes. To pursue our work on pyrrolamides, we recently examined the strain *Streptomyces netropsis* DSM40843, known to produce distamycin. We showed that this strain also produces congocidine and a congocidine/distamycin hybrid we named disgocidine. Isolation of the genes involved in the biosynthesis of these molecules revealed two clusters that are both required for the synthesis of any of three pyrrolamides. Analysis of these clusters allowed us to propose a biosynthetic pathway for congocidine and distamycin biosyntheses. It also revealed that disgocidine biosynthesis results from natural combinatorial biosynthesis (combination of enzymes from different pathways). We are currently undertaking the development of a combinatorial biosynthesis and mutasynthesis-based approach to synthesise new pyrrolamide derivatives.

Keywords: pyrrolamide, biosynthesis, Streptomyces, combinatorial biosynthesis

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THE UPCOMING ROLE OF ACTINOMYCETES IN THE STRAIN COLLECTION OF THE HELMHOLTZ CENTRE FOR INFECTION RESEARCH (HZI)

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The focus of the HZI has been on Myxobacteria for a long time. As Actinomycetes are still today a very important and successful group for the detection and isolation of novel biological active compounds especial antibiotics. So since 2012 a number of Actinomycetes strains and Projects were incorporated into the work of the microbial strain collection group MISG which was founded in 2013. The collection includes about 2000 reference strains of the class Actinobacteria and more than 600 new isolates of uncommon genera which have been isolated in-house or are part of external cooperation's.

Aims of the strain collection are:

1. Myxobacteria and Actinobacteria

   Novel isolates as source for new antibiotics

   Development of new isolation methods
2. Induction of "silent" genes
3. Establishing incubation systems in microtiter format for induction of silent gene clusters
4. Taxonomy within the "Oldie approach
5. Compendium of Actinobacteria

The function of the microbial strain collection Actinobacteria within the HZI structure is shown and examples of the different aims are given in the talk.

Keywords: Secondary metabolites, Antibiotics, Taxonomy, Induction

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ACTINOMYCETES MICROBIAL COLLECTIONS REMAIN UNTAPPED SOURCES FOR THE DISCOVERY OF NOVEL NATURAL PRODUCTS

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MEDINA is a non-profit research center focused on the discovery of novel drug candidates from microbial natural products with more than 50-years of inherited experience in drug discovery from the former Basic Research Center at MSD and one of the richest and most diverse microbial collections currently in expansion.

One of the objectives at Fundación MEDINA is to enrich our natural products libraries with new and diverse strains from untapped environments, and especially Natural Parks in Andalucía, and to continue to exploit the existing microbial collection through culture-based approaches in order to succeed in maximizing the chemical diversity of our libraries. Current NPs libraries harbor more than 130,000 fungal and bacterial extracts derived from only a fraction of our 116,000 strains microbial collection, and are at the origin of our collaborative research programs for the discovery of new leads.

Our research in microbial natural products is focused on the identification of novel compounds with biological activity as potential new leads to respond to unmet needs in four major therapeutic areas including infectious and neglected parasitic diseases, cancer and neurodegeneration. As a result of these screening programs, we have identified novel molecules with interesting new chemistry and biological activities. A selection of success cases that support the approaches developed in our center will be discussed.

Keywords: natural products, actinomycetes, screening, diversity

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RATIONAL ENGINEERING FOR PRODUCING OLD AND NOVEL GLYCOPEPTIDE ANTIBIOTICS

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Glycopeptide antibiotics (GPAs) are considered drugs of last resort for the treatment of life-threatening infections caused by relevant Gram-positive human pathogens such as *Staphylococcus aureus, Enterococcus* spp. and *Clostridium difficile*. The emergence of GPA resistant clinical isolates first among enterococci and then in staphylococci has prompted the research for second generation GPAs and a flurry of activity aimed at understanding resistance mechanisms and their evolution. GPAs are glycosylated non-ribosomal peptides produced by a diverse group of soil actinomycetes. They target Gram-positive bacteria by binding to the acyl-D-alanyl-D-alanine (D-Ala-D-Ala) terminus of the growing peptidoglycan on the outer surface of cytoplasmic membrane. GPA resistant organisms avoid such a fate by replacing D-Ala-D-Ala terminus with D-alanyl-D-lactate (D-Ala-D-Lac) or D-alanyl-D-serine (D-Ala-D-Ser), thus markedly reducing antibiotic affinity for cellular target. In most GPAs-producing actinomycetes, antibiotic biosynthetic genes are clustered and co-regulated with resistance genes, that encode proteins reprogramming cell wall biosynthesis, thus evading the self-produced antibiotic action. We are investigating regulation of genes devoted to antibiotic production and self-resistance, focusing on *Actinoplanes teichomyceticus* that produces teicoplanin and *Nonomuraea* sp. ATCC 39727 which is the producer of A40926. A40926 is a teicoplanin-like antibiotic that is the natural precursor of the second generation semi-synthetic dalbavancin, recently approved for clinical use in acute bacterial skin and skin structure infections. Our results demonstrate that a thorough understanding of the molecular mechanisms regulating antibiotic production and self-resistance may enable knowledge-based approaches for rational engineering, replacing or complementing classical strain improvement (based on recursive mutagenesis and massive phenotype-based screening), thus facilitating old and novel GPAs-production processes.

Keywords: Glycopeptide antibiotics, rational engineering, strain improvement, self-resistance

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STREPTOMYCES AS BIOACTIVE COMPOUNDS PRODUCERS: AN IMPORTANT GENUS IN MICROBIAL COMMUNITY

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It is well known that members of the genus *Streptomyces*, a very interesting genus of the Actinobacteria family, are the producers of the most of the known bioactive compounds, which include antibiotics, enzymes, growth promoters, agents for plant protection, antiparasitic agents and herbicides. The need for more natural products is profound and amongst the microbiologists is clear that the answer is the extensive study of the microbial community in the planet. Companies and scientists collect samples from particular habitats, from different parts of the world in order to screen for antibiotic/or other products. Data from researches have showed that Greek strains were the most active ones compared with other isolates from European countries. The Microbiology group of the University of Athens has studied the *Streptomyces* diversity in Greek habitats for over 25 years and a collection of very important isolates have been selected from ecosystems which display either typical Mediterranean climatic conditions or extreme conditions like volcanoes or polluted areas. The isolates were identified at the strain level; they were studied phenotypically, physiologically and genetically and were examined for their biotechnological applications, including the potential of their usage on the field of biological control of soil borne diseases. Almost one third of the Greek *Streptomyces* isolates found to be antagonistic against the common phytopathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum*. The isolates with the stronger antifungal activity *in vitro* proved to be very effective biocontrol agents during *in vivo* processes, as they could protect target plants from the examined fungi. The culture extracts of the selected *Streptomyces* isolates were biochemically analyzed in order to isolate and examine their antifungal bioactive compounds.

Keywords: Streptomyces, soil borne diseases, biological control

CHROMOSOME SEGREGATION IN *STREPTOMYCES COELICOLOR*

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The reconciliation of monodirectional tip extension with bidirectional chromosome segregation represents a key challenge to organisms that undergo polarized growth such as *Streptomyces coelicolor*. In order to understand this process, we have developed Fluorescence Reporter Operator System (FROS) in *S. coelicolor*. We used *in vitro* transposon mutagenesis to deliver 120 copies of the *tet*operator (*tetO*) arrayed in tandem to an *oriC*-proximal site within the *S. coelicolor* chromosome. By fusing the cognate repressor protein (TetR) to mCherry and eGFP, we have visualized binding of TetR to the tandem *tetO* array and, as a result, the location of *oriC* during chromosome replication through tip extension, erection of aerial hyphae and sporulation. Using time lapse fluorescence microscopy we have generated a model describing chromosome segregation in streptomycetes that permits colonization chromosome colonization of the extending tips as well as apex-distal branches.

Keywords: Chromosome segregation, Fluorescence reporter operator system

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DISCOVERY OF NOVEL NATURAL PRODUCTS VIA SYNTHETIC BIOLOGY

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Microorganisms are a major source of new therapeutic agents. Sequenced genomes and metagenomes provide a tremendously rich source for discovery of novel gene clusters involved in natural product biosynthesis. However, due to the lack of tools to efficiently identify a complete biosynthetic gene cluster, determine the role of each involved gene, and subsequently designate a function to the target gene cluster, only a tiny fraction of those putative natural product biosynthetic gene clusters have been characterized (1). To overcome this limitation, my group has developed a new genomics-driven, synthetic biology enabled method to discover and produce novel natural products from sequenced genomes and metagenomes. By taking advantage of the highly efficient yeast in vivo homologous recombination mechanism, this method refactors the target cryptic pathway together with the genetic elements needed for DNA maintenance and replication in S. cerevisiae, E. coli, and a target heterologous host using a plug-and-play scaffold and a set of heterologous promoters that are functional in the target heterologous host. As proof of concept, we awakened the silent polyketide spectinabilin pathway from Streptomyces spectabilis in Streptomyces lividans (2) and activated a cryptic pathway containing a polyketide synthase-non-ribosomal peptide synthetases from Streptomyces grieseus in Streptomyces lividans, which led to the discovery of two novel tetramic acid natural products that have never been reported in literature (3). Our method bypasses the traditional laborious processes to elicit pathway expression and represents a new platform for discovering novel natural products (4). Currently, in collaboration with other research groups, we are establishing a fully integrated robotic system to automate all the steps in gene cluster refactoring and product detection, which should significantly increase the throughput of our method and discover novel pharmaceutically important natural products.

Keywords: synthetic biology, natural product discovery, biosynthesis

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INDUCIBLE GENE EXPRESSION SYSTEMS IN S. COELICOLOR BY MEANS OF SYNTHETIC RIBOSWITCHES

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We will report about the application of synthetic theophylline-dependent riboswitches for conditional gene expression in *Streptomyces coelicolor.* Application of the method requires a sequence of only ~85 nt to be inserted between the transcriptional start site and the start codon of a gene of interest. No auxiliary factors are needed. All tested riboswitch variants worked well in concert with the promoters *galP2, ermEp*1 and SF14. Moreover, they allowed theophylline-dependent expression not only of the heterologous *b*-glucuronidase reporter gene but also of *dagA*, an endogenous agarase gene. The right combination of the tested promoters with the riboswitch variants allows for the adjustment of the desired dynamic range of regulation in a highly specific and dose-dependent manner and underlines the orthogonality of riboswitch regulation. We anticipate that any additional natural or synthetic promoter can be combined with the presented riboswitches. Moreover, this system should easily be transferable to other *Streptomyces* species, and most likely to any other genetically manipulable bacteria.

Keywords: Synthetic riboswitches, theophylline aptamer, conditional gene expression

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TOWARDS BIOTECHNOLOGY 2.0: SYNTHETIC BIOLOGY OF BIOACTIVE MOLECULES

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Our ability to readily sequence complete genomes and to manipulate/re-design them on a large scale enables the design and construction of organisms with new functionalities of unprecedented scope (“synthetic biology”). We explore these possibilities in the context of bioactive molecule production. Many microorganisms already have the machinery to produce diverse bioactive molecules. As a first step towards re-engineering bioactive molecule biosynthesis for enhanced productivity and diversity, we aim to understand the possibility of catalytic parts exchange. In addition, we are expanding our collection of computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters, to enrich our library of parts and building blocks for pathway engineering. Furthermore, we are using computational modelling (constraint-based descriptions of bacterial metabolism) to identify suitable overproduction hosts and pinpoint biosynthetic bottlenecks to target for further cellular engineering in a synthetic biology strategy.

Keywords: Synthetic biology, antibiotic production

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GENOMICS OF MARINE ACTINOMYCETES FROM THE TRONDHEIM FJORD, NORWAY: TRANSFER AND EVOLUTION OF SECONDARY METABOLITE BIOSYNTHESIS GENE CLUSTERS

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Marine sponges are known to host a wide variety of bacteria, which can represent up to 35% of their biomass [1]. Among these bacteria, actinomycetes deserve special attention due to their ability to produce bioactive secondary metabolites. In the course of a bioprospecting program, over 900 actinomycete bacterial isolates were obtained from sponges collected at the bottom of the Trondheim fjord, Norway. 16S rRNA-based phylogenetic analysis of a part of this collection revealed representatives of at least 12 actinomycete genera. Complete genome sequence analyses of two *Actinoalloteichus* spp. from sponges *Geodia barrette* and *Antho dichotoma* suggested recent acquisition of their ancestor by the sponges. However, clear differences, possibly related to host adaptation or interaction with the sponge microbiome could be revealed. The same trend was observed after analyses of the genomes of *Streptomyces albus* isolates originating from sponges *Geodia barrette* and *Phakelia ventilabrum*[2]. Comparison of genomes of 9 *Streptomyces* species isolated from *Antho dichotoma* strongly suggested their relatedness to certain terrestrial streptomycetes. At the same time, sponge-derived *Streptomyces* displayed distinct profiles of secondary metabolite biosynthesis gene clusters. Based on these and other observations, possible implications of host-dependent adaptation and gene transfer for evolution of secondary metabolite biosynthesis in sponge-associates actinomycetes will be discussed.

Keywords: Marine actinomycetes, Comparative genomics, Secondary metabolite biosynthesis gene clusters

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Nevzat Sahin, Turkey

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Russell Kerr, Canada

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ORAL PRESENTATIONS

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PHYLOGENOMIC ANALYSIS OF *SALINISPORA* SPECIES

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Salinispora is a marine actinomycete and an important producer of diverse secondary metabolites including anticancer and antibiotic compounds. This genus is comprised of 3 species (*S. tropica, S. arenicola*, and *S. pacifica*) that are closely related based on the highly conserved 16S rRNA gene. Multilocus sequence analyses (MLSA) of 5 housekeeping genes revealed a well-supported concatenated tree providing strong support for the delineation of the three species and the ancestral relationship of *S. arenicola* to the more recently diverged sister taxa *S. tropica* and *S. pacifica*. Recently, our laboratory obtained 86 draft *Salinispora* genomes, which provide an unprecedented opportunity for comparative genomic analysis. Genome analysis revealed that 2479 single-copy genes are present in each of the 86 strains, representing a core genome of approximately 49% of the average gene content of the 3 species. Eliminating genes that showed evidence of recombination resulted in a pool of 1286 genes (recombination-free) that were used to generate a concatenated phylogeny for the genus. This detailed phylogeny reveals new intra-species relationships and represents a unique opportunity to explore in more detail the genetic diversification of *Salinispora* species. The genome sequences are also being used to identify shared genes that have been duplicated or occur near or within secondary metabolite biosynthetic pathways to address the hypothesis that these genes confer resistance and can be used to help identify the molecular targets of secondary metabolites. Results from these analyses will be presented.

Keywords:*Salinispora*, Phylogenomics, Core genome

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INSIGHT INTO THE RELATIONSHIP BETWEEN ANTIBIOTIC PRODUCTION AND PHYLOGENETIC-CLASSIFICATION OF *RHODOCOCCUSERYTHROPOLIS* STRAINS

Wataru Kitagawa

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Antibiotic production ability of genus *Rhodococcus* has been recently reported and investigated. Some antibiotic compounds isolated from the genus such as aurachin RE and the biosynthesis genes have been also studied. Of these antibiotic-producing *Rhodococci*, 15 strains were identified in *Rhodococcus erythropolis*. These 15 strains can be divided into 3 groups by its antibiotic property. Strains in each of the 3 groups showed growth inhibition activity against the other 2 groups, but not against members in the same group. These findings indicate that they produce group's respective antibiotics, and also imply that intraspecies competition between *R. erythropolis*. However, the detailed phylogenetic relationship of them remained unknown. In order to analyze the phylogenetic classification and intraspecies diversity, and also to understand the relationship between the antibiotic production and the classification, phylogenetic analyses were demonstrated in detail.

In this study, 16S rRNA gene nucleotide sequence, multi-locus sequence analysis (MLSA), and MALDI-MS were employed for the analysis. In addition to the 15 antibiotic producers, 6 non-antibiotic producing strains (a total of 21 strains) of *R. erythropolis* were used. The 16S rRNA gene analysis demonstrated that these 21 strains shared almost identical sequence (over 99.5 % identity). It indicated that the resolution of 16S analysis is too low to see the intraspecies diversity. Moreover, multiplicity and intragenome heterogeneity of the gene sequences were discovered. Therefore, we concluded that 16S rRNA gene analysis could not be applied for the detailed classification. For MLSA analysis, the nucleotide sequences of 5 independent housekeeping genes (*gyrB*, *rpoB*, *recA*, *trpB*, and *atpD*) were analyzed and combined in silico. The concatenated gene sequences of these strains were used for the phylogenetic analysis. For MALDI-MS analysis, ribosomal proteins of each strain were prepared and subjected to the molecular mass analysis. The mass peak patterns were then used for cluster analysis followed by construction of dendrograms.

As the results, both MLSA and MALDI-MS analysis gave good resolution for the intraspecies classification. The topologies of the phylogenetic trees were quite similar with each other. On top of that, the phylogenetic classification was well matched to the antibiotic groupings; the members in the same antibiotic group falls into same branch in the trees. These results demonstrated that the 21 strains of *R. erythropolis* were actually close relatives, in particular, members between in the same antibiotic group were highly close relatives. This is the first report that several strains in the same species, sharing same 16S rRNA gene, produce different antibiotics. By MLSA analysis, *gyrB* or *rpoB* sequences mainly contributed to the resolution of intraspecies classification. Only the nucleotide sequence analysis of *gyrB* or *rpoB* may help to identify some novel antibiotic producers in genus *Rhodococcus*.

Keywords:*Rhodococcus*, Antibiotics, Intraspecies diversity, Intraspecies competition, MLSA

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SINGLE AMINO ACID SUBSTITUTIONS IN SSGB HAVE MAJOR CONSEQUENCES FOR THE CONTROL OF CELL DIVISION IN *STREPTOMYCES COELICOLOR*

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Streptomycetes are multicellular bacteria with a complex life cycle. During vegetative growth, occasional cross-walls are formed that divide the vegetative hyphae into connected compartments. During the reproductive phase, sporogenic aerial hyphae differentiate into chains of up to a hundred spores via a highly coordinated cell division process that involves the formation of many septa in a short time span. This can be visualized as spectacular ladders of FtsZ (Schwedock 1997). Our research focuses on understanding the regulatory mechanisms that govern this process. The SsgA-like proteins are a family of developmental regulatory proteins that occur exclusively in morphologically complex actinomycetes. SsgA plays a major role in all processes that involve cell-wall remodelling, namely germination, branching, tip growth and cell division. We have shown that - in contrast to in unicellular bacteria like *E. coli* and *B. subtilis* - cell division in streptomycetes is positively controlled, whereby FtsZ is actively recruited to the septum sites by the SsgB protein (Willemse 2011). In turn, the localization of SsgB is mediated by SsgA, although the precise mechanism is yet unknown. SsgB also stimulates the polymerization of FtsZ into protofilaments, and co-localises with FtsZ on the divisome throughout the entire divison process.

SsgB is an extremely well-conserved protein in streptomycetes, whereby only aa residue 128 is variable: T128 occurs in streptomycetes that sporulate in submerged cultures, while Q128 occurs in those that form pellets. A similar level of conservation is found in other actinomycete genera, but the conservation between the orthologues of different genera is surprisingly low (Girard 2013). To investigate the effect of aa substitutions on cell division in *Streptomyces coelicolor*, and to elucidate how SsgB interacts with SsgA and FtsZ, an SsgB mutant library was created and introduced into the *ssgB* null mutant for functional analysis. 40 of these mutants with a single aa change were selected. Eight changes resulted in a complete block of sporulation, whereas all other transformants carrying *ssgB* point mutants showed major defects in spore morphology. Analysis with transmission electron microscopy (TEM) revealed defects in spore-wall thickness, spore-size distribution, DNA segregation and condensation, spore shape and/or septum placement. Surprisingly, we identified specific aa changes that resulted in rotation of the septum, up to a point where 90 degree rotation of the septum plane was achieved, resulting in unique longitudinal division of spores. The implications of these findings for the control of sporulation and a model for the function of SsgB and its interaction with FtsZ and SsgA will be presented.

Keywords: Cell division, Sporulation, FtsZ, SALP, Microscopy

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NOVEL FUNCTION OF CHROMOSOME SEGREGATION PROTEINS IN *STREPTOMYCES HYPHAE* - CHROMOSOME ORGANISATION AT HYPHAL TIPS

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In rod shaped bacteria chromosomes segregation is initiated soon after duplication of the origin of chromosomal region (*oriC*). Segregation proteins ParA (ATPase) and ParB (DNA binding protein) are responsible for a rapid movement of the newly replicated *oriC* regions toward the new locations in the cell. In *Streptomyces* ParA and ParB were shown to assist chromosome segregation during sporulation. ParA forms filaments along sporogenic hyphae, which facilitate formation of ParB nucleoprotein complex at the *parS* sites clustered around *oriC* and guarantee their placement in between developing septa.

Here, using the time lapse microscopy, we have studied the function of ParAB in the vegetative hyphae and their role in chromosome organisation at hypal tips. Our data show that *parAB* deletions affect the position of chromosomes and the rate of hyphae extension. Thus, they reveal novel function of segregation proteins, namely anchoring the chromosome at the growing tip. Our data also suggest that proper organisation of the tip chromosomes is important for the efficient hyphal growth.

Keywords: Tip growth, Chromosome segregation

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***Greg Amos*,**

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PRODUCTION OF ANTIBIOTICS AND OTHER SPECIALIZED METABOLITES IN SOIL AND SAND MICROCOSMS OF *STREPTOMYCES COELICOLOR*

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Streptomycetes are soil dwelling filamentous bacteria, known for their production of bioactive 'specialized' metabolites, previously referred to as secondary metabolites. Genome sequencing of the model species *Streptomyces coelicolor* identified >26 known and predicted (cryptic) specialized metabolite gene clusters, involved in the biosynthesis of multiple classes of bioactive compounds, e.g. antibiotics, siderophores, lipids, pigments and ribosomally-synthesized and post-translationally-modified peptides. Most studies investigating microbial specialized metabolite production are usually undertaken in liquid or on solid media. Information is lacking on gene expression and antibiotics production in soils. The aim of this study was to investigate primary and specialized metabolism in a model *Streptomyces* grown under pseudo-natural conditions in sterile microcosms.

The results from proteomic, transcriptomic, and metabolomic studies of *S. coelicolor* grown in sand or soil microcosms with differing carbon and nitrogen content will be discussed. Interestingly and contrary to the consensus of specialized metabolism commencing after reduction or cessation of growth, this study revealed expression of specialized metabolite biosynthetic genes and proteins at the onset of and during exponential growth in sand and soil. Some specialized metabolite genes/proteins were expressed constitutively, which was further supported by metabolic data. The blue-pigmented antibiotic actinorhodin could be detected throughout the growth phases in sand cosms. 'Secondary' metabolism as a term should therefore be reconsidered under environmental conditions as this study suggests it may play an important specialized, but primary, role in nature.

Keywords: Soil metabolomics, Soil proteomics, Soil transcriptomics, Soil microcosms, Antibiotics, Specialized/secondary metabolism, *Streptomyces*

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UNRAVELLING THE REGULATORY NETWORKS THAT CONTROL SPORULATION IN *STREPTOMYCES*

Matthew Bush, Maureen Bibb, Govind Chandra, Mark Buttner, Kim Findlay

John Innes Centre

The filamentous bacteria *Streptomyces* has a complex yet fascinating life-cycle, culminating in the formation of specialised reproductive structures called aerial hyphae that extend upwards out of the substrate mycelium into the air. Subsequently, the orderly arrest of aerial growth and a remarkable synchronous septation event with concomitant separation of chromosomes occurs to form long chains of 50-100 uni-genomic spores. These two key processes require the developmental master regulators WhiA and WhiB. *whiA*and *whiB* mutants have near identical phenotypes, leading to the suggestion that there exists some level of cooperation in their function. Here, we reveal for the first time, the role of these non-classical transcription factors in *Streptomyces* development and present our ongoing research into the cooperation between WhiA and WhiB.

To better understand the role of WhiA and WhiB in *Streptomyces*, we have identified the WhiA and WhiB regulons through a combination of chromatin immunoprecipitation-sequencing (ChIP-seq) and microarray transcriptional profiling, exploiting a new model organism for the genus, *Streptomyces venezuelae*, which sporulates in liquid culture. *whiA* and *whiB* are both required for the initiation of sporulation septation and chromosome segregation in *S. venezuelae*, and several genes encoding key proteins of the *Streptomyces* cell division machinery, such as *ftsZ*, *ftsW* and *ftsK*, were found to be directly activated by both WhiA and WhiB during development.

Interestingly, the genomes of *Streptomyces* species appear to lack clear homologues of many of the key components of the cell division machinery. Determining the precise position of 50-100 cytokinetic rings presumably requires additional proteins that may be absent in other bacteria. Many of the genes encoding such proteins are likely to be under the regulatory control of WhiA and WhiB. One such gene that I have identified, *flpA,* is critical for proper cell division in *Streptomyces*. High resolution imaging and timelapse microscopy is revealing the role that these proteins play in sporulation.

Keywords: Development, Cell biology, Regulatory neworks, *Streptomyces*

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YLMG CONTROLS THE NUCLEOID SHAPE AND THE LOCALIZATION OF THE CELL DIVISION ACTIVATOR SSGB DURING SPORULATION IN *STREPTOMYCES COELICOLOR*

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During sporulation-specific cell division in *Streptomyces*, the aerial hyphae differentiate into chains of spores via a highly coordinated cell division process whereby many septa are produced in a short time span. Our research focuses on understanding the regulatory mechanisms that govern this process. In contrast to in unicellular bacteria like *E. coli* and *B. subtilis* - cell division in streptomycetes is positively controlled, whereby FtsZ is recruited to the septum sites by the divisome protein SsgB [1]. SsgB is a member of the family of SsgA-like proteins that occur exclusively in morphologically complex actinomycetes and control processes related to peptidoglycan (PG) synthesis and remodelling. It is yet unclear how the localization of the cell division activator SsgB is controlled.

YlmG is a small membrane protein of unknown function. In bacteria, the *ylmG* gene is located between the important cell division genes *sepF* and *divIVA* in the *dcw* cluster of division and cell-wall related genes. Perhaps surprisingly, *ylmG* null mutants of *Streptococcus pneumoniae* did not show major defects in the cell division process. However, YlmG plays a role in controlling nucleoid distribution in chloroplasts of the plant *Arabidopsis thaliana* and in *cyanobacteria*[2]. In this study, we analysed the role of YlmG in *Streptomyces coelicolor*,in particular during sporulation-specific cell division. Deletion of *ylmG* resulted in developmental defects, with the null mutant producing primarily non-differentiated aerial hyphae, while the majority of the spores that were formed were irregularly shaped. The most notable difference between wild type and *ylmG* mutant spores was a dramatic change in spore nucleoid shape. Rather than a well-condensed nucleoid in the center of the spores, transmission electron microscopy combined with STED high-resolution fluorescence microscopy revealed that the *ylmG* null mutant spores had a less condensed nucleoid that localized towards the edge of the spores, whereby the nucleoid had a donut-like shape. During sporulation-specific cell division, YlmG-eGFP localised at sites of active PG synthesis or remodeling, and DNA and YlmG were found to be mutually exclusive. Furthermore, preliminary evidence suggests a direct interaction between YlmG and SsgB, and SsgB failed to localise to septum sites in *ylmG* null mutants, providing a plausible explanation for the highly reduced and aberrant sporulation. Our data suggest a role for the nucleoid in controlling the localization of SsgB during the initiation of sporulation-specific cell division in streptomycetes, whereby YlmG ensures that nucleoids are kept away from sites of active peptidoglycan synthesis or remodeling sites to avoid DNA damage during cell division. A model to explain our data is presented.

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CELL ARCHITECTURAL RESPONSES TO OSMOTIC STRESS IN *STREPTOMYCES*

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It is essential for bacterial life to be able to adapt quickly to environmental stress. Osmotic stress is one of the most common stresses that bacteria encounter, and can cause fatal consequences such as shrinkage or bursting of cells upon hyper-osmotic or hypo-osmotic stress, respectively. Bacteria have been shown to mount sophisticated responses to deal with environmental stress, but differently from eukaryotic cells, little is known about how cell architecture is affected by stress, and the role of cytoskeleton in coping with stress, in bacteria.

In this study, we examined cell-biological responses of *Streptomyces coelicolor* upon up- and down-shift of osmotic pressure of the growth medium, including the roles of the intermediate filament-like protein FilP, and the growth determinant DivIVA.

Upon hyper-osmotic stress (addition of 1M sucrose or 0.5M NaCl) we observed by using time-lapse imaging, that hyphal growth at existing tips was arrested for 2-3 hours, and re-initiation of growth occurred exclusively via establishment of multiple new branches along lateral walls, in a synchronized fashion throughout the sample.

To understand this observed phenomenon better, sub-cellular localization of DivIVA-EGFP and FilP-Ypet during hyper-osmotic stress response was investigated using fluorescence microscopy. Interestingly, these two cytoskeletal proteins remained at their pre-stress localizations (tips for DivIVA, apical gradients for FilP) during the entire growth arrest period, and underwent dynamic rearrangement of their sub-cellular localizations only right before hyphae exhibited lateral re-growth. We also visualized nucleoids during stress response by using a strain expressing HupA (*Streptomyces* nucleoid associated protein) fused to EGFP. We observed condensation of nucleoids soon after hyper-osmotic shift, which was released just before regrowth. DNA relaxation temporally coincided with the dynamic spatial arrangement of DivIVA and FilP.

Then, we examined if known transcriptional regulators in hyper-osmotic stress response are involved in the establishment of the re-growth pattern that we have observed. To our surprise, mutants lacking regulators such as *sigB*,*sigH*, or *osaB* performed identically to wildtype under hyper-osmotic stress.

We also show and discuss results concerning hypo-osmotic stress response of *Streptomyces* hyphae.

In summary, we have observed a unique cellular response of *Streptomyces* upon hyper-osmotic stress, leading to redistribution of polar growth sites. This cell-biological stress response can be divided into three phases. First, there is a long phase of growth arrest, during which the cells seem to stay in a non-dynamic, "frozen" state, despite known changes in gene expression pattern. Secondly, just before growth resumes, the cells again enter a dynamic state, enabling structural proteins and nucleoids to be rearranged for re-growth. Old polarisomes and FilP gradients at existing tips are dismantled, new polarity centers are established at lateral walls, and nucleoids resume a normal uncondensed state. Thirdly, growth resumes by extensive synchronous lateral branching.

Keywords:*Streptomyces*; Coiled coil; Osmotic stress; Polar growth; Nucleoid

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SSBB PLAYS AN IMPORTANT ROLE IN CHROMOSOME SEGREGATION DURING REPRODUCTIVE GROWTH OF *STREPTOMYCES COELICOLOR*

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Single stranded DNA binding proteins (SSB) proteins are essential for cell survival in all domains of life. The SSB proteins bind ssDNA with a high affinity and in a sequence independent manner. Thus these proteins protect transiently formed ssDNA in the cell during DNA metabolism and prevent formation of unproductive secondary structures. In addition, during DNA replication, recombination and repair SSB proteins interact and modulate the functions of various proteins involved in all aspects of DNA metabolism. Our recent bioinformatics analysis of available bacterial genomes revealed the presence of the SSB parologues in many bacteria and indicated that evolution of SSB proteins in Eubacteria is highly dynamic. However the role of duplicated SSB proteins is poorly studied. In this study we selected *Streptomyces coelicolor*, bacterium with complex life style that also possesses two SSBs, as a good model system to study biological role(s) of paralogous SSB proteins. Differential regulation of paralogous *ssb* genes in *S. coelicolor* suggested different roles for their products. Expression of *ssbA* is constant and high during life cycle, decreasing towards late stationary phase, while the expression of *ssbB* is at all-time low. In minimal medium the *ssbB* is significantly upregulated. Promoters for both genes are determined. Surprisingly, promoter of *ssbB* possesses unusual features and is active in taxonomically distant *Escherichia coli*. Results of the gene disruptions strongly indicated that SsbA is essential for survival while SsbB is important during the sporulation process. To get a better insight into the role of SsbB and the complex mechanism of cell division in *Streptomyces* we have constructed double and triple mutant strains carrying mutations in *ssbB* gene and genes reported previously to be important for the chromosomal segregation (*parB* and *smc*). By fluorescence microscopy we showed the effect of these mutations on chromosome segregation. In addition to genetic analysis we solved the 3D structure of SsbB and showed an interesting variation in comparison to quaternary structure of the previously published *S. coelicolor* SsbA protein. To the best of our knowledge this is the first example of solved crystal structures of two paralogous SSB proteins from the same organism.

Keywords: Paralogous SSB proteins, Chromosome segregation, *S. coelicolor*

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DIVERSITY AND NATURAL PRODUCTS FROM MARINE SPONGE-ASSOCIATED ACTINOMYCETES

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University of Wuerzburg

Actinomycetes are known for their unprecedented ability to produce novel lead compounds of clinical and pharmaceutical importance. This presentation focuses on the diversity, abundance, and methodological approaches targeting marine sponge-associated actinomycetes. Novel qPCR data on actinomycete abundances in different sponge species and other environmental sources are presented. Sponge-associated actinomycetes are further a good source for novel bioactive compounds. Methodological approaches encompassing co-cultivation and elicitation experiments, bioassay-guided isolation, as well as –omics (genomics, metabolomics) are employed towards this goal. Our results highlight marine sponges as a prolific resource for taxonomically novel and rare actinomycetes with potential for drug discovery.

Keywords: Sponges, Actinomycetes, Diversity, Abundance, Natural products

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ABUNDANCE OF ACTINOBACTERIAL CELLULASE GENES IS CORRELATED WITH CARBON FRACTIONS IN WOODLAND SOIL

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Cellulose is the most abundant form of fixed carbon (C) in the biosphere, and fungi are thought to play a greater role in its degradation in terrestrial environments than bacteria. However, detailed investigation of the role of different microorganisms in cellulose degradation is hindered by the complexity of cellulotlytic systems and the genes involved. Glycoside hydrolase family 48 gene (GH48) is one of the few glycoside hydrolase gene families that consistently function as a cellulase gene in bacteria. In this study, primers were designed to target the GH48 gene from actinobacteria, which are abundant in soils. The GH48 primers were used to quantify diversity and abundance of actinobacteria with potential for cellulose-degradation in 3 paired pasture-woodland sites for which soil biogeochemical parameters, including organic C fractions, were also measured. Phylogenetic analysis of clones generated revealed a significant diversity of uncultured GH48 genes in the soils, and 76 out of 80 putative cellulase gene sequences obtained were actinobacterial. T-RFLP analysis showed that GH48 gene diversity was significantly affected by land-use. Quantification by qPCR showed that the abundance of GH48 genes was correlated to particulate, recalcitrant and humic organic carbon fractions predicted by mid-infrared spectroscopy in most of the woodland soils. The high abundance of this gene and its correlation to soil C suggests that actinobacteria may contribute to cellulose degradation in soil, and that this group of bacteria might thus compete with fungi for access to and turnover of C in plant litter in terrestrial environments.

Keywords: Cellulases, Carbon cycle, Soil, Glycoside hydrolases

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TRANSCRIPTOME SEQUENCING FOR DECIPHERING REGULATORY NETWORKS IN ACTINOMYCETES

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Center for Biotechnology, Bielefeld University

High-throughput sequencing has recently also revolutionized transcriptome analysis. The techniques known as RNAseq deliver single-nucleotide resolution and an unmatched dynamic range to transcript analysis and quantification in a genome-wide manner. RNAseq is also suitable to discover hitherto unknown transcripts, providing numerous small RNAs and novel regulatory elements throughout a bacterial genome.

We have recently optimized transcriptome sequencing methods and applied them to different actinobacterial species such as *Corynebacterium glutamicum*, *Mycobacterium tuberculosis*, *Actinoplanes* sp. and *Streptomycetes*. The results obtained were used to improve genome annotation and describe several transcriptomic features of Actinobacteria in great detail. Among other findings, all analyzed species show high numbers in leaderless mRNAs, with *C. glutamicum* (33% of all mRNAs) as an extreme example. Further findings include high numbers of antisense transcripts, an example of which was functionally analyzed.

Besides providing a 'static' view on the transcriptome (i.e. description of all RNAs), RNAseq is also a perfect tool for analyzing transcriptome dynamics and proved to be superior to microarrays due to the features described above. We applied RNAseq on mutants in sigma and anti-sigma factor genes of *C. glutamicum* and were able to obtain deep insights into their regulatory networks. Even sigma factor crosstalk at promoters was resolved by developing an *in vitro* approach, a genome-wide Run-Off transcription/SEquencing method (ROSE). ROSE allows to identify all promoters of a specific sigma factor in a single experiment.

The presentation will address the key issues of the RNAseq method, application examples from different actinobacteria, and a detailed description of ROSE and the findings made on ECF sigma factor-networks in *C. glutamicum*.

Keywords: Transcriptome, RNAseq, *Corynebacterium glutamicum*, Sigma factor

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IPSA, A NOVEL LACI-TYPE REGULATOR REQUIRED FOR CELL WALL BIOSYNTHESIS IN CORYNEBACTERIA AND *MYCOBACTERIA*

Meike Baumgart,Kerstin Luder, Shipra Grover , Cornelia Gätgens, Gurdyal S Besra, Julia Frunzke

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Introduction: The development of new drugs against tuberculosis and diphtheria is focused on disrupting the biogenesis of the cell wall, the unique architecture of which confers resistance against current therapies. The enzymatic pathways involved in the synthesis of the cell wall by these pathogens are well understood but the underlying regulatory mechanisms are largely unknown. While inositol is an essential polyol in eukaryotes, in eubacteria it is present only in the order *Corynebacteriales* where it serves both as a cell wall component and as a building block of mycothiol, the major antioxidant in *Mycobacteria.*

Objectives: IpsA, a LacI-type transcriptional regulator conserved among *Mycobacteria* and *Corynebacteria,* was chosen as a target for our studies because a *Corynebacterium glutamicum*Δ*ipsA* mutant displayed a drastically altered cell morphology hinting towards a function in the control of cell wall biosynthesis. The aim of this study was the elucidation of the underlying regulatory mechanisms leading to the described phenotype.

Methods: We applied a wide range of molecular biology and protein biochemical methods including DNA microarrays, fluorescence microscopy, electrophoretic mobility shift assays (EMSAs), high-throughput growth analysis, promoter studies using fluorescent proteins as well as analysis of cell wall lipids and lipoglycans by thin layer chromatography.

Results: IpsA was shown to play an important role in the regulation of cell wall biogenesis. IpsA triggers *myo*-inositol formation by activating *ino1*, which encodes the inositol-phosphate synthase. An *ipsA* deletion mutant of *C. glutamicum* grown on glucose displayed significantly impaired growth, an elongated cell morphology and uneven DNA distribution, all of which could be complemented by inositol instead of glucose as carbon source. Further studies revealed the absence of inositol-derived lipids in the cell wall and a complete loss of mycothiol biosynthesis. The phenotype of the *C. glutamicum ipsA* deletion mutant was complemented to different extent by homologs of *Corynebacterium diphtheriae* (dip1969) and *Mycobacterium tuberculosis* (rv3575), indicating the conserved function of IpsA in these pathogenic species. Additional targets of IpsA with putative functions in cell wall biogenesis were identified and IpsA was shown to bind to a conserved palindromic motif within the corresponding promoter regions. *Myo*-inositol was identified as an effector of IpsA, causing the dissociation of the IpsA-DNA complex *in vitro*.

Conclusion: This characterization of IpsA function and of its regulon sheds light on the complex transcriptional control of cell wall biogenesis in the mycolata taxon and generates novel targets for drug development.

Keywords:*Corynebacteria*, *Mycobacteria*, Cell wall biosythesis, Mycothiol, Inositol

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EVOLUTION OF VIRULENCE IN *RHODOCOCCUS EQUI*, A PARASITE OF MACROPHAGES

Raúl Miranda-CasoLuengo[1,*], Alexandra A. Miranda-CasoLuengo[1,*], Garry B. Coulson[2,*], Xiaoguang Wang[1], Jenna Adair[2], Jennifer M. Willingham-Lane[2], Mary K. Hondalus[2] and Wim G. Meijer[1]

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The actinomycete *Rhodococcus equi* is a facultative intracellular pathogen, infecting humans, foals and a range of other animals, resulting in pyogranulomatous cavitating pneumonia, ulcerative enteritis and occasionally osteomyelitis and cerebral infections. It is a parasite of macrophages, preventing phagosomal maturation in a process that is dependent on a pathogenicity island (PAI) present on a virulence plasmid. The PAI encodes a family of virulence associated proteins (Vap), one of which is essential for virulence (VapA). We recently solved the structure of these proteins and identified a protein interaction network in which VapA is central. The PAI has a different G+C content than the remainder of the chromosome, suggesting it was most likely acquired by the avirulent ancestor of R. equi via lateral gene transfer. Although the PAI encoded Vap proteins play a critical role in virulence, their presence alone is not sufficient to render an avirulent strain virulent. A genome wide transcriptomic analysis revealed that the presence of the virulence plasmid affects approximately 20% of the transcriptome. We have examined the DNA-protein interaction of a PAI encoded transcriptional regulator with the chromosome, showing that this regulator takes over from chromosomally encoded regulators to dramatically alter gene expression. These data indicate that the emergence of virulence in a non-pathogenic ancestral bacterium was a gradual process that was initiated by the acquisition of a virulence plasmid, followed by alteration of chromosomal gene expression patterns resulting in an adjustment of bacterial physiology to suit the environment of the infected host.

Keywords: Virulence, Evolution, Macrophage, Transcriptome, Virulence plasmid

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ENDOLICHENIC ACTINOMYCETES AND THEIR ROLE IN THE LICHEN SYMBIOTIC ASSOCIATION

Oddur Vilhelmsson

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Although generally described as bipartite mutualistic associations of a fungus and an alga or cyanobacterium, lichens are also known to harbour species-specific communities of endolichenic bacteria. Among these, actinomycetes, including members of the Frankineae, Corynebacterineae and Micrococcineae, are typically found in large numbers. The functional roles and colonization mechanisms of these endolichenic bacteria are largely unknown.

We investigated the bacterial community associated with *Peltigera membranacea* using metagenomic and culture-dependent methods. Analysis of 28,000 bacterial contigs from the *Peltigera membranacea* metagenome indicated the presence of a diverse community that, while dominated by Proteobacteria, also contained a sizable population of actinomycetes. Among these, members of the Frankineae, Corynebacterineae and Micrococcineae are prominent. Functional analysis yielded multiple hits on several genes with putative roles in biopolymer degradation, lichen secondary metabolite resistance, inorganic phosphate mobilization, and several other potentially important functions in thallus colonization and symbiosis. We have cultured and isolated from lichens representatives of several of the most prominent actinobacterial taxa indicated by the metagenomic analysis to be present in the *P. membranacea* thallus. Phenotypic analysis of selected isolates indicated that biopolymer hydrolytic activity is common among these bacteria, as is inorganic phosphate mobilization and nitrogen fixation, suggesting nutrient scavenging and degradation of senescing thallus as likely major roles of the endolichenic actinomycetes.

Keywords: Lichens, Symbiosis, Metagenomics, Frankineae

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DEATH BY WORM-STAR: *LEUCOBACTER* ARE DIVERSE NATURAL PATHOGENS OF CAENORHABDITIS

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Three novel nematopathogenic *Leucobacter* have been isolated from nematodes, and display fascinating modes of pathogenicity.

An unknown actinomycete, CBX151, was isolated from nematodes living on a rotting banana trunk in the Cape Verde islands and identified as a novel subspecies of *Leucobacter celer*. When introduced to the laboratory nematode *Caenorhabditis elegans*, CBX151 adhered to the nematode cuticle in a thick “fur coat” and impaired worm movement on solid medium. Infected worms showed upregulation of an antimicrobial peptide but otherwise tolerated the infection and had a normal lifespan. However, when infected worms were added to an aqueous medium they immediately began to aggregate by their tails into a rosette-like formation, or “worm-star”, from which it was almost impossible to escape. Worms trapped in stars died within 24 hours and CBX151 proliferated using the worm carcases as a nutrient source. This remarkable phenomenon relies on exceedingly robust adhesion between the bacteria and the worm cuticle, and may be linked to the biofilm-forming capabilities of the bacterium. Intriguingly, the closely-related *L. celer* does not induce worm-star formation suggesting that this ability may be a recent adaptation of CBX151 to exploit the nematode host.

Another strain, CBX152, was isolated as a co-infection with CBX151 but identified as a distinct novel species of *Leucobacter*. CBX152 does not adhere to the worm cuticle but instead causes a distinctive rectal swelling and is usually lethal within 24 hours of infection. Infection with CBX152 induces a strong immune response in the worms which includes the formation of large vesicles in the body cavity. A third strain, CBX130, was isolated from nematodes found in an aviary in Kakegawa, Japan and causes a similar rectal infection to CBX152. The Japanese strain, however, is slower-growing than CBX152 and elicits a weaker immune response and significantly less worm mortality. Phylogenetic analysis showed that CBX152 and CBX130 are subspecies forming a novel *Leucobacter* species that is most closely related to the non-nematopathogenic *Leucobacter chromiiresistens*.

All three strains have been extensively characterised using chemotaxonomic techniques and whole-genome sequencing. The remarkable adhesion and pathogenicity of the bacteria have been studied in *in-vivo* assays using wild-type *C. elegans* as well as a variety of mutant nematode strains which show varying levels of susceptibility to *Leucobacter* infection. Surprisingly, *C. elegans* mutations which confer resistance to CBX152 frequently induce hypersusceptibility to CBX151.

The fascinating worm-trapping strategy exhibited by CBX151 opens new avenues of inquiry into the mechanisms by which actinomycete pathogens exploit host resources. In light of these recent discoveries it is becoming clear that *Leucobacter* is an important genus for the study of nematode pathogens, and the wider field of host-pathogen interactions.

Keywords:*Leucobacter,* Nematode, Pathogen, Worm-star

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NOVEL SECONDARY METABOLITES FROM *SALINISPORA* THROUGH MASS SPECTROMETRY-GUIDED GENOME MINING APPROACHES - NEW STRATEGIES TOWARDS MORE RATIONAL AND HIGH-THROUGHPUT NATURAL PRODUCT DISCOVERY

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Marine bacteria present a prolific source of natural products. *Salinispora*, an actinomycete from marine sediments, has yielded several bioactive and structurally unique compounds that were isolated by traditional isolation approaches over the years. The recent genome sequencing and analysis of over 100 *Salinispora* strains revealed a plethora of unprecedented natural product biosynthetic gene clusters of over 20 per genome, unraveling the huge, yet mostly unexplored metabolic potential of these bacteria[1].The development of mass spectrometry-based concepts, including molecular networking[2], peptido- and glycogenomics[3,4] in our labs allows rapid screening of secondary metabolomes and subsequent connection of metabolites to their biosynthetic gene clusters. Additionally, pattern-based genome mining is introduced as a novel concept, that extends the MS-based genome mining approaches to non-peptidic and non-glycosylated compounds. All of these methods have the potential for automation.

In this study, all genome sequenced *Salinispora* strains were systematically cultured in small scale, sequentially extracted with solvents and analyzed by HPLC-MSMS for the generation of metabolomic profiles. Molecular networking of the data allowed dereplication and annotation of known compound families and revealed numerous new secondary metabolites. Through application of peptido- and glycogenomics and pattern-based genome mining certain novel compounds could be connected to their gene clusters. Candidate compounds were then isolated by MS-guided fractionation and subjected to full NMR structure elucidation and bioactivity testing. Additionally, efforts to automate the discoveries of peptidic and glycosylated compounds from large datasets are highlighted.

The presented results illustrate the huge discovery potential of this approach and pave the way to future, fully-automated genome-based natural product discovery platforms.

Keywords:*Salinispora*, Natural products, Genome mining, Mass spectrometry

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A CLEAR SIGNAL: ANTIBIOTICS ARE WEAPONS

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The discovery and development of antibiotics to fight bacterial diseases is one of the great triumphs in modern medicine. However, despite their tremendous relevance for humans, there is a surprising lack of consensus on the role of antibiotics in nature for the bacteria that produce them. What ecological and evolutionary functions do antibiotics serve in nature? And under what conditions are antibiotics expressed? Two divergent views have emerged to answer these questions. One argues that antibiotics are weapons used by bacteria to kill competitors, while the other proposes that they are cooperative social signals used to coordinate the behaviors of coexisting bacterial strains. Although these dichotomous views have helped to frame questions about the ecological and evolutionary role of antibiotics, there are few experimental tests of these ideas. Moreover, partitioning the roles of antibiotics into these strict classes fails to consider important evolutionary alternatives, e.g. that antibiotics are used as cues or mechanisms of bacterial behavioural manipulation. Here we experimentally distinguish these possibilities, using the antibiotic-producing bacteria genus *Streptomyces* as our model.

Streptomycetes are ubiquitous saprophytes in soil, where multiple species coexist and compete for limited resources. Antibiotic production in this group and the diverse responses to these secreted products may depend on this competitive context, particularly on the presence of competitors that may be killed or inhibited by antibiotics. To address this, we measured how interspecies interactions modify antibiotic production among co-cultured streptomycetes. We find strong evidence that these interactions have a critical role in regulating antibiotic production, leading to significant changes in the capacity to inhibit competitors. While some interactions between strains lead to joint increases in antibiotic production and may be viewed as cooperative, most interactions are competitive. Consistent with the idea that antibiotic production is sensitive to cues produced by other strains, the inhibitory capacity of the strains in our collection was increased two-fold as a consequence of growth in the presence of competitors. In addition, consistent with the idea of widespread manipulation, we found that competitor bacteria can strongly suppress antibiotic production in other strains, leading to an average two-fold reduction in inhibitory capacity. Using simulations, we show that these strain-specific alterations to antibiotic production can dramatically increase the number of antagonistic strategies that coexist in a community setting; thus competitive cues and manipulation are potential drivers of bacterial diversity. The widespread use of cues and manipulation, as a component of the competitive strategies of coexisting bacteria, is consistent with the view that antibiotics are used as weapons, and argues against their general use as cooperative signals.

Keywords: Interspecific interactions, Signals, Cues, Antibiotics, Competition

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SYSTEMATIC ANALYSES OF THE TRANSCRIPTOME AND TRANSLATOME OF *STREPTOMYCES COELICOLOR*

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Streptomyces produce various secondary metabolites including antibiotics, and many of these are used in medical and agricultural fields. The production of these compounds occurs after passing through the transition from primary metabolism to secondary metabolism, and involves complex morphological differentiation. Despite the significant attributions of *Streptomyces* to biotechnological area, their complex organizational structures and gene regulation mechanisms are not fully discovered. Here we show the architecture of *Streptomyces coelicolor* genome and gene expression patterns at transcriptional and translational level along with different developmental phases, by integrating genome-wide data of transcription start sites (TSSs), mRNA abundance and ribosome-protected mRNA fragment (RPF) abundance. Total 2,776 TSSs of genes were identified, and 18% of them represented leaderless genes. Based on the identified TSSs, the length distribution of 5' untranslated regions (UTRs) was calculated and conserved promoter motif was elucidated. We also found 151 novel transcripts, 42 of which exhibited translational expression, indicating novel open reading frames (ORFs). Analyses of transcriptional and translational expression at four different growth phases showed increasing expression pattern of genes in secondary metabolism to the later developmental stages. Using clustering analysis we were able to find genes which have same expression patterns with secondary metabolite genes. Finally, expression level comparison of protein complex subunits revealed strong correlation between protein stoichiometry and translational expression. Thus, we offer comprehensive information on genome architecture, and transcriptional and translational gene expression patterns which allows better understanding of *Streptomyces coelicolor.*

Keywords: RNA-seq, Ribo-seq, dRNA-seq

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REGULATION OF MICROBISPORICIN BIOSYNTHESIS IN THE RARE ACTINOMYCETE *MICROBISPORA* SP.

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Microbisporicin is a potent type I lantibiotic produced by the rare actinomycete *Microbispora sp* that has proven very successful against multi-drug resistant infections in preclinical trials. The gene cluster for microbisporicin, which contains unusual post-translational modifications, including 5-chlorotryptophan and 3, 4-dihydroxyproline, has been cloned and analysed (Foulston and Bibb, 2010) revealing a novel regulatory mechanism that involves a pathway-specific extracytoplasmic function (ECF) sigma (MlbX)/anti-sigma (MlbW) complex. A model for the regulation of microbisporicin biosynthesis derived from mutational and quantitative RT-PCR analyses suggests that MlbR, a LuxR homologue, functions as an essential master regulator to trigger microbisporicin production while MlbX and MlbW induce feed-forward biosynthesis and producer immunity (Foulston and Bibb, 2011). Preliminary results show that over-expression of either of the two positive regulatory genes, *mibR* or *mibX*, leads to enhanced microbisporicin production. Further studies to unveil the interplay between the different components of the system have revealed the role of the stringent factor ppGpp and microbisporicin itself as triggers of this complex regulatory pathway. Understanding microbisporicin production and its regulation should result not just in increased yields, but also enable the development of variants with improved clinical activity and broaden our knowledge of lantibiotic biosynthesis in general.

Keywords: lantibiotic, Sigma factor, Regulation

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INCORPORATION OF SUCCINATE DURING POLYKETIDE ASSEMBLY

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Succinate is proposed to be a building block in the biosynthesis of several polyketide streptomycetes natural products including macrotetralide nonactin and several other macrodiolide compounds. However, the mechanism of succinate incorporation into the polyketide is unclear. Here we report the identification of a macrodiolide antibiotic biosynthesis gene cluster by alignment the genomes of two producing strains. This cluster contains PKS genes encoding seven discrete KS enzymes and one ACP encoding gene. Using a cosmid containing the entire set of genes required for this antibiotic biosynthesis we obtained its production in the heterologous host. Through genetic experiments and *in vitro* reconstitution we have determined the complete macrodiolide biosynthetic pathway including the mechanism of succinate incorporation.

Keywords: Polyketide, PKS, Macrodiolide

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CHARACTERIZATION OF CONDENSATION ENZYME SUBUNITS INVOLVED IN BIOSYNTHESIS OF LINCOMYCIN

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Lincomycin is clinically used antibiotic synthesized from an aminosugar and an amino acid precursor. The crucial step of lincomycin biosynthesis, the condensation of these precursors into N-demethyllincomycin is catalysed by a multimeric enzyme N-demethyllincomycin synthetase (NDLS). Based on comparative analysis of biosynthetic clusters of lincomycin and related celesticetin together with gene inactivation experiments a set of 5 to 6 putative NDLS subunits was predicted. Two of them are homologous to regular domains of nonribosomal peptide synthetases (NRPS): adenylation domain and carrier protein (CP).

LmbC, adenylation domain from lincomycin biosynthesis, recognizes unusual amino acid precursor 4-propyl-L-proline, while homologous CcbC from celesticetin biosynthesis recognizes proteinogenic L-proline. Phylogenetic analysis revealed that LmbC and CcbC evolved from a common L-proline specific ancestor. Nevertheless, their substrate specificity is completely different reflecting the incorporated amino acid precursor. CcbC has narrow L-proline specificity while in LmbC evolution the substrate binding site was adapted by point mutations to refuse proteinogenic L-proline and highly prefer 4-propyl-L-proline. The enlargement of the substrate binding pocket during evolution of LmbC resulted in relaxed substrate specificity enabling even efficient activation of synthetic 4-propyl-L-proline derivatives with prolonged side chain, 4-butyl-L-proline and 4-pentyl-L-proline. This finding has remarkable biotechnological potential. Site directed mutagenesis of the substrate binding pocket residues of LmbC and CcbC now also enables the identification of residues important for further genetic engineering of the LmbC substrate specificity.

The CP coding sequences were detected by gene analysis of the lincomycin and celesticetin gene clusters as portions of larger fusion genes *lmbN* and *ccbZ*, respectively. Surprisingly, the fusion partners of both CPs are not homologous, but involved in different steps of the aminosugar precursor biosynthesis. Immunodetection documented not only two-domain LmbN consisting of CP and sugar isomerase domains, but unexpectedly also stand alone isomerase domain as a protein present in lincomycin producer *Streptomyces lincolnensis*. We proved that stand alone isomerase does not originate from some cleavage of LmbN into two separately acting proteins, but from the second *lmbN* translation start, which could be a relic of the gene fusion. Therefore NDLS contains the CP only in the form of bifunctional LmbN. A phosphopantetheinyl transferase essential for attachement of phosphopantetheine cofactor on the CP was found encoded outside of the lincomycin gene cluster. This protein called Slp was proved to attach the cofactor to the CP of LmbN. Subsequently, LmbC tethered 4-propyl-L-proline only on the CP with cofactor. LmbC together with CP domain of LmbN thus fulfill function of initiation modul of NRPS within the NDLS.

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Keywords: Lincomycin, Celesticetin, Biosynthesis, Adenylation domain, Carrier protein

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TOWARDS A NOVEL INTEGRATED BIODISCOVERY NATURAL PRODUCTS GENOME (EVO) MINING APPROACH

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The advent of the genomic era has facilitated the discovery of an increasing number of Natural Products biosynthetic pathways. However, the current genome mining approaches only allow for the exploration of a limited chemical space. We have designed a strategy for the discovery of new chemical classes of bacterial metabolites, by combining modern and classic bioprospection methods, including exploration of rare actinomycetes from a unique oligotrophic environment, yet mega diverse with a great degree of endemism, in a Mexican dessert. We selectively isolated mycelliated actinomycetes, and after taxonomical molecular analysis (16s) we selected strains from underexploited genera, such as *Actinokineospora* and *Lentzea*, which have large genomes. We then proceeded to sequence their genomes, which were analyzed through EvoMining, a new bioinformatic tool based in phylogenomics that leads to systematic identification of novel biosynthetic gene clusters, complementary to those identified by sequence similarity searches (e.g. AntiSMASH). EvoMining is not biased towards the identification of well-known NP biosynthetic enzymes such as PKSs or NRPSs, opening the possibility for the discovery of novel biosynthetic pathways and unknown chemical classes of compounds. The results of our bio-prospection approach will be presented, including an assessment of this method, plus examples of interesting novel biosynthetic systems.

Keywords: Bioprospection, Genome mining, Rare actinomycetes

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COMPETITIVE EFFICACY FROM POLYKETIDE PRODUCTS OF *STREPTOMYCES* SP. MG1

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Bacteria have evolved numerous adaptive strategies for survival in competitive environments. These strategies include production of extracellular enzymes and bioactive metabolites. To identify molecules and enzymes that promote competitive fitness, our laboratory has developed a model system using two soil bacteria, *Streptomyces* sp. Mg1 (S. Mg1) and *Bacillus subtilis*. When cultured together on an agar surface, both organisms display visible changes in colony growth and development. The visible responses to competition suggest complex exchange of chemical information between the species. In this study, we focus on a family of linear polyketides (Mg1-pk1-3) produced by *S.* Mg1. Using imaging mass spectrometry, we find different patterns of localization for individual forms of Mg1-pk1-3. Two forms are colony localized and one form diffuses away from the colony. Structural characterization of the different forms suggests that chemical transformation leads to different activities and different localization patterns for the compounds. The different forms also show distinct activities toward *B. subtilis*, including a lytic-degradative activity (LDA). We have acquired mutants of *S.* Mg1 that are stably resistant to LDA and sequenced their genomes to identify potential targets and mechanisms of action. We discuss new mechanisms of action for the polyketide LDA toward competing *B. subtilis*, which may provide *Streptomyces* sp. Mg1 a food source in nutrient-limited environments.

Keywords:*Streptomyces* sp. Mg1, Secondary metabolism, Lysis, *Bacillus*, Competition

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INSIGHTS FROM GENOMIC APPROACHES TO UNDERSTAND CELL WALL PROCESSES AFFECTED BY PROTEIN O-MANNOSYLATION IN *STREPTOMYCES*

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Protein glycosylation is a phenomenon shared by all domains of life. Within eukaryotes it is a widespread process with roles ranging from cell-signaling and gene regulation to protein sorting and stability. Although protein glycosylation is now widely reported in prokaryotes, its precise role is often unknown. A previous screen for a phiC31 phage receptor in *Streptomyces coelicolor* A3(2) identified *SCO3154* and *SCO1423* as encoding the protein *O*-mannosyltransferase (Pmt) and polyprenol phosphate mannose synthase (Ppm1), respectively, of a putative *O*-mannosylation system. Further biochemical analysis of *ppm1*- and *pmt*- mutant strains elucidated a pathway in which mannose is transferred from GDP-mannose to a polyprenol by Ppm1 to generate polyprenol phosphate mannose which is then 'flipped' across the membrane by an unknown mechanism before final extracellular transfer of mannose to target proteins by Pmt.

Mutants in both *ppm1* and *pmt* had a slow growth rate and were surprisingly hypersensitive to multiple antibiotics that act mainly on cell wall biosynthesis. Two of these antibiotics, vancomycin and imipenem, are often 'last resort therapies' against antibiotic-resistant pathogens. Multiple approaches are being undertaken to elucidate the molecular processes affected by protein glycosylation within this organism and understand why they have such an impact on innate antibiotic resistance.

RNAseq based transcriptional studies are underway that aim to reveal genes that are expressed to compensate for the absence of Ppm1 activity and therefore why these strains have an increased antibiotic susceptibility profile. With a similar aim, studies on the *S. coelicolor* glycoproteome are adding to our inventory of glycoproteins. We are also identifying genes in *S. coelicolor* that act before the synthesis of polyprenol phosphate mannose eg GDP-synthases and additional glycosyl transferases that act after Pmt to extend the glycan chain. A fourth approach is the isolation of (second site) suppressor strains that have become less susceptible to antibiotics; some of these can be explained by mutations in *vanS*, but others are in unknown loci that we are identifying by genome re-sequencing. It is hoped that a better understanding of this process in *S. coelicolor* may help identify possible targets for antibiotic 'potentiators' that could increase the longevity of the current crop of antibiotics.

Keywords:*Streptomyces* glycosylation antibiotics

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OXYR INTERPLAYS WITH A NOVEL SIGMA FACTOR IN THE REGULATION OF THE OXIDATIVE STRESS RESPONSE AND IRON METABOLISM IN *S. TSUKUBAENSIS*

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Introduction: Iron is an essential nutrient for all living organisms; however iron overload can be harmful due to the formation of hydroxyl radicals via the Fenton reaction. Given this toxicity, bacteria have developed a strict control and regulation of iron homeostasis that interplays with the oxidative stress response in oxic environments. OxyR is a hydrogen peroxide sensing LysR protein that regulates bacterial response to oxidative stress and is described to be involved in metal ion homeostasis as a response and adaptation to peroxide stress. In *Streptomyces coelicolor*, OxyR has been described as a positive transcriptional regulator of the *ahpCD* operon. Streptomycetes present a common genomic organization of the *oxyR-ahpCD* region, with *ahpC* and *ahpD* genes being transcribed in an operon divergently from the OxyR encoding gene. In this work we characterize the role of the *oxyR-ahpCD* system in *S.tsukubaensis* NRRL 18488.

Results:*In silico* analysis showed that the *S. tsukubaensis* presents a different genomic organization of the *oxyR* region: OxyR encoding gene is located 3.9 kb upstream from the *ahpCD* operon and three novel genes are present, including STSU_11560 that encodes a novel extracellular sigma factor that shares 67% homology with *S. clavuligerus* extracytoplasmic function (ECF) sigma factor SigG and is divergently transcribed from *oxyR*.

Transcription analysis of the *S. tsukubaensis* wt, D*sig*, D*oxyR* and D*ahpC* strains showed a cross-regulation between $OxyR_{STSU}$ and the sigma factor STSU_11560. As expected, *oxyR* and *ahpC* transcription was induced in the wt upon an H_2O_2 insult. Furthermore, EMSA analysis showed that $OxyR_{STSU}$ binds to the *STSU_11560-oxyR* intergenic and to the *ahpC* promoter regions in both its reduced and oxidized form. However, *sig* transcription was not induced by H_2O_2 addition suggesting that the sigma factor STSU_11560 is not involved in the immediate response to induced oxidative stress by H_2O_2. Further characterization of the D*sig* strain, defective on the sigma factor STSU_11560, revealed a hampered iron uptake although siderophore production levels in iron limiting conditions were similar to the wt.

Conclusions: We were able to establish that STSU_11560, encoding an ECF sigma factor, controls the uptake of ferrous iron in *S. tsukubaensis*. Additionally, the ferrous iron uptake and oxidative stress response are being independently regulated by Sig and OxyR, respectively.

Keywords: Oxidative stress, Iron metabolism, *Streptomyces*

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NEW SYNTHETIC PROMOTER CASSETTES: ACTIVATION OF LANDOMYCIN CLUSTER LEADS TO THE PRODUCTION OF NEW COMPOUNDS

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Actinomycetes as producers of valuable natural products have been regarded as an exhausted source. Recent whole genome sequencing programs have revealed that the biosynthetic potential of actinomycetales has been greatly underexplored relying on traditional approaches. A common feature of all actinomycetes genomes is a length of about 8 – 10 Mb and the presence of approximately 20 – 30 gene clusters potentially encoding the synthesis of secondary metabolites. Activation of such cryptic gene clusters is of great interest especially under increasing threat of multidrug resistant pathogens.

According to the current state of our knowledge about physiology and genetics of actinomycetes, expression of cryptic secondary metabolite pathways is triggered by complex environmental factors which are difficult to reproduce under laboratory conditions. This prompted us to look for tools for clusters activation mainly through utilization of synthetic cis-regulatory elements like promoters, ribosome binding sites and terminators which allow avoiding the native regulatory networks controlling gene expression in nature.

Here we report the development of synthetic promoter cassettes for activation of cryptic gene pathways. These cassettes consist of synthetic promoters of different strength and antibiotic resistance markers flanked with *P-GG* and *B-CC* sites between these promoters. The cassettes can be introduced in front of genes to be activated through RedET recombination. *P-GG* and *B-CC* sites allow excision of antibiotic resistance marker from the final construct by the expression of the phiC31 integrase.

The efficiency of constructed cassettes was demonstrated by activation of the silent landomycin gene cluster. The H2-26 cosmid containing the silent landomycin gene cluster without a SARP regulator *lanI* was used. An insertion of the strong synthetic promoter “21” in front of the *lanE* gene led to the cluster activation in *Streptomyces albus* J1074. Production of at least seven different compounds was detected with tetrangulol and rabelomycin among them. Remaining five compounds were purified and attempts to solve their structures were undertaken. To our best knowledge three compounds are new, while the structures of the remaining two remain unsolved. These results indicate that the constructed cassettes are a powerful tool for the activation of cryptic gene clusters and generation of new compounds.

Keywords: Synthetic promoters, Cluster activation, *Actinomycetes*

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DISCRETE ACYLTRANSFERASES - PROMISING TOOLS FOR POLYKETIDE ENGINEERING

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Trans AT type I polyketide synthases (PKS) are loaded with extender units by discrete acyltransferase (AT) enzymes. In most trans-AT-type biosynthetic pathways that were characterized so far, only loading with malonyl-CoA units was observed. Recently it was demonstrated, that the kirromycin biosynthetic gene cluster contains two functional discrete ATs. The AT KirCI is responsible for loading malonyl-units to the PKS assembly line[1], whereas the other AT, KirCII, is loading ethylmalonyl-units specifically to one acyl carrier protein of the assembly line[2,3], resulting in the installation of the characteristic ethyl branch in the kirromycin carbon scaffold. Surprisingly, KirCII was also able to transfer non-native extender units including allyl-, propargyl-, and azidoethylmalonate to this carrier protein in vitro[4].

There properties now are exploited to make the enzyme a novel tool for synthetic biology approaches to designer-polyketides.

Keywords: Kirromycin, Polyketide, PKS, Discrete AT, Acyltransferase, Engineering

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STOCHASTIC INFERENCE OF THE COMPLEX MAPK-ERK PATHWAY WHEN THE SYSTEM HAS IMPULSIVE CHANGES

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The modelling of the system is a way to describe and understand the actual behaviour of the system mathematically. There are a number of approaches to construct a model for a network and the stochastic modelling is an only way to represent the actual randomness of a system by a model. In this study we perform the diffusion approximation in order to stochastically model a realistic pathway. We use the MAPK-ERK pathway in our analysis since it is one of the major pathways, which controls the growth control of the cell. As the novelty in this study we initially simulate the system via the exact Gillespie algorithm while the system has impulses. The impulses are generated under different scenarios. In the first scheme we accept that certain species in the system have abrupt changes under predetermined time intervals. On the other hand in the second scheme we consider that when certain genes exceed a threshold value, their numbers of molecules in the system change by a fixed amount.

Furthermore as the second novelty of the study, we implement the diffusion bridge method in order to estimate the model parameters, which are the stochastic reaction rate constants while the measurements of all species in the system at the selected time points are taken from the Gillespie outputs. Hereby by those listed calculations we both determine the effect of the impulses in a large system under its stochastic simulation and evaluate the performance of the diffusion bridge algorithm within the diffusion model when measurements of the species are fully observed at the given time intervals while the system is under impulse. In the analysis we assess the performance of the outputs in terms of the computational demand and the accuracy of the estimates.

Acknowledgement: The author would like to thank the Scientific and Technological Research Council (TÜBİTAK Project No: TBAG-112T772) and Middle East Technical University, Academic Development Programme (AGEP Project No: BAP-08-11-2014-007) for their financial supports.

Keywords: Stochastic simulation algorithm, impulses, diffusion bridge method, diffusion approximation, MAPK-ERK pathway, system biology

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PATHWAY-SPECIFIC CCAR REGULATION OF CEPHAMYCIN C BIOSYNTHESIS IN *STREPTOMYCES CLAVULIGERUS*

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Streptomyces clavuligerus (*S. clavuligerus*) is a fasnicating bacterium capable of producing a wide range of secondary metabolites such as cephamycin C, clavulanic acid, holomycin and a tunicamycin related antibiotic. CcaR, a SARP-type (***S****treptomyces* **A**ntibiotic **R**egulatory **P**rotein) positive regulatory protein, is encoded by *ccaR* gene located in cephamycin C gene cluster. In this study, the effect of CcaR regulation in the expression of cephamycin C genes was examined by qRT-PCR experiments in a time dependent manner in three strains; the wild type, *ccaR*-disrupted *S. clavuligerus* and *ccaR* overexpressing *S. clavuligerus*. In the absence of CcaR, the most effected genes were found as *lat* and *cmcI* with 2,200- and 1,087-fold reduced expression levels compared with the wild type. Expression of other biosynthetic genes, *pcbAB–pcbC–cefD–cefE–cmcJ–cmcH* and *blp* was 225- to 359-fold lower in the *ccaR*-disrupted strain. However, expression of the remaining genes in the cluster; *pcbR–pbpA–bla* and *orf10* were slightly affected. On the other hand, *ccaR* overexpression in *S. clavuligerus* resulted with a 5.1-fold increase in its own expression, and 4.3 and 5-fold increases in the expression of *lat* and *cmcI* genes, respectively. Expression patterns of other cluster genes were increased in a range of 2- to 3-fold. Furthermore, cephamycin C production was determined in strains containing an additional *ccaR* gene integrated in the chromosome, *S. clavuligerus* pSET-PC, or multiple copies of *ccaR* expressed from *gly* promoter, *S. clavuligerus* pAK23 as compared to the wild type. And pAK23 fermentation resulted in a maximum of 6.1-fold increase in specific cephamycin C production relative to the wild type. Taken together, this work represents an extensive study of the effects of the positive regulator CcaR on the expression of 16 genes involved in cephamycin C biosynthesis.

Keywords: Cephamycin C, *Streptomycesclavuligerus*, CcaR, regulation

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POSTER PRESENTATIONS

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ENVIRONMENTAL MICROBIOLOGY

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PHYLOGENETIC DIVERSITY OF ACTINOMYCETES AND BIOGEOCHEMICAL CHARACTERISTICS IN SEDIMENTS OF EASTERN MEDITERRANEAN SEA

Ilknur Tuncer and Nihayet Bizsel

Actinomycetes are very important due to their high diversity and production of secondary metabolites. They are phenotypically and phylogenetically highly diverse and can be found in adverse environments. Discovery from terrestrial actinomycetes has declined recently and thus marine environment has been opened to the research area. In the present study, isolatation, phenotypic and phylogenetic analysis of actinomycetes from sediments of Eastern Mediterranean Sea in association with biogeochemical analysis of sediments was aimed.

Totally 10 stations with 72 – 1235 m depths in Eastern Mediterranean Sea were sampled in March 2012. Sediment samples were analyzed to determine grain size and carbon, nitrogen, phosphorus contents. Isolation of actinomycetes was achieved using seven different sediment processing methods and six isolation media prepared with sterile seawater and then incubation at 26 – 28 °C upto 2 months. They were physiologically analyzed using API ZYM and BBL GP ID commercial kits. 16S rRNA gene sequences of the isolates were deposited into GenBank database and the phylogenetic tree including their accession numbers was constructed.

The geochemical composition of the study area was generally in the range of Aegean Sea and reflected the regional variability. The isolation and diversity of actinomycetes were much higher from stations closer to coastal areas in North Aegean Sea compared to deeper stations in South Aegean Sea. According to 16S rRNA gene analysis, 23 isolates had high phylogenetic affiliation with the families *Nocardiopsaceae*, *Pseudonocardiaceae* and *Streptomycetaceae*. The majority of the strains formed a highly diverse clade having members belonging to the genus *Streptomyces*. Similar to previous culture-dependent study in Eastern Mediterranean Sea, the phenotypic and phylogenetic diversity of the *Actinobacteria* were found high. The physiological tests of the isolates showed that they utilized proteins rather than carbohydrates which supported the previous study giving the correlation of benthic bacteria biomass and sedimentary protein in Eastern Mediterranean Sea.

In this study, it was indicated that phenotypic and phylogenetic diversity of actinomycetes changed in accordance with geographical differences and environmental factors. And also for further studies i.e. identification of new species and production of secondary metabolites, the present study provided highly diverse actinomycetes.

Keywords: Phylogenetic diversity, Actinomycetes, Sediment, Eastern Mediterranean Sea

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GROWTH PROMOTION OF THE SEAWATER-IRRIGATED HALOPHYTE *SALICORNIA BIGELOVII* BY ENDOPHYTIC AUXIN-PRODUCING ACTINOMYCETES

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Salicornia bigelovii is a promising halophytic crop for saline soils, and can therefore contribute to arid-zone management. *S. bigelovii* has substantial economic value as a bioresource, in addition to its importance as a culinary product. Endophytic actinomycetes were isolated from *S. bigelovii* roots to evaluate their potential for plant growth promotion and investigate the mechanisms involved. Twenty-four endophytic isolates obtained from *S. bigelovii* roots were initially selected based on their ability to produce indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA) *in vitro*. An isolate of *Spirillosporaalbida* produced the highest levels of both IAA and IPYA. The addition of L-tryptophan (L-TRP) to broth inoculated with *S. albida* enhanced the production of IAA and IPYA several fold. Under greenhouse conditions using saline soils amended with or without L-TRP, *S. bigelovii* seedlings inoculated with *S. albida* exhibited significant increases in the fresh and dry weights, and lengths of roots and shoots. The growth promotion observed by *S. albida* was most pronounced in the presence of L-TRP treatment compared to the absence of L-TRP. This growth promotion was supported by the significant increases in the levels of *in planta* IAA and IPYA, compared with control plants. A mutant isolate of *S. albida* that was incapable of producing IAA *in vitro*, failed to increase the endogenous levels of IAA and IPYA and failed to promote plant growth, compared to plants inoculated with the wild type strain. The pure culture of *S. albida* was incapable of producing gibberellic acid, isopentenyl adenine, or zeatin indicating that the mechanism of growth promotion was likely to be due to IAA production. Our findings suggest that yields of *S. bigelovii* can be enhanced by the field application of this endophytic isolate. This study is the first to demonstrate the potential of endophytic actinomycetes to promote plant growth under saline conditions.

Keywords: Auxins; Endophytes; Plant growth promotion; *Salicornia*

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ENHANCEMENT OF GROWTH OF *SALICORNIA BIGELOVII* SEEDLINGS BY RHIZOSPHERE-COMPETENT PLANT GROWTH REGULATORS-PRODUCING MARINE ACTINOMYCETES

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Saline habitats cover a wide area of our planet and halophytes are increasingly being used for human benefits. *Salicornia bigelovii* is a promising halophyte found along the coastline of many countries including the United Arab Emirates. This study aimed to isolate and identify rhizosphere-competent plant growth promoting marine actinomycetes from *Salicornia* rhizosphere and to evaluate their potential as biological inoculants to promote the growth of *Salicornia* seedlings. Seventy-three actinomycete isolates were initially screened for their ability to colonize *Salicornia* roots *in vitro* and to be rhizosphere-competent under a naturally competitive environment. Nine promising rhizosphere-competent isolates were subsequently evaluated for their ability to produce *in vitro* plant growth regulators (PGRs) (auxins, cytokinins and polyamines), and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Three rhizosphere-competent isolates namely, *Streptomyces* sp., *Microbispora* sp. and *Pilimelia* sp. were selected based on their superior abilities to produce PGRs and ACC deaminase. Greenhouse experiments were conducted to investigate the effects of these three isolates, individually or in combination, on *Salicornia* seedlings growth and endogenous levels of auxins, cytokinins and polyamines in both roots and shoots. While the individual application of each strain resulted in a significant plant growth promotion compared to the control, the combination of the three isolates resulted in superior levels of plant growth promotion. This was evident from the significant increases in the levels of photosynthetic pigments, *in planta* auxins, cytokinins and polyamines and the significant reduction of the endogenous levels of ACC, in roots and shoots compared with control plants or plants grown in soils inoculated with only individual strain. These three isolates are considered to have the potential to perform as plant growth promoters for *Salicornia* production in nutrient impoverished soils in arid coastal areas. This study is the first report to indicate the potential of marine actinomycetes to promote *Salicornia* growth under saline conditions.

Keywords: Plant growth promotion, Plant growth regulators; Rhizosphere-competency; *Salicornia*

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INFLUENCE OF THIOLUTIN PRODUCED BY *SACCHAROTHRIXALGERIENSIS* NRRL B-24137 ON THE EXPRESSION OF VASCULAR WILT OF FLAX

Merrouche Rabiaa

Microbiology

Thiolutin is an antibiotic produced by *Saccharothrix algeriensis* NRRL B-24137. This actinomycete strain was isolated from Algerian Saharan soil. *It* produces several antibiotics which belong to dithiolopyrrolone group, with strong antifungal and antibacterial activities. This group consists of two-cycles (resulted from the condensation of two cystines) containing nitrogen and sulfur. The major antibiotic, thiolutin, was known to be produced by some *Streptomyces* species, but it was discovered for the first time in the genus of *Saccharothrix*.

The objective of this work is to produce a large quantity of Thiolutin by *S. algeriensis*, in order to purify it and study its influence on the vascular wilt of flax. This antibiotic was produced on a semi-synthetic medium (SS) supplemented with yeast extract and extracted from the culture filtrate by dichloromethane. The bioautography of this extract showed two active bands named, AJ and PS with retention factor (Rf) values of 0.52 and 0.59, respectively. However, the HPLC analysis by C18 column showed the presence of seven dithiolopyrrolones: thiolutin, formyl-pyrrothine, tigloyl-pyrrothine, senecioyl-pyrrothine-, butanoyl-pyrrothine and isobutyryl-pyrrothine. The major antibiotic, thiolutin, (yellow compound), which was purified by HPLC, showed high activity against the bacterium *Bacillus subtilis* and the fungus *Umbelopsis ramanniana*, in addition to other fungi belonging to other genera such as *Fusarium* as well.

The influence of thiolutin on the expression of vascular wilt of flax was also studied at a concentration of 5 mg of antibiotic per 100 g of dry soil, where the wilting in flax was markedly decreased. The effectiveness (% decrease of the disease) reached 82% compared to the untreated plants (control). Furthermore, when introduced into soil, thiolutin allowed a decrease in population of pathogenic agent, *Fusarium oxysporum* f. sp. *Lini*, 113 times compared to the control. Several parameters were considered, such as, the dose of antibiotic (2 mg/gds or 5 mg/gds), presence or absence of soil microflora (sterile or non-sterile soil) and the influence of thiolutin on germination and plant growth.

Keywords: Thiolutin, *Saccharothrix algeriensis*, Dithiolopyrrolones, *Fusarium oxysporum* f. sp. lini, *Fusarium* wilt, Flax.

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BIODIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF NON-PATHOGENIC ACTINOBACTERIA IN ANTHROPOGENICALLY DISTURBED ECOSYSTEMS

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Background: The critical environmental situation leads to the increasing number of hostile habitats for organisms. Biotopes with elevated levels of mineral salts, heavy metals and/or oil contamination are dominated by actinobacteria of the genera *Dietzia*, *Gordonia*, *Tsukamurella*, and *Rhodococcus*. An integrated study on the biology of ecologically important actinobacterial taxa and their adaptation mechanisms for survival under unfavorable environmental conditions is of interest for a wide range of issues relevant to different biotechnological areas. Investigation of natural and synthetic multi-component hydrocarbon-oxidizing communities makes it possible to elucidate the interrelations and metabolic connections of microorganisms involved, to evaluate and explore their biotechnological potential. It also allows better knowledge on gene expression and dominance mechanisms of particular actinobacterial species.

Objectives: 1. Rapid differentiation of actinobacteria in situ using direct species-specific PCR detection. 3. Thorough study of genomes and phenomes of natural strains isolated from various habitats to determine their ecological specialization. 4. Investigation of ultra-structural and nanomechanical properties of actinobacterial cells exposed to toxic ecopollutants. 5. Investigation of actinobacterial functional genes and adaptation factors to harsh environmental conditions.

Results & Conclusions: 1. Novel data on biology of ecologically important actinobacterial taxa *Dietzia*, *Gordonia*, T*sukamurella*, and *Rhodococcus* were obtained, and the morphological-functional response of actinobacterial cells to the ecotoxicatnt exposure was evaluated. 2. Genes coding the oxidations of oil hydrocarbons, nitrogen-containing heterocycles, polycyclic triterpenoides and organic sulfides were determined. 3. Novel data were obtained on the nature of heavy metal ion uptake by *Rhodococcus* actinobacteria predominant in oil-contaminated biotopes. It was found that adaptation mechanisms of rhodococcal non-specific resistance to heavy metals were due to their ecological confinement and ability to synthesize glycolipid biosurfactants. Hydrocarbon-oxidizing strains with high emulsifying activity and resistant to elevated (>250 mM) heavy metal concentrations were selected. 4. Biotechnology relevant strains were selected and used to develop laboratory prototypes of biocatalysts useful for transformation and degradation of β-sitosterol, betulin, tioanizole, drotaverine hydrochloride, etc. to synthesize biologically active compounds. 5. The taxonomic profile of the Regional Specialized Collection of Alkanotrophic Microorganisms (official acronym IEGM, WFCC # 768, www.iegm.ru/iegmcol) was enlarged, and the IEGM collection holding was significantly replenished with actinobacterial cultures isolated from soil samples taken from anthropogenically-disturbed areas. Strains deposited in the collection are characterized by high oxygenase activities, expressed biodegradative abilities towards individual hydrocarbons, oil and oil products. These strains can also synthesize glycolipid biosurfactants and are resistant to heavy metal salts.

Keywords: Biodiversity, *Dietzia*, *Gordonia*, *Tsukamurella*, *Rhodococcus*

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EXPLORING METAGENOMES FROM DIVERSE ENVIRONMENTS FOR NOVEL NATURAL PRODUCT GENE CLUSTERS

Chiara Borsetto[1], Gregory Amos[1], Paris Laskaris[2], David Pearce[3], Rabah Forar Laidi[4], Carlos Vallin[5], Christophe Corre[1], David Hodgson[1], Stefano Donadio[6], Elizabeth Wellington[1]

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Recent studies have shown that resistance to multiple antibiotics is increasing and spreading among bacteria, thus there is an urgent need to discover and develop new compounds with antimicrobial activity. The production of active secondary metabolites in bacteria is often correlated with the presence of thiotemplate systems such as polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS). Metagenomics allows the exploitation of the unculturable fraction of bacterial population present in soil by extracting DNA from the total bacterial community. The main aim of this research is to explore this uncultured majority present in diverse and extreme environments such as Antarctic, sub-tropical (island off Cuba) and Sahara desert soil using functional metagenomics in order to discover genes clusters related to the production of new active secondary metabolites. A PCR screen based around the marker genes PKS and NRPS was used to find biosynthetic clusters. Additionally next generation sequencing (NGS) for PKS, NRPS and general bacterial 16S rRNA amplicons was performed to assess the bacterial communities and the potential related to antibiotic biosynthetic diversity present in different soils.

Two fosmid libraries were created for Antarctic and Sub-tropical soils. A PCR screening with already existing probes described in the literature and newly designed degenerate probes led to the identification of 19 NRPS, PKS and hypothetical hybrid PKS/NRPS clusters in the two libraries. The use of the new primers sets in tandem with the published ones increased threefold the identification of new cluster genes. According to bioinformatics analysis performed with AntiSMASH, the most relevant and unique clusters were transferred into suitable heterologous hosts *Streptomyces coelicolor* M1152 and *Pseudomonas aureofaciens* in order to express and chemically characterize the compounds using Liquid Chromatography – Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. The initial pEpiFOS-5 vector used for the libraries was engineered using PCR-targeting system in order to allow the integration of fosmids with clusters into the heterologous superhost *S. coelicolor* M1152.

NGS analysis allowed the construction of PKS and NRPS biodiversity phylograms, which will be used to identify the samples and enrichment methods generating the highest secondary metabolite diversity.

In conclusion, this study has identified several gene clusters which can be related to hypothetical new active compounds with antimicrobial activity. The screening of metagenomic libraries with newly designed degenerate primers for PKS and NRPS genes proved a successful strategy for cluster recovery. These results also confirmed the potential of functional metagenomics to discover new active secondary metabolites from the unexploited bacterial fraction present in environments for which selective isolation methods gave very different diversity. This study has demonstrated the key characteristics of worldwide soil communities related to the highest hit rate for antimicrobial secondary metabolites biodiversity.

Appropriate permission to study soils in Antarctic, Cuba and Algeria was granted.

Keywords: Metagenomics, Natural product discovery, Biogeography, Gene clusters

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ENDOPHYTIC ACTINOBACTERIA ASSOCIATED WITH TWO INDIGENOUS SOUTH AFRICAN PLANTS, ASPALATHUSLINEARIS AND ALOE FEROX

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The Cape Floral Region, encompassing the fynbos biome, is situated in the southern most tip of Africa. The fynbos biome is consider to be a biodiversity hotspot and 70% of the over 9 000 plant species found within the biome are endemic. South Africa has a long oral history of using fynbos species for medicinal applications and several fynbos-derived natural products have been commercialized. *Aspalathus linearis* and *Aloe ferox* are indigenous fynbos species that are sold worldwide for their wide ranging health benefits. *A. linearis* is high in antioxidants and is sold as an herbal tea, rooibos while *Aloe ferox* is used to treat skin conditions such as eczema and burns, as well as constipation. While these plants have been extensively studied for their pharmacological properties, little is known of the microbial communities associated with these plants. This study aims to explore the diversity of endophytic and rhizospheric actinobacteria associated with *A. linearis* and *A. ferox*. Diversity was assessed by culture dependent and metagenomic methods.

Leaf and root samples were collected from several commercial farms within the Western Cape Province, and included cultivated and wild plants. Actinobacteria were isolated using traditional methods. Metagenomic DNA was extracted from leaf and root tissue, as well as rhizopheric soil. The 16S rRNA gene was amplified with universal and actinobacteria-specific primers targeting the V3-V4 region and sequenced on a MiSeq. Actinobacterial isolates were screened for antimicrobial activity against a range of plant- and human pathogens.

Several actinobacteria were isolated from the A. ferox leaves, presenting the genera *Gordonia,Nocardia* and *Streptomyces.* Relatively few endophytes were cultivated from the *A. linearis* leaves which may be attributed to the high phenolic content of rooibos leaves. Large numbers of actinobacterial were isolated from the roots/rhizophere. Metagenomic analysis identified highly diverse communities were present in the roots of both plants. Several OTUs identified may represent novel actinobacterial genera. Many of the leaf and root endo/epiphytes displayed antimicrobial activity. For several strains antimicrobial activity was enhanced by the inclusion of plant extract in the growth medium.

Keywords: Rooibos, Aloe ferox, Fynbos

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BIOREMEDIATION OF A PESTICIDES MIXTURE BY A *STREPTOMYCESCONSORTIUM* IN DIFFERENT SYSTEMS

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Pesticides are usually applied simultaneously or one after another for crop protection, and their application often leads to a combined contamination of soils by these residues. Furthermore, it is important to characterize these mixtures due to their persistence, composition and geographical distribution. Organochlorine pesticides are persistent pollutants, and currently are still ubiquitous in the environment. The bioremediation offers the possibility to remove these toxic pollutants by using biological activity and the use of actinobacteria consortia is an attractive approach because of their ability to degrade pesticides and to increase the metabolic pathways to achieve it.

The aim of this work was to study the ability of an actinobacteria consortium to remove an organochlorine pesticides mixture (lindane, chlordane and methoxychlor) under control laboratory conditions, in slurry and soil systems.

The consortium *Streptomyces* sp. A2, A5, A11 and M7, previously selected for its ability to remove lindane, were pre-cultivated 72h as pure cultures in trypticase soy broth. Then these strains were inoculated as a defined consortium (2 g Kg^{-1} of soil or slurry), in sterile and non-sterile soil and in sterile slurry systems, and incubated for 16 days at 30 °C. The soil used was classified as silty clay loam. All tests were carried out in presence of the organoclorine pesticides mixture (1.66 mg Kg^{-1} each one). Respectively controls were carried out. Residual pesticides concentrations were measured by gas chromatography. The effect of the kind of system used for the mixture removal was analyzed by the main effect plot.

The removal of the mixture was different for each pesticide and among the different systems. In the case of sterile soil the removal percentages were 38.9% for methoxychlor, 31.2% for lindane and 22.5% for chlordane. In non-sterile soil the removal percentages were 21% and 25.4% for methoxychlor and lindane. For the slurry system the removal percentages were 10.2% and 35.9% for methoxychlor and lindane respectively. Chlordane removal was no detected in both systems.

The main effect plots analysis showed that the kind of system has a significant effect on the removal of methoxychlor and chlordane, showing statistically significant differences (*PP* > 0.05).

The type of systems used in the bioremediation process has a significant effect on the removal of the organochlorine pesticides mixture. The actinobacteria consortium has the ability to remove organoclorine pesticides in soil and slurry systems and could be a promising tool for *ex-situ* or *in-situ* bioremediation of this toxic compounds mixture.

Keywords:*Streptomyces consortium*; Organochlorine pesticides; Mixed

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PREDATORY BEHAVIOR IN THE GENUS *STREPTOMYCES* AND RELATED GENERA

Charushila Kumbhar, Milind Watve

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Streptomyces sp. are non obligate predator of bacteria and predatory abilities are widespread in this genus. This indicates that predatory growth is restricted to solid surfaces and is not detectable in liquid cultures. Further there was no obligate association between the ability to produce antibiotics in conventional shake flask cultures and the ability to exhibit predation. Genes involved in secondary metabolite pathways were differently expressed during predation specific to prey species. The demonstration of predatory activity answers a number of unanswered questions and resolves many paradoxes in the biology of antibiotics.

Keywords: Predatory *streptomyces*, Secondary metabolite, Ecology

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF FIVE ACTINOBACTERIA STRAINS ISOLATED FROM HYDROCARBON-CONTAMINATED SOIL SAMPLES

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Hydrocarbon contaminated soil has a great number of substrates suitable for the growth of complex microbial community. Isolation and characterization of bacteria involved in transformation of these substrates helps optimize the process of bioremediation.
In this study chemotaxonomic and biochemical methods were used to compare five Gram positive bacterial strains labeled RNP05, CHP-ZH25, CHP-NR31, CHP-315 and NS094. The strains were isolated from contaminated soil samples taken near oil refineries in Pancevo and Novi Sad (Serbia).
The strains were identified by 16S rRNA gene sequencing. Fatty acid composition was determined by GC/MS after derivatization in methanol: toluene: sulphuric acid mixture. Utilization of different carbon sources (phenanthrene, phenol, 4-hydroxybenzoic acid, 3,4-hydroxybenzoic acid, sodium benzoate, diesel fuel, motor oil) was examined on mineral medium. Tolerance to heavy metals was studied on Mueller-Hinton agar with increasing concentrations of $CuSO_4x5H_2O$, $Cd(CH_3COO)_2$, $NiCl_2$, $Zn(CH_3COO)_2$ and $K_2Cr_2O_7$.. Specific enzyme activities were detected using API ZYM test.
The strains RNP05 and CHP-NR31 were identified as members of *Rhodococcus* genus, while strains CHP-ZH25, CHP-315 and NS094 represent *Oerskovia*, *Gordonia* and *Micromonospora spp.* respectively. *Rhodococcus sp.* CHP-NR31 is rich in palmitic, myristic, oleic and tuberculostearic (10-methyloctadecanoic) acid. It shows the highest tolerance to nickel (Ni^{2+}) and tested positive for esterase C4, esterase lipase C8, lipase C14, leucine and cysteine arylamidase, acid phosphatase, α-glucosidase, α-galactosidase and β-galactosidase. *Rhodococcus sp*. RNP05 contains more than 30% of 10-methyloctadecanoic acid. It has the highest tolerance to zinc (Zn^{2+}). It tested positive for alkaline and acid phosphatase, esterase C4, esterase lipase C8, leucine, valine and cysteine arylamidase, trypsine, α-chymotrypsine, naphtol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase. *Oerskovia sp.* CHP-ZH25 has a high amount of branched chain fatty acids (12-methyltetradecanoic, 13-methyltetradecanoic and 15-methylhexadecanoic acid). It has the highest tolerance to nickel (Ni^{2+}). *Micromonospora sp.* NS094 is rich in palmitic, octadecenoic and 13-methyltetradecanoic acid. It shows the highest tolerance to chromium (Cr^{3+}). It tested positive for esterase lipase C8, leucine and valine arylamidase, and β-galactosidase. *Gordonia sp.* CHP-315 is rich in 14-methylpentadecanoic, 10-methyloctadecanoic and octadecenoic acid. It has the highest tolerance to copper (Cu^{2+}) and positive reaction for alkaline and acid phosphatase, esterase lipase C8, lipase C14, leucine and valine arylamidase and α-glucosidase. All the strains were capable of using phenol, 4-hydroxybenzoic acid, diesel fuel and motor oil as a sole source of carbon. *Rhodococcus sp*. RNP05 could use 3,4-hydroxybenzoic acid. Phenanthrene was used by all the strains except *Gordonia sp.* CHP-315 and sodium benzoate by *Rhodococcus sp.* CHP-NR31, *Rhodococcus sp*. RNP05 and *Micromonospora sp.* NS094.

On the basis of hydrocarbon utilization and metal tolerance tests, *Rhodococcus* strains have the highest biodegradation potential. The cellular fatty acid profiles of all tested strains are in accordance with data previously reported in the literature.

Keywords: Hydrocarbon contamination, Bacterial characterization

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ACTINOBACTERIAS COMMUNITY'S STRUCTURES DURING COMPOSTING PROCESS AND AGRICULTURAL'S WASTES RELATED

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Management of agricultural wastes allows the generation of organic amendments such as compost, whose quality is determined by optimal stabilization and maturation of organic material used for their generation. The decomposition process for producing compost is characterized in four stages according to the temperature reached during the process; Thermophilic stage determines the onset of degradation of recalcitrant compounds and removing mesophilic pathogens; whose efficiency depends on the microbial activity of enriched microorganism from the materials selected for the fertilizer generation. The actinobacterias group is known for its metabolic capacity to synthesize extracellular enzymes capable of degrading lignocelluloses composites, in addition to being characterized as plant growth promoters by production of phytohormones and/or the production of metabolites with antimicrobial activity. So the distribution of community belonging to this group during the composting process provides the opportunity to optimize the composting process and add value to the final product.

The aim of this research was to determine the dominant groups's richness of the actinobacterias community in relation to physic - chemical parameter those affecting the composting process and the actinobacterias community's structure of the compost original materials.

Three rows tumble composting as an open system type were constructed, each representing a replica with a carbon/nitrogen (C/N) ratio equal to 20:1 generated from green grass, oat straw, wheat straw, cow manure and mature compost. Each stage of the composting process were physical - chemically characterized, considering; moisture, organic matter, organic carbon and total nitrogen. The total bacterial count was determined by epifluorescence microscopy and the actinobacterias's richness through the study of the V3 region of the 16S ribosomal gene by PCR - DGGE. Bioinformatics cluster analysis of DGGE bands was analyzed using the Multivariate Statistical Package software and 16 S rDNA sequences with the Basic Local Alignment Search Tool. The temporal change of microbial richness and the effect of the variables on it were evaluated by ANOVA and linear correlation coefficients, respectively.

Polymorphism pattern associated with the thermophilic stage was significantly different (P *Streptomyces sp*., *Nocardia sp*., *Promicromonospora sp*. and *Corynebacterium sp*.

The dominance and richness of the actinobacterias's community significantly changed in the thermophilic stage. The temperature variable gives the greater contribution in explaining this variation. The actinobacterias's community from the cow manure could contribute differentially to optimize the composting process highlighting members of the *Streptomyces sp.*and *Promicromonospora sp*.

Keywords: Actinobacterias, Composting, Agricultural raw, DGGE

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SPECIES RICHNESS IN THE COMMUNITY OF ENDOPHYTIC AND RHIZOSPHERIC ACTINOBACTERIAS FROM CHILEAN NATIVE POTATO (*SOLANUMTUBEROSUM*)

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Endophytic actinobacterias represents a potential source of microorganisms capable for synthesizing secondary metabolites of pharmacological interest and antibacterial activity, due to they has been proposed as potential biological control agents. The use of endophytes represents a new strategy for controlling phytopathogens on crops of agricultural importance. The study of the microbial communities associated with the Chilean native potato, represents a unique and unexplored search for potential biocontrol agents The particular interest is the study of actinobacterias associated to the root system and Chilean native potato tuber for finding new agents that could have competent capacity to establish symbiotic relationships and enhance plant growth commercial potato crop.

The aim of this study was to determine Species richness in the community of endophytic and Rhizospheric actinobacterias from Chilean native potato (*Solanum tuberosum*).

Plant material was collected from native Chilean native potato (S*olanum tuberosum)* cultured on Chiloe Region, (south of Chile) which corresponding to a crop origin center. To study the endophytic community a physical disinfection using sonicator was performed, followed of a chemical disinfection using chlorine 3%, ethanol 70% and PBS tween 20 0.01%. For studying rhizosphere was carefully retirement community soil particles attached to the root with distilled water. For studying of actinobacteria specific richness the V3 region of the 16S ribosomal DNA gene was analyzed by PCR-DGGE. Bioinformatics cluster analysis of DGGE bands was analyzed using the Multivariate Statistical Package software and 16S rDNA sequences with the Basic Local Alignment Search Tool and the significance of the microbial richness changes was evaluated by ANOVA.

The polymorphisms pattern of rhizospheric and endophytic actinobacterias communities founded on Chilean native potato throw a poor significance wealth (P *Streptomyces sp*. and *Micromonospora s*p.

The richness of the endophytes community was significantly poor (P *Streptomyces s*p. and *Micromonospora sp*. This is the first report of the distribution of wealth of actinobacterias in native potatoes.

Keywords: Actinobacterias, Endophytes, Native potato, DGGE.

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SUBTERRANEAN LIFE: MINING MOONMILK-DERIVED MICROBIAL DIVERSITY

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Caves are subterranean ecosystems that are considered to be unusual and extreme environment for life (Rooney et al, 2010). They are geologically isolated from surface energy inputs and also devoid of the sunlight, which makes them highly oligotrophic habitats (Barton, 2007). On the other hand, caves are characterized as geologically stable environment with quite constant physical parameters, which limits potential disturbance of microbial diversity and provide an ideal habitat for studying microbial interactions and adaptations to near-starvation conditions. Surprisingly, those extreme habitats are full of life as recent geomicrobiological studies revealed presence of heterogeneous microbial communities in the subterranean systems worldwide (Barton, 2007). Indeed microorganisms have been considered to play an important ecological role in reshaping cave environment, which is supported by recent investigations which provide evidence of contribution of microbes in the formation of various speleothems including moonmilk (Cacchio et al, 2012). Unexpectedly, in the most prevalent carbonate caves, which are primarily inorganic, nutrient-limited habitats, the dominance of Actinobacteria have been reported (Cheeptham et al, 2013). The abundance of this main bio-decomposers of organic matter in strictly inorganic habitat is intriguing. Elucidation of the adaptations that could evolve in a response to highly oligotrophic environment, can lead to discovery of novel bioactive molecules, which may be a main weapon of bacteria to outcompete the nutritional rivals. Therefore, our goal is to establish microbial community structure of secondary cave deposit – moonmilk and determine what are the biological, chemical and physical factors enabling those bacteria to survive and how they are metabolically adapted to such extreme conditions. A multi-directional approach combining cultivation-dependent and –independent methods as well as phylogenetic analyses and microscopic techniques is applied in order to achieve our target.

Keywords: Caves, Moonmilk, Actinobacteria, Diversity

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STREPTOMYCES SPECIES ISOLATED FROM FOREST SOIL AND THEIR ENZYME ACTIVITY

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In this study, forest area located in the province of Sakarya city soil bacteria of the genus Streptomyces isolated and made computer-assisted diagnosis and various enzyme activities of the isolates were determined. All isolates of phenotypic and biochemical characteristics of the data being transmitted to the computer program determined IDENTAX, diagnosis is made by determining their position in the genus. Isolates in the color group analysis based on aerial and substrate mycelium color is divided into four main groups.

Diagnostic test results of Streptomyces isolates are completed by a computer program MINITAB and were created similarity dendrograms. In addition, all isolates protease, amylase, lipase, cellulase and asparaginase enzyme activity was determined. As a result of enzyme assay of 15% of all isolates positive for the enzyme asparaginase, 5% positive for protease enzymes, cellulase enzymes also isolates D0047 is determined to be positive for positive for amylase and lipase were found. In particular, the enzyme asparaginase chemotherapy of neoplastic agent usage and 15% of our isolates showed positive results for the enzyme asparaginase is very important.

Keywords:*Streptomyces*, Enzyme, Characterization

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GENOME CHARACTERISTICS OF THE ENDOPHYTIC ACTINOBACTERIUM *MICROMONOSPORA LUPINI* STRAIN LUPAC 08: ON THE PROCESS OF ADAPTATION TO AN ENDOPHYTIC LIFE STYLE?

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Introduction: Endophytic microorganisms live inside plants for at least part of their life cycle. According to their life strategies, bacterial endophytes can be classified as "obligate" or "facultative". *Micromonospora* is a Gram positive actinobacterium that was first isolated from soil. Reports that *Micromonospora* inhabits nitrogen-fixing nodules in a systematic way, has opened up a question as to what is the potential ecological role of this bacterium on the plant and whether it is in a process of adaptation from a terrestrial to a facultative endophytic life. In an effort to identify the genomic traits which make possible adaptation from a soil to an endophytic habitat, the aim of this work was to sequence a representative strain, *Micromonospora lupini* Lupac 08 isolated from a nitrogen fixing nodule of *Lupinus angustifolius*.

Materials and Methods: The genome sequence of *M. lupini* Lupac 08 was determined using the 454 FLX system and Titanium platform (454 Life Sciences). Sequences were assembled in 50 contigs and four scaffolds ranging from 583 to 7,083,659 and annotation was performed using the MaGe (Magnifying Genomes) interface.

Results: The results obtained show that the genome of *M. lupini* contains a diverse array of genes that may help its survival in soil or in plant tissues, while the high number of putative plant degrading enzyme genes identified is quite surprising since this bacterium is not considered a plant-pathogen. Functionality of several of these genes was demonstrated *in vitro*, showing that Lupac 08 degraded carboxymethylcellulose, starch and xylan. Furthermore, we have provided experimental data which supports the hypothesis that strain Lupac 08 is a plant growth promoting bacterium. In addition, the production of chitinases detected *in vitro*, indicates that strain Lupac 08 may also confer protection to the plant.

Conclusions: The genome corresponding to a representative *Micromonospora* strain isolated from a nitrogen fixing nodule of a legume is presented. *Micromonospora* appears as a new candidate in plant-microbe interactions with an important potential in agriculture and other biotechnological applications. The current data strongly suggests that a beneficial effect is produced on the host-plant.

Keywords:*Micromonospora*, Legume, Genome, Hydrolytic enzymes

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BIOPROSPECTING FOR CULTURABLE ACTINOMYCETES WITH ANTIMICROBIAL PROPERTIES IN RIVERS FROM THE COLOMBIAN ORINOQUIA

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Actinomycetes have been the origin of the largest number of new antibiotic drug candidates and lead molecules with applications in many other therapeutic areas. However, the emergence of antimicrobial resistance developed in various bacterial pathogens and the increase in numbers of new diseases has led to a resurgence of interest in finding new biologically active compounds produced by culturable strains isolated from aquatic environments. The aim of this study was to isolate and characterize actinomycetes strains from sediments of rivers in the Colombian Orinoquia, and screen them for antibacterial and antifungal activity. This region was chosen, as it represents an unexplored area mainly comprised of tropical forests. Fifteen samples were taken from several points along Guaviare River, including water, and sediments at 15 cm and 1 m depth. All samples were enriched for actinomycete-like bacteria by using either $CaCO_3$ or inoculation in ISP3, SC and ACA media with subinhibitory concentrations of cicloheximide and nalidixic acid. These approaches allowed the isolation of 374 strains, which were subsequently dereplicated by using REP/BOX-PCR. Isolates were then identified by using 16S rRNA sequencing, and their antimicrobial properties were evaluated against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Acinetobacter baumannii, Colletotrichum sp and Fusarium oxisporum.* Our results suggest that abundance of Actinomycete-like isolates was higher at 1 m depth, whereas very few strains were obtained from water samples. Furthermore, culturable-species diversity was assessed, with a significant number of *Streptomyces* spp strains, several of them active against bacteria and fungal strains. To date, this is the first study of this type in the Colombian Orinoquia and could contribute to identify new bacterial species with antimicrobial properties in this region.

Keywords: Actinomycetes, Colombian orinoquia, Antibacterial and Antifungal activity

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A NOVEL β-OXIDATIVE DEACETYLATION PATHWAY OF P-HYDROXYCINNAMATE CATABOLISM İN *RHODOCOCCUS JOSTII* RHA1

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University of British Columbia

p-Hydroxycinnamates, such as ferulate and *p*-coumarate, are components of plant cell walls and have a number of commercial applications. Previously, we had shown that *Rhodococcus jostii* RHA1 (RHA1) catabolizes ferulate via vanillate and the β-ketoadipate pathway. Here, we used transcriptomics to identify genes in RHA1 that are up-regulated during growth on ferulate versus benzoate. The up-regulated genes included three transcriptional units predicted to encode the uptake and β-oxidative deacetylation of *p*-hydroxycinnamates, and a MarR-family transcriptional regulator: *couHTL*, *couMNO* and *couR*. Neither Δ*couL* nor Δ*couO* mutants grew on *p*-hydroxycinnamates, but grew on vanillate and dihydrocoumarin. Purified CouL catalyzed the thioesterification of several *p*-hydroxycinnamates. Among the tested substrates, the best were *p*-coumarate and caffeate (k_{cat}/K_M ~400 $mM^{-1}s^{-1}$). CouL did not transform sinapate, similar to two 4-coumaric acid:CoA ligases from plants, and contains the same bulged loop that is a determinant of substrate specificity in the plant homologues. Of these *p*-hydroxycinnamates, *p*-coumarate was also RHA1's preferred growth substrate. CouO is a member of the amidohydrolase superfamily that we predict to catalyze the last step of the β-oxidative catabolism of *p*-hydroxycinnamates, effectively replacing the canonical β-ketothiolase that the pathway lacks. Consistent with this prediction, the Δ*couO* mutant accumulated 4-hydroxy-3-methoxyphenyl-β-ketopropionate when incubated in the presence of ferulate. Furthermore, purified CouO catalyzed the C-C bond cleavage of 4-hydroxy-3-methoxyphenyl-β-ketopropionyl-CoA to vanillate and acetyl-CoA. This reaction has never been reported for a member of the amidohydrolase superfamily. Overall, our study reveals a novel β-oxidative deacetylation pathway of *p*-hydroxycinnamate catabolism that recruits an unprecedented amidohydrolase reaction. The study also augments our understanding of the bacterial catabolism of aromatics from renewable feedstocks.

Keywords: p-Hydroxycinnamate, *Rhodococcus*, β-oxidation, Biodegradation

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GENOMIC AND POST-GENOMIC TOOLS

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TOWARDS THE MOLECULAR DESCRIPTION OF THE SACCHAROPOLYSPORA ERYTHRAEA METABOLIC SWITCH: A SYSTEMS APPROACH

Cuauhtemoc Licona-Cassani, Esteban Marcellin, Lars Nielsen[1], Sooa Lim, Tim Mercer

The University of Queensland

Saccharopolyspora erythraea is a soil-dwelling filamentous actinomycete used for the industrial production of erythromycin, one of the most popular antibiotics in human medicine. Despite extensive industrial exploitation of *S. erythraea*, its metabolism remains largely unexplored, with more than 20 potentially bioactive secondary metabolites of un-known chemistry and function. In this thesis, a systems biology platform was developed and used to delineate the control mechanisms around the metabolic switch in *S. erythraea*.

A set of systems biology tools was developed to explore the developmental cycle of *S. erythraea* in bioreactors at transcriptional, translational and post-translational level. First, a genome-scale metabolic network was constructed and used to increase erythromycin production by 50% using a precursor supply strategy. Reconstruction of the metabolic network highlighted a large number of singletons and the presence of 'uncommon' pathways. These results suggested that a re-annotation of the genome was required. Thus, we proceeded to sequence mRNA across the developmental cycle in bioreactors. RNA-seq data was combined with LC-MS/MS proteomics and *in silico* simulations to report the first functional genome annotation of an actinomycete. Through this process we confirmed the presence of 164 hypothetical proteins and annotated 59 new proteins that mapped to previously un-annotated regions. Furthermore, deep characterisation of the transcriptome across the developmental cycle enabled the study of transcriptional dynamics of genomic macro domains, more importantly, to identify a targeted mRNA degradation phenomenon during the metabolic switch of *S. erythraea*. Moreover, using quantitative phosphoproteomics, we described potential regulatory events on enzymes from central metabolism which are involved in secondary metabolic functions. We identified for the first time dynamic phosphorylation of proteins involved in the metabolic switch. Finally, we used the collected biological data sets to propose and discuss a cellular mechanism of the metabolic switch of *S. erythraea*.

The work presented here represents the first attempt to systematically characterize and understand the mechanisms underpinning the control of the metabolic switch in *S. erythraea*. This work is a relevant contribution to the current knowledge of the biology of *S. erythraea*, and provides a solid foundation for the design of strategies to improve the production of erythromycin.

Keywords: Multi-omics approach, *S. erythraea*, Metabolic switch

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BREAKING THROUGH BARRIERS - REAL-TIME DETECTION OF TOPOISOMERASE-DEPENDENT DNA RELAXATION USING THE SINGLE-MOLECULE MAGNETIC TWEEZER

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Inside the bacterial cell DNA needs to be highly condensed to fit the space limited by the cell wall. Despite its compaction bacterial chromosome is also a dynamic structure. Through the cell cycle the chromosome is topologically rearranged to allow the proper occurence of DNA replication, transcription and segregation. The chromosome topology maintaining involves two crucial cellular enzymes - gyrase (GyrAB) and topoisomerase I (TopA) which catalyze DNA condenstation and relaxation respectively. Topoisomerases enable the movement of replication/transcription forks along chromosome breaking through topological barriers. Many fast growing bacteria besides TopA possess an additional gene encoding for topoisomerase III (TopB) which supports DNA relaxation and decatenation during chromosome replication and segregation. Interestingly, *Streptomyces coelicolor*, filamentous bacteria which grow as a branched and sporulating hyphae, possess only topoismerase I which moreover differs remarkably from other bacterial homologs. We characterized the DNA relaxation in the presence of TopA using magnetic tweezer technique allowing us nanomanipultions on single DNA molecules and real-time detection of relaxation process. We observed, that TopA although shares similarities with other TopA homologs it also exhibits features assigned to TopB protein (multistep relaxation accompanied by high procesivity and removal of several supercoils in single relaxation cycle).

Summarizing, in our work we provide a new approach for analysis of DNA dynamics *in vitro* using magnetic tweezers on single-molecule level. The technique seems to be sutibale for analysis not only DNA-modyfing enzymes but also for monitoring the processivity of polymerases, NAP proteins or DNA damage-repair machinery eliminating the disturbances and/or limitations provided by bulk experiments.

Keywords: Topoisomerase, Single molecule magnetic tweezer, Topa, *Streptomyces*

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ENHANCED SALINOMYCIN PRODUCTION BY ADJUSTMENT OF THE SUPPLY OF EXTENDER UNITS

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Salinomycin, a polyketide compound produced by *Streptomyces albus*, plays a vital role in animal husbandry. The biosynthesis of the polyketide carbon chain is initiated by loading of a malonyl-CoA to SlnA1 and then followed by condensation steps with 5 malonyl-CoA, 6 methylmalonyl-CoA and 3 ethylmalonyl-CoA extender units. Further oxidation and reduction render the final compound. We aim to enhance salinomycin production by increasing the precursors supply through redirecting the precursor metabolic flux from other PKS to salinomycin biosynthesis.

Firstly, we obtained the complete genome sequence of *S.albus*DSMZ41398. The linear chromosome (8,384,669bp) of strain DSMZ41398, with an average G+C% mol content of 72.6%, comprises 7,340 predicted protein-coding sequences (locus tagged as SLNWT). There are nine Type-I PKS or Type-I PKS-NRPS gene clusters in the genome predicted to share the same precursors (malonyl-CoA and methylmalonyl-CoA) with salinomycin biosynthesis. RT-PCR assay showed seven out of nine gene clusters were transcribed under the fermentation condition of salinomycin. Subsequently, each of the seven gene clusters was disrupted, and the mutants LCY-2 (PKS-NRPS-2 deletion) and LCY-6(PKS-6 deletion) both showed enhanced salinomycin production for about 8-fold and 2.5-fold compared with the wild-type. Meanwhile, LC-MS/MS quantification indicated more intracellular free malonyl-CoA and methylmalonyl-CoA were accumulated in both mutants. These data proved that the disruption of undesired PKSs led to the enhanced supply of precursors for salinomycin biosynthesis.

In the mutants and the wild-type, however, the concentration of ethylmalonyl-CoA is almost the same. Therefore, we further increased the intracellular concentration of ethylmalonyl-CoA by over-expressing crotonyl-CoA reductasegene *ccr* (*SLNWT464*) in the PKS-disrupted mutants. As expected, a higher production level was obtained in LCY-2 by over-expressing *ccr* and the production reached 6 g/L whereas the wild-type produced 0.6 g/L.

The engineering strategy described here can be implemented in a variety of microorganisms to rationally overproduce various polyketide secondary metabolites.

Keywords: Salinomycin, Supply of extender units, Production

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2NDFIND: A WEB-BASED SUPPORT TOOL TO FIND SECONDARY METABOLITE BIOSYNTHETIC GENE CLUSTER

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Natl. Inst. Infect. Dis., Japan

The accumulation of genome sequence data has led to a significant understanding of the nature of microorganisms, such as their physiology, evolution, and pathogenicity. The discovery of many natural products from biosynthetic gene clusters in microbial genomes would be counted among these achievements. In this work, we inspected four well-annotated actinomycete genomes (*Streptomyces coelicolor*A3(2), *S. avermitilis* ATCC 31267, *S. griseus* NBRC 13350 and *Saccharopolyspora erythraea* NRRL 23338), and found that there are some Pfam domains only or frequently appeared in secondary metabolite biosynthetic proteins. Then, we tried to develop a web-based support tool (2ndFind) to find secondary metabolite biosynthetic genes by using such Pfam domains. The 2ndFind performs gene prediction from actinomycete or fungal genome sequences by MetaGeneAnnotator or AUGUSTUS, and then searches secondary metabolism-specific Pfam domains by the HMMER package. We believe this tool facilitates finding of secondary metabolite biosynthetic gene clusters from genome sequence.

URL:http://biosyn.nih.go.jp/2ndfind/

Keywords: Secondary metabolism

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RECONSTRUCTION OF THE GRECOCYCLINE BIOSYNTHETIC PATHWAY FROM *STREPTOMYCES* ACTA 1362 USING TRANSFORMATION-ASSOCIATED RECOMBINATION IN YEAST

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The implementation of *Actinobacteria* biosynthetic potential involves the expression of orphan secondary metabolite gene clusters in heterologous host. Therefore, construction of vectors carrying entire biosynthetic gene clusters is of high interest. The yeast recombination system was successfully exploited for assembly of long stretches of DNA fragments. It was shown that transformation-associated recombination (TAR) can be employed to clone large DNAs fragments via homologous recombination with a TAR vector that has short (~ 60 bp) 5' and 3' gene targeting sequences The “capture” vector arms and homologous target DNA undergo recombination to yield a stable plasmid containing the targeted genomic region. Originally TAR method was developed to facilitate cloning large genomic fragments without having to construct and screen genomic DNA libraries. Advantage of the method is elimination of in vitro enzymatic reactions such as restriction and ligation and reducing the amount of DNA handling[1,2]. In TAR cloning protocols, genomic DNA and a shuttle vector with short homology arms corresponding to sequences flanking the region of interest are cotransformed into yeast cells. A TAR-based genetic platform that allows cloning and heterologous expression of a silent biosynthetic pathway was reported recently[3].

Here we present successful reassembly and heterologous expression of 37-kb long grecocycline biosynthetic gene cluster (*gre*) using TAR method. Fragments of the *gre* cluster were obtained using PCR and transformed into *Saccharomyces cerevisiae* together with a “capture” vector, resulting in a construct, where the entire cluster is located on the shuttle vector. Obtained construct was successfully expressed in *S. albus* J1074.

Keywords: Transformation-associated recombination, Yeast, Grecocycline, Actinomycetes

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NEW PCR PRIMERS FOR HIGH THROUGHPUT SCREENING OF NRPS GENES IN ACTINOMYCETES

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Targeted sequencing used for detection of new biosynthetic clusters, as well as individual genes encoding nonribosomal peptide synthetases (NRPS), is a promising attitude to novel bioactive compound discovery and development. In order to reveal the occurrence of these biosynthetic systems in microbial genomes, as well as metagenome libraries, the set of degenerated primers specifically targeting adenylation domains (ADs) of NRPS was published in 2005[1]. This widely used set of primers was designed according to DNA sequences of AD conserved regions A3 and A7 from six actinomycetal NRPS genes that include 17 AD sequences. However, the increasing number of sequenced NRPS genes revealed higher variability of respective AD conserved regions, indicating certain limitations of A3/A7 primer pair. In this study a pair of new degenerated primers (UniF/UniRab) for NRPS AD identification was designed in order to extend the amount of ADs identified by A3/A7 pair. Conserved regions for primer design were identified according to alignment of 28 amino acid sequences representing actinomycetal ADs from 20 biosynthetic clusters with a special focus on ADs specifically activating proline or proline derivatives. The primers were subsequently used for AD screening of reference strains and tested samples by 454 GS Junior pyrosequencing.

Both primer sets were used for identification of adenylation domains in chromosomal DNA of 20 environmental actinomycete strains isolated from soil and marine sediments. In the second part of experiment two actinomycete strains with already sequenced genomes and thus well defined number of ADs, *Salinispora tropica* CNB-440 (representative of marine environment) and *Streptomyces avermitilis* MA-4680 (soil bacteria), were further used as reference strains in order to determine the efficiency of both sets of primers, the previously published A3/A7 and newly designed UniF/UniRab, as well as their mutual combinations. The overall number of ADs identified by UniF/UniRab set of primers using 454 GS Junior pyrosequencing was lower when compared to A3/A7 pair, however the total number of identified ADs was extended by 10-20 % when using UniF/UniRab in addition to A3/A7 set.

Keywords: Adenylation domain, NRPS, Degenerated primers, PCR, NGS

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THIS DOCUMENT IS ONLY THE ABSTRACT OF THE SYMPOSIUM BOOK WHICH AIMS TO CONTROL OF THE ABSTRACTS

GENOME-WIDE INVESTIGATION OF TRANSLATION IN *STREPTOMYCES COELICOLOR*

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With the advance of high-throughput technologies to elucidate the cell biology, quantifying the cellular components across a variety of scales, ranging from transcripts to metabolites, paves the way of understanding cell metabolism. Development of sequencing technology allows generation of fast, inexpensive, and accurate genome sequencing data. Furthermore, it has revolutionized transcriptomics by providing genome wide expression data. However, proteomics and metabolomics do not provide genome wide data due to limitations of detection sensitivity and resolution.A current study of ribosome profiling that is based on the deep sequencing of ribosome protected mRNA fragment enables genome wide investigation of translation with single-base pair resolution.

Ribosome profiling was performed to monitor translation in *Streptomyces coelicolor*, a soil-dwelling filamentous bacteria responsible for producing most natural antibiotics. This study identified more than 80% of annotated genes, which had a larger coverage than previous proteomics data with less than 20%. We also analyzed and elucidated the high correlations of transcription and translation by integrating RNA-seq data.

Keywords:*Streptomyces coelicolor*, Ribosome profiling, Proteome

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IMPACT OF CHROMOSOMAL POSITION EFFECT ON HETEROLOGOUS PRODUCTION IN *S. ALBUS* J1074

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Streptomyces albus J1074 is well known for its outstanding potential for heterologous production. For the last decade it was used for heterologous expression of numerous antibiotic biosynthetic clusters, *e. g.*, thiocoraline, cyclooctatin and steffimycin. Numerous genetic tools recently developed and perfect heterologous host properties of the strain make it potentially a good candidate as a universal heterologous host. However, employment of this potential requires optimization of antibiotic production. One of the factors that can influence expression of heterologous genes is a chromosomal position effect [1]. This term describes differences in a gene expression caused by location of the genes on the chromosome. It may affect expression of native genes as well as transgenes inserted into different regions of genome. Despite their huge importance as natural products producers, the position effect was not yet investigated in streptomycetes due to the lack of effective tools for genetic manipulations. With this aim the β-glucuronidase reporter gene[2] was placed on a mariner transposon[3] between two *fd*-phage terminators and transposon mutant library with randomly distributed reporter gene was obtained. The GusA-activity of 25 mutants was measured and 6-fold variation of activity had been observed. Obtained results were also checked for correlation with factors that may impact position effect phenomenon (distance to *oriC* and strength of local promoters).

To obtain the strain with improved antibiotic production the *attB* site of φC31 was introduced into the mariner transposon and transposon mutant library with randomly distributed *attB*-containing transposons was generated. After the integration of the cosmid, containing aranciamycin biosynthetic cluster into genomes of obtained mutants *via attB*x*attP* recombination, production levels of this antibiotic in 26 mutants were compared. It was observed that relocation of aranciamycin biosynthetic cluster through over the chromosome led mostly to significant decrease of aranciamycin production. Meanwhile, strains with the increased number of *attB*-sites demonstrated higher antibiotic production.

As a result, the impact of chromosomal position on expression of heterologous genes had been explored and factors that may cause this effect were analyzed.Using combination of system for transposon mutagenesis and ***attB×attP*** recombination system the aranciamycin biosynthetic cluster was introduced randomly into the *S. albus* genome. Analysis of aranciamycin producers demonstrated that copy number of antibiotic biosynthetic cluster is crucial for the increase of antibiotic production.

Keywords:*S. albus*J1074, Position effect, Heterologous production

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GENOMIC CHARACTERISTICS OF ADVANCED OXYTETRACYCLINE PRODUCTION STRAINS OF *STREPTOMYCESRIMOSUS*

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The original isolate of the oxytetracycline (OTC) producing organism *Streptomyces rimosus* (G7 (ATCC 10970)) underwent a "black box" strain improvement Programme through chemical mutagenesis coupled with assaying for Increased OTC production and fermenter performance. This process, which took over 30 years and was being being carried out by Pfizer Central Research, led to a lineage of strains with OTC Increased production ranging from.

Keywords: Antibiotic production strains, Genome sequence

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CLUSTOM SEQUENCING – A NEW WAY FOR THE IDENTIFICATION OF TAILORING ENZYMES

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In recent years the development of new antibiotics became of great importance, because of the growing number of resistant bacteria like MRSA and VRE. A way of overcoming this problem is the method of combinatorial biosynthesis. Therefor different tailoring enzymes are used to add chemical active groups to a core structure to improve its bioactivity. A very interesting group of tailoring enzymes is the group of glycocyltransferases.

Glycosyltransferases are enzymes responsible for the attachment of an activated nucleotid sugar to the aglycon as target molecule. Many bioactive natural products are glycosylated with saccharide moieties of different length, in which the sugars contribute to specific interactions with the biological target [1]. Examples include the therapeutically important antibiotic erythromycin A, the antifungal agent amphotericin B and the anticancer drug doxorubicin [2]. In all three cases the loss of the sugar moiety causes the loss of biological activity.

In most instances it has been shown that tailoring enzymes, especially glycosyltransferases, are valuable tools for the formation of novel natural compounds[3]. To increase the pool of suitable glycosyltransferase genes clustom sequencing was performed. Cosmid-libraries of 122 different actinomycetes strains were screened using pobes for PKSI genes, sugar biosynthetic genes and glycosyltransferase genes. Positive hybridizing cosmids were pooled and sequenced (clustom sequencing).

Sequencing data revealed a pool of around 60 glycosyltransferase genes. 18 of these glycosyltransferase genes have not been described in the database before.

In this poster we will present further information about the tailoring enzymes we found, especially the uncharacterized glycosyltransferase genes.

Keywords: Clustom sequencing, Tailoring enzymes

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RECOMBINATIONAL CLONING OF THE ANTIBIOTIC BIOSYNTHETIC GENE CLUSTERS IN LINEAR PLASMID SCP1 OF *STREPTOMYCES COELICOLOR*A3(2)

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The model organism *Streptomyces coelicolor*A3(2) harbors a 356-kb linear plasmid, SCP1. We report here development of a recombinational cloning method for deleting large segment from one telomere of SCP1 followed by replacing with the telomere of pSLA2 and sequentially inserting with the overlapping cosmids in vivo. The procedure depends on homologous recombination coupled with cleavage at telomere termini by telomere terminal protein. Using this procedure, we cloned the 81-kb avermectin and the 76-kb spinosad biosynthetic gene clusters into SCP1. Heterologous expression of avermectin biosynthetic gene cluster was succeeded in *S. coelicolor*. These results demonstrate the utility of SCP1 for cloning large DNA segments such as antibiotic biosynthetic gene clusters.

Keywords:*Streptomyces*; Linear plasmid; Recombinational cloning

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GROWTH AND DEVELOPMENT

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COMPLEX REGULATION OF STRESS-RESPONSE SIGMA FACTORS BY A MULTIPLE PATHWAYS THROUGH PLEIOTROPIC ANTI-ANTI-SIGMA FACTOR BLDG İN *STREPTOMYCES COELICOLOR*A3(2)

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Introduction: In their natural habitats, bacteria are exposed to various stresses. The stress response is regulated mainly by stress-response sigma factors of RNA polymerase, which govern expression of genes essential to overcome the adverse conditions. In Gram-positive bacterium *Bacillus subtilis* and other close bacteria, the activity of such sigma factor SigB is regulated by a "partner-switching" phoshorylation mechanism by the anti-sigma factor RsbW, anti-anti-sigma factor RsbV, and by two PP2C phosphatases, RsbP and RsbU [1]. The regulation of stress response in Gram-positive soil bacteria *Streptomyces* is different to that one in *B. subtilis.* The genome of *Streptomyces coelicolor* contains genes encoding 65 sigma factors including 9 homologues of SigB (SigB, F, G, H, I, K, L, M, N), 60 homologues of anti-sigma factor RsbW, 18 homologues of anti-anti-sigma factor RsbV, and 44 homologues of PP2C phosphatases RsbU/RsbP [2] that indicate rather complex picture of regulation comparing to *B. subtilis*. We previously found that a pleiotropic anti-anti-sigma factor BldG is involved in the osmotic stress activation of SigH by sequestering its anti-sigma factor UshX, and it also interacts with additional anti-sigma factor ApgA. However, both anti-sigma factors were unable to phosphorylate BldG [3]. Recently we found that SigF-specific anti-sigma factor RsfA phosphorylates BldG, however the study suggested that there are likely more kinases which phosphorylate this anti-anti-sigma factor *in vivo*[4].

Objectives: Identification andcharacterization of the RsbW-family anti-sigma factors interacting and phosphorylating anti-anti-sigma factor BldG.

Methods: Bacterial two hybrid system was done as described previously[3]. *In vitro* phosphorylation of BldG by anti-sigma factor kinases was performed as described in[3].

Results: Bacterial two-hybrid system was used to investigate interactions of 27 candidates of proposed RsbW-family anti-sigma factor kinases with BldG. At least 13 anti-sigma factors were found to interact differentially with BldG. The interaction was investigated also by native PAGE. Two approaches, radioactive and non-radioactive, were used to investigate phosphorylation of BldG. Almost all interacting anti-sigma factors specifically phosphorylated BldG in a conserved phosphorylable Ser57 residue.

Conclusions:

1. Bacterial two-hybrid system revealed that 13 putative RsbW-family anti-sigma factors interact with anti-anti-sigma factor BldG.

2. Almost all of them were able to specifically phosphorylate BldG.

3. The results revealed a complex way of regulation of several stress-response sigma factors through a pleiotropic anti-anti-sigma factor BldG that is phosphorylated by rather large group of anti-anti sigma factors.

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Keywords: Sigma factor, Stress-response, Differentiation, Protein kinases

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DISRUPTION OF A CELL WALL DEACETYLASE IN THE PATHOGEN *CORYNEBACTERIUM DIPHTHERIAE* AFFECTS OVERALL CELL WALL ARCHITECTURE

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University of Strathclyde

Modification of the bacterial cell wall is one of the most fundamental processes that bacteria use to adapt and to survive in changing environmental conditions. The ability to modify the bacterial cell wall, through modification and decoration is a key adaptation that bacteria use to avoid host defences (such as hydrolytic enzymes) and enhance survival in host cells. These modifications can be highly complicated such as the covalent and non-covalent attachment of proteins, lipids and polysaccharides. Important human pathogens such as *Corynebacterium* and *Mycobacterium* have taken cell wall decoration to extraordinary levels with the attachment of highly specialised mycolic acids, arabinomannan, glycolipids and proteins, which have been relatively well studied in these organisms. Some of these components, such as the mycolic acids are vital components and are essential for pathogenicity and in some cases viability. Other modifications of cell wall polymers such as N-deacetylations have received relatively little attention. We have identified a putative polysaccharide deacteylase in *Corynebacterium diphtheriae* that is vital for cell growth and maturation of the overall cell wall. Mutants are unable to assemble a completely mature cell envelope, and have altered mycolic acid and glycolipid profiles. In addition disruption of the deacetylase alters antibiotic and lysozyme sensitivity and reduces virulence. These data indicate the complex interaction between the cell wall components of mycolated pathogens and reveals details of the hierachy of cell wall assembly

Keywords:*Corynebacterium*; Cell wall biosynthesis; Deacetylation

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CHARACTERIZATION OF SCO1395 AND SCO4885, TWO DEVELOPMENTALLY REGULATED AND CONSERVED GENES REPRESSING SPORULATION AND HYDROPHOBIC COVER FORMATION IN *STREPTOMYCES COELICOLOR* SUBSTRATE HYPHAE

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Streptomyces species produce many clinically important secondary metabolites and present a complex developmental which includes physiological and morphological differentiation and culminates in sporulation. In this work, the biological function of SCO1395 and SCO4885, two developmentally regulated genes highly overexpressed during the vegetative stages, was analysed by means of mutagenesis, confocal and electron microscopy, transriptomics, and QPCR. SCO1395 encodes for a putative mutT-like protein and SCO4885 for a putative lipoprotein. Both genes are highly conserved in the *Streptomyces* genus (90% average similarity). Our results demonstrate that both genes are repressing secondary metabolite production and sporulation in *S. coelicolor* vegetative hyphae, modifying the expression of several genes, including key genes related with hydrophobic cover formation and sporulation. This study contributes to the post-genomic work necessary to mine the huge amount of transcriptomic/proteomic data obtained during the last decade in order to characterize the function of unknown genes and proteins differentially expressed during development.

Keywords:*Streptomyces*, Conserved genes, Sporulation

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CHROMOSOME ORGANIZATION IN VEGETATIVE HYPHAE IS AFFECTED BY TOPOISOMERASE I AND SEGREGATION PROTEINS

Agnieszka Strzalka[1], Agnieszka Kois-Ostrowska[1], Marcin Szafran[2], Dagmara Jakimowicz[3], Jolanta Zakrzewska-Czerwinska[3]

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Topological stress is caused by supercoils formation during such processes as transcription or replication. Optimal level of DNA supercoiling in cells is maintained by topoisomerases, which can be divided in two distinct groups. *Streptomyces* chromosome contains only one gene encoding type I topoisomerase – *topA*. Deletion of *topA* is lethal and low level of this enzyme affects growth and stops sporulation. In aerial hyphae TopA takes part in chromosome segregation and cooperates with segregation proteins ParAB[1]. ParA is ATPase while ParB is DNA binding proteins which forms large complexes along the hyphae. TopA is recruited by ParB nucleoprotein complexes and is required for their distribution along the hyphae. Little is known about the organization of chromosomes in vegetative hyphae. To date, it was reported that replication in *S. coelicolor* in vegetative hyphae is asynchronous and replisomes have been observed to follow after the tip of growing hyphae[2].

Main goal of our work is to examine how a decrease of TopA and ParA/ParB proteins affects chromosome organization, particularly replisome position and trafficking in vegetative hyphae of *S. coelicolor*. To achieve this, we have constructed the number of strains with reduced level of TopA, deletion of one or two genes from *parAB* system and producing fusion protein DnaN-EGFP (allows observation of replisomes). Replisome position was analyzed by fluorescent microscopy and monitored in real time using time-lapse microscopy.

Our results show that depletion of TopA affects the mean distance between the tip of hyphae and the replisomes. The position of replisome was more disturbed in the strains with additional deletion of *parB* or *parA*. Inducing TopA expression resulted in return to normal distance between the tip and replisome. We also observed increased frequency of two or more replisomes appearing within short distance of each other. Moreover depletion of TopA impaired ability of replisome to follow the growing tip.

Thus, our data indicate that lower level of TopA, which leads to increased negative DNA supercoiling, affects chromosome organization within vegetative hyphae. Lack of segregation proteins ParAB and higher DNA condensation probably lead to impaired chromosome separation after replication. Our results suggest that optimal level of DNA supercoiling and presence of ParAB are necessary for proper replisome formation and trafficking in vegetative hyphae.

Keywords: Fluorescent microscopy, Topoisomerase I, Replication, ParAB, *Streptomyces coelicolor*

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CELL IMMOBILISATION OF *STREPTOMYCES COELICOLOR* IMPROVES DIFFERENTIATION AND ACTINORHODIN PRODUCTION

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Streptomycetes are mycelium-forming bacteria that produce two thirds of clinically relevant secondary metabolites. Despite the fact that secondary metabolite production is activated at specific developmental stages of the *Streptomyces* life cycle, different streptomycetes show different behaviours, and fermentation conditions need to be optimised for each specific strain and secondary metabolite. Cell-encapsulation constitutes an interesting alternative to classical fermentations, which was demonstrated to be useful in *Streptomyces*, but development under these conditions remained unexplored. In this work, the influence of cell-encapsulation in hyphae differentiation and actinorhodin production was explored in the model *Streptomyces coelicolor* strain. Encapsulation led to a delay in growth, to a reduction of mycelium density and cell death; the high proportion of viable hyphae duplicated extracellular actinorhodin production in the encapsulated cultures with respect to the non-encapsulated ones.

Keywords:*Streptomyces*; Encapsulation; Differentiation; Antibiotics; Cell death

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CHARACTERIZATION OF SCO4439, A DEVELOPMENTALLY REGULATED TRANSPEPTIDASE AFFECTING SPORE RESISTANCE AND GERMINATION

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The aim of this work was the characterization of the biological function, expression and structure of *SCO4439*, a developmentally associated transpeptidase overexpressed during the aerial mycelium and sporulation stages. SCO4439 encodes for two active domains: a D-alanyl-D-alanine carboxypeptidase (transpeptidase) at the carboxyl end, and a putative cytosolic transcriptional regulator domain located at the N-terminal end. Both domains are separated by a putative transmembrane region. *SCO4439* is involved in the resistance of the spores to heating, acid and sonication as well as in the swelling of the spores after germination. The D-alanyl-Dalanine carboxipeptidase domain, together with the putative hydrophobic transmembrane domain are highly conserved in the *Streptomyces* genus (average similarity of 70%), and the presence of both domains is essential to restore germination and spore resistance in the ΔSCO4439 mutant strain. The conservation of the putative transcriptional regulator domain is much lower (average similarity of 44%), and it is not necessary to restore the phenotype detected in the spore resistance and germination. In our knowledge this is the first time that germination and the resistance of the spores was related with a DD-peptidase, which is suggesting the existence of DD-peptidases as SCO4439 specialized in the modification of spore cell walls during germination.

Keywords:*Streptomyces*, Transpeptidase, Differentiation, Germination,

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THE PAROLOGOUS PYRUVATE KINASES IN *STREPTOMYCES COELICOLOR* HAVE DISTINCT ROLES IN GROWTH AND ANTIBIOTIC PRODUCTION

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Streptomyces species are prolific producer of bioactive metabolites, nevertheless analysis of complete genomes still shows that there are many biosynthetic clusters present that are silent under normal cultivation conditions. The current increase in clinical antibiotic resistance requires the discovery of new antibiotics, but also a greater understanding of antibiotic production for industrial exploitation. Our interest is in studying the interaction of primary metabolites into secondary metabolism. We focus on the Phosphoenolpyruvate-Pyruvate-Oxaloacetate node of central carbon metabolism using *Streptomyces coelicolor* as a model and we have identified pyruvate kinase as a potential target that influences the production of antibiotics. The genome encodes two parologue pyruvate kinase genes - SCO2014 (pyk1) and SCO5423 (pyk2). Phenotypic analysis of the two mutants revealed differences in their physiological role, *pyk2* exhibits altered growth on glucose, whereas *pyk1* mutants shows altered actinorhodin production. We used cross-species complementation experiments with the *E.coli* pyruvate kinase mutants ΔpykF, ΔpykA and ΔpykAΔpykF double mutant and complemented these with pyk1 and pyk2 from *S. coelicolor* on different media to clarify the physiological role. Furthermore pyk1 and pyk2 were overexpressed in *E. coli* and studied on their biochemical properties. The results indicate that pyk2 is the housekeeping enzyme whereas pyk1 is activated under low energy state condition as gluconeogenesis for example, as it requires AMP for activity. Our data show that parologous genes in primary metabolism have distinct physiological roles in *Streptomyces* that impact significantly on growth and the production of antibiotics, which could be used for industrial strain improvement in the biotechnology industry.

Keywords: Metabolic engineering, Genetic redundancy, Primary metabolism, Secondary metabolism

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GLYCOPEPTIDE RESISTANCE IN *AMYCOLATOPSIS BALHIMYCINA*

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Glycopeptides, produced by various *Amycolatopsis* species, are drugs of last resort for the treatment of infections caused by multi-resistant gram-positive pathogens. They block key steps in the peptidoglycan biosynthesis by binding to the terminal D-alanyl-D-alanine (D-Ala-D-Ala)-residue of the cell wall precursor lipid II. However, glycopeptide resistance evolved over the last two decades.

The resistance mechanism is based on the synthesis of an alternative cell wall, such as the alteration of D-Ala-D-Ala to D-Ala-D-lactate (D-Lac) in lipid II, which results in a 1000-fold decrease of the glycopeptide binding affinity to its target. In many bacteria this modification is executed by the VanRSHAX enzymes: VanH converts pyruvate to D-Lac, VanA ligates D-Ala and D-Lac and VanX cleaves the ubiquitous D-Ala-D-Ala dipeptide. Expression of these genes is under the genetic control of a two-component regulatory system consisting of the response regulator VanR and the membrane spanning receptor histidine kinase VanS. However, the origin of the *vanRSHAX* genes remains a matter of discussion. Since glycopeptide producers have to protect themselves against their own product, one may speculate that resistance has coevolved in the glycopeptide producers together with the ability to synthesize glycopeptides.

In order we used *Amycolatopsis balhimycina,* the producer of the vancomycin type glycopeptide balhimycin, to analyse the self-resistance mechanism. In the genome of *A.balhimycina* we identified genes with high homology to *vanRS* (referred as $vlnRS_{Ab}$) and *vanHAX* (referred as $vanHAX_{Ab}$) from pathogenic Enterococci.

Mutational analysis of $vlnR_{Ab}$ and RT-PCR analyses revealed that expression of $vanHAX_{Ab}$ is independent of $vnlR_{Ab}$ and furthermore independent of the presence of balhimycin. Surprisingly, a *vanHAX*-deletion mutant in *A. balhimycina* still produces resistant cell wall precursors ending on D-Ala-D-Lac identified by LC-MS analyses. Furthermore, the $vanHAX_{Ab}$-deletion mutant is still able to synthesize balhimycin.

These results indicate that *A. balhimycina* can build up a resistant cell wall without use of $VanHAX_{Ab}$. We therefore screened the genome for genes, which may take over the function of $vanHAX_{Ab}$. Thereby $DdlX_{Ab}$ was identified and characterized as an alternative D-Ala-D-Lac ligase. $VanY_{Ab}$, whose gene is located in the balhimycin biosynthetic gene cluster can remove the terminal D-Ala from lipid II. Whether D-Lac is produced by a specific reaction or occurs as side product during *Amycolatopsis* metabolism is under investigation.

Keywords: Actinomyces, Antibiotic, Vancomycin, Peptidoglycan, Resistance

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THE RADICAL COPPER OXIDASE GLXA IS DEPENDENT ON TATB FOR SECRETION AND ACTS COOPERATIVELY WITH CSLA DURING *STREPTOMYCES* GROWTH AND DEVELOPMENT

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Exopolysaccharides are produced by many microorganisms and play critical roles in various aspects of their biology. The cellulose synthase-like protein (CslA) synthesizes a β-(1,4)-glycan at hyphal tips in the filamentous bacterium *Streptomyces.* Here we show that the downstream located *glxA*, which encodes a radical copper oxidase, acts in conjunction with *cslA* in the production of this glycan. In liquid-grown cultures, *cslA* and *glxA* mutants did not form pellets but rather produced open mycelial networks. Moreover, pellet formation is also abolished in a strain lacking the lipoprotein Sco1, which is responsible for the delivery of the copper ion to GlxA. In addition, open mycelial growth is present in the *tatB* and *tatC* mutants affected in the twin-arginine-translocation (TAT) pathway, which we show, is involved in GlxA secretion. Notably, the addition of copper to the growth medium largely restored pellet formation in the Sco1 mutant, indicating that GlxA can be maturated in a Sco1–independent manner. Unexpectedly, we could also restore pellet formation in a *tatC* mutant with increased amounts of copper. An explanation is that immature GlxA is transported to the extracellular environment independent of the Tat translocon. However, pellet formation was not restored in a *tatB* mutant. We therefore speculate that TatB facilitates in targeting GlxA to the Sec translocon in case of Tat malfunction.

Keywords: TAT secretion, Radical copper oxidase GlxA, Cellulose synthase-like protein CslA, Liquid morphology

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OFF THE WALL: FROM FILAMENTOUS GROWTH TO PRIMORDIAL CELLS AND BACK AGAIN

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Streptomycetes are filamentous bacteria that grow by apical tip extension. This process is orchestrated by the tropomyosin-like protein DivIVA, which is present at hyphal tips. DivIVA interacts with various proteins, among which the cellulose synthase-like protein CslA. This protein synthesizes a β-(1,4)-glycan, which is thought to protect growing apices that are continuously being remodeled. To obtain further insight in the role of DivIVA and CslA in polar growth and morphogenesis, we have recently generated so-called *Streptomyces* L-forms that can grow without peptidoglycan. As a consequence, such cells are round and lack any obvious form of polarity. L-form cells have recently been suggested to resemble primordial cell, based on the observation that their growth and proliferation do not require the canonical cytoskeletal or cell division proteins. Instead, their proliferation can merely be explained by physical processes. However, our work on *Streptomyces* L-forms suggests that these cells require glycans, such as those formed by CslA, for their growth. Such glycans might have served for protection of early life forms, before the modern cell wall was invented. We have recently isolated an L-form mutant strain, which readily switches back and forth between mycelial and L-form growth. This mutant with the capability to re-synthesize peptidoglycan is crucial to understand which genes play an essential role in proliferation of L-forms, but also to unravel the mechanism underlying filamentous growth.

Keywords:*Streptomyces*, Filamentous growth, CslA, DivIVA, L-form

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THE INVOLVEMENT OF MTRAB-LPQB GENE CLUSTER IN CELL DIVISION AND ANTIBIOTIC PRODUCTION IN *STREPTOMYCES* SPECIES

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The MtrAB-LpqB pathway is a highly conserved signal transduction system in the Gram-positive phylum Actinobacteria that is implicated in the regulation of osmoprotection, cell envelope homeostasis and cell cycle progression. The *mtrAB-lpqB* operon is found in all but one sequenced actinobacterial species and is essential in unicellular mycobacteria, including *Mycobacterium tuberculosis*.

LpqB is a lipoprotein that interacts with MtrB outside the cell and negatively modulates its activity. However, despite recent advances, studies in unicellular actinobacteria have been hindered because components of the MtrAB-LpqB pathway are essential, probably because cell division itself is essential in unicellular bacteria. We are using filamentous multicellular *Streptomyces* species to study MtrAB-LpqB function which is advantageous because cell division is non-essential in streptomycetes.

In-frame deletion of *mtrA* and *mtrB* in *Streptomyces coelicolor* resulted in strains that form small, whitish-grey colonies that produce very few spores relative to wild-type. Furthermore, deletion of *mtrA* or *mtrB* results in over-production of the antibiotic actinorhodin which is usually coordinated with the onset of sporulation, suggesting antibiotic production and differentiation are uncoupled in these mutants.

Our data support the hypothesis that MtrAB-LpqB has a conserved role in regulating cell division in actinobacteria and also suggest that manipulating MtrA activity could increase antibiotic production in industrially important *Streptomyces* strains. Our data suggest that MtrA regulates *ftsZ* expression in *S. coelicolor.*

To find out more about the role of the MtrAB operon it is crucial to identify the MtrA target genes. The targets of MtrA can be investigated by Chromatin immunoprecipitation and sequencing (ChiP-seq). We are attempting to do this in *S. venezuelae* which is closely related to *S. coelicolor* but has several advantages. First the developmental cycle is faster but more important *S. venezuelae* sporulates in liquid culture whereas *S. coelicolor* does not. Thus we constructed an *S. venezuelae* strain producing FLAG tagged MtrA to investigate MtrA targets using ChiP-seq and the first ChiP experiments have been sent for sequencing.

In contrast to *S. coelicolor* we were not able to delete *mtrA* in *S. venezuelae* but can delete *mtrB* and *lpqB*. *S. venezuelaemtrA* appears to be essential but the *mtrB* and *lpqB* deletion mutants showed a comparable phenotype to the same deletion mutants in *S. coelicolor* with their colonies showing poor growth and no green spore pigment.

Keywords:*Streptomyces*, Two component system, Cell division

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PRODIGININES, SECONDARY METABOLITES ASSOCIATED WITH THE PROGRAMMED CELL DEATH OF *STREPTOMYCES COELICOLOR*

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Prodiginines (PdGs or prodigiosin-like pigments) are natural red-pigmented compounds produced by a series of microorgansims, with *Streptomyces coelicolor* and *Serratia marcescens* as best studied model species. PdGs are promising molecules in medicine due to their immunosuppressive and anticancer activities besides to their previously described antibacterial, antifungal, antiprotozoal, antihelmintic, antiviral, and antimalarial properties. Numerous studies describe the role and the molecular targets of PdGs in eukaryotic cancerous cell lines. In light of the known anti-proliferative properties of PdGs, we questioned whether these molecules could display similar activities and, in consequence, would be associated with the programmed cell death (PCD) process of the producing microorganism.

S coelicolor is known to undergo several rounds of PCD during its complex life cycle and produces PdGs, namely undecylprodigiosin and streptorubin B, in a conditional and developmental manner prior to the sporulation process. By monitoring the Red Auto Fluorescence (RAF) of PdGs, we show that PdGs production localizes in the dying filaments of a confluent culture. Furthermore, we observed that a mutant unable to produce PdGs (**Δ***redD*) showed a higher proportion of viable filaments compared to the wild type strain (WT). This PdGs –deficient mutant also accumulated more intracellular total proteins, RNA, and DNA than the parental strain *S. coelicolor* M145 suggesting that PdGs participate in the destruction of all macromolecules. Our results suggest that PdGs participate in the PCD process of *S. coelicolor*.

Keywords: Programmed cell death

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ARCHITECTURE OF *STREPTOMYCES* REPLICATION INITIATION COMPLEX

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In bacteria, chromosome replication is initiated by binding of the initiator protein, DnaA, to DnaA boxes within the *oriC* region, which leads to DNA unwinding at the AT- rich region called DUE (DNA Unwinding Element). Among bacteria, the mechanism of initiation of chromosome replication is best understood in *Escherichia coli*while little is known about this process in *Streptomyces*. *Streptomyces* are Gram-positive soil bacteria that grow as vegetative mycelia, which differentiate to aerial mycelium and spores. They differ from other bacteria not only in their complex life cycle but also in possessing a large (10 Mb), linear, GC-rich (72%) chromosome. Replication of the linear chromosome proceeds bidirectionally from the centrally located *oriC* towards the ends of the chromosome. Compared to other bacterial origins, *Streptomyces oriC* region is longer and more complex: it possesses numerous DnaA boxes organized in two clusters. *Streptomyces oriC* contains also three sequences for AdpA binding. AdpA is a global transcriptional factor and its binding to the 5'-end of the origin of replication inhibits chromosome replication at the initiation step.

Mapping of DnaA binding sites using different *in vitro* footprinting methods (DMS, Dnase I) revealed the presence of high-, medium-, and low-affinity DnaA boxes. In order to localize origin-specific DUE, we applied WebSIDD tool, which allows identifying short DNA sequences that are prone to strands opening under negative superhelical stress. Two SIDD sites were predicted within the *Streptomyces oriC* region, but *in vitro* attempts (P1 nuclease) to unwind DNA by the DnaA failed suggesting involvement of additional element(s) in this process. However, *in vivo* mappingusing RIP (replication initiation point) indicated that replication initiation start co-localizes with one of the *in silico* identified DUE region.

To investigate further the biological role of AdpA in the regulation of chromosome replication we constructed the *S. venezuelae* Δ*adpA* strain. By using this species as a model organism we were able to observe the complete growth cycle – from the vegetative mycelium to spore formation – in the liquid medium and compare it to the wild type. Our results show that Δ*adpA* mutant grows faster in the liquid medium than the wild-type and its sporulation is limited.

Keywords: Replication, DnaA,*Streptomyces*, AdpA, Chromosome

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ALTERED DESFERRIOXAMINE-MEDIATED IRON UTILIZATION IS A COMMON TRAIT OF BALD MUTANTS OF *STREPTOMYCES COELICOLOR*

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Streptomyces coelicolor is an important model organism for developmental studies of filamentous GC-rich actinobacteria. The genetic characterization of mutants of *S. coelicolor* blocked at the vegetative mycelium stage, the so-called bald (*bld*) mutants that are unable to erect spore-forming aerial hyphae, has opened the way to discovering the molecular basis of development in actinomycetes. Desferrioxamine (DFO) production and import of ferrioxamines (FO; iron-complexed DFO) are key to triggering morphogenesis of *S. coelicolor* and we show here that growth of *S. coelicolor* on the reference medium for *Streptomyces* developmental studies is fully dependent on DFO biosynthesis. UPLC-ESI-MS analysis revealed that all *bld* mutants tested are affected in DFO biosynthesis, with *bldA*, *bldJ*, and *ptsH* mutants severely impaired in DFO production, while *bldF*, *bldK*, *crr* and *ptsI* mutants overproduce DFO. Morphogenesis of *bldK* and *bldJ* mutants was recovered by supplying exogenous iron. Transcript analysis showed that the *bldJ* mutant is impaired in expression of genes involved in the uptake of FO, whereas transcription of genes involved in both DFO biosynthesis and FO uptake is increased in *bldK* mutants. Our study allows proposing altered DFO production and/or FO uptake as a novel phenotypic marker of many *S. coelicolor bld* mutants, and strengthens the role of siderophores and iron acquisition in morphological development of actinomycetes.

Keywords: Siderophore, Bald, Iron, Development, Desferrioxamine

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ANALYZING THE FUNCTION OF *STREPTOMYCES COELICOLOR* PARH IN THE PROCESS OF CHROMOSOME SEGREGATION AND IDENTIFICATION OF A NOVEL SEGREGATION COMPONENT

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Background: Chromosome segregation in bacteria is of central importance in microbial cell and molecular biology. In *Streptomyces coelicolor*, there are five known components of the partition (*par)* system needed for developmentally-associated genome segregation: the *cis*-acting centromere-like sites (*parS*) and four *trans*-acting proteins (ParA, ParB, ParJ and Scy). *parA* encodes a Walker-type ATPase that is essential for proper DNA segregation and the placement of the evenly spaced ParB-*parS* nucleoprotein complexes in aerial hyphae. The purpose of this study was to characterize the function of a second ParA homologue ParH and investigate interactions between ParH with known segregation, condensation, and cell division proteins and to screen a genomic library to find novel interacting proteins.

Methods: A *parH* null mutant was isolated and strains containing ParH-EGFP, and ParB-EGFP in a *DparH* strain were constructed and analyzed by fluorescence microscopy. Site-directed mutagenesis of *parH* was used to change a conserved lysine residue (K99E) in the ATPase Walker A box. To determine if ParH interacts with known segregation, condensation and division proteins, an *E. coli* bacterial two-hybrid system based on adenylate cyclase was used. Plasmids carrying *parH*, *parH* variants, and target genes fused with both T25 and T18 fragments of *cyaA* and were screened on MacConkey indicator plates. A random genomic library of *S. coelicolor* M145 was constructed to screen for novel ParH interacting proteins.

Results/Conclusion: In aerial hyphae of the Δ*parH* mutant,»5% of spores are anucleate compared to 1% of spores in wild type and 24% in *parA* null mutant. In predivisional aerial filaments, ParH-EGFP occasionally localized into a bright band or bands of fluorescence in apical compartments or as increased diffuse fluorescence toward the tip in these filaments. Different localization patterns of ParH-EGFP in aerial hyphae suggest that localization of ParH might be dynamic. This may suggest that evenly-spaced ParH bands are either localized over evenly-spaced ParB-*parS* complexes or ParH co-localizes with FtsZ at the evenly-spaced sites of cell division. As judged by two hybrid analyses, ParH interacts with itself and ParB. However, the Walker A motif K99E mutation in ParH and N-terminal in-frame deletion in ParH impaired the two-hybrid interaction between ParH and ParB. No evidence was obtained to indicate there is an interaction between ParH with ParA (heterodimer), ParJ, SMC, ScpA, ScpB, or FtsZ. By screening a random genomic library, the highly conserved actinobacterial signature protein HaaA (ParH and ParA-associated protein A) was found as a novel interacting partner of ParH. Interestingly, HaaA, also interacts strongly with ParA in bacterial two-hybrid system. Preliminary characterization indicates that a *haaA*-null mutant exhibits slight developmental defects. These data suggests that a previously unidentified protein that may play a role in developmental chromosome segregation of *S. coelicolor*.

Keywords:*Streptomyces coelicolor*, Chromosome segregation, ParH, HaaA

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ROS AS A KEY PLAYER IN THE *STREPTOMYCES NATALENSIS* MORPHOLOGICAL DIFFERENTIATION

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Introduction: *Streptomyces* are aerobic Gram-positive bacteria that undergo a complex life cycle. After spore germination a first vegetative mycelium (MI) is formed characterized by highly septated hypha and an active primary metabolism; upon nutrient starvation certain hyphal compartments undergo programmed cell death (PCD) and a second vegetative mycelium (MII) arises from the viable compartments; along with the formation of MII a shift from primary into secondary metabolism occurs and the developmental program is activated; then the superficial layer of MII differentiates into aerial mycelium, while the innermost mycelium goes through a second round of PCD; finally spores are formed after aerial hypha septation.

The identification and characterization of proteins that are part of both the stress response network and *Streptomyces* development program shows that these physiological processes are intimately connected. Additionally there are a number of studies that associate the accumulation of reactive oxygen species (ROS) to physiological stresses and to PCD. In this study we provide further evidences regarding the role of ROS in streptomycetes morphological differentiation.

Results: *S. natalensis* D*katA1* and D*catR* were constructed, defective on the monofunctional catalase and on the fur-like repressor that controls *katA1* expression, respectively. Both strains presented morphological differentiation impaired phenotypes; D*katA1* displayed a bald phenotype, while D*catR* formed scarce aerial mycelium.

Characterization of the mutant strains showed an early activation of molecular defences against hydrogen peroxide and free ferrous iron (Fe^{2+}) as well as an induction of primary metabolism. The early activation of these protective mechanisms in D*katA1* can be related to an adaptive response to the lack of an important antioxidant mechanism, the monofunctional catalase. Concerning antioxidant defences activation in D*catR,* the over-expression of the catalase *katA1* was a direct consequence of *catR* deletion. Curiously, D*katA1* vegetative mycelium seems to be kept at the MI stage, since the typical highly septated hyphae are still observed at late stages of cultivation. This correlated with an extension of vegetative mycelium life-span in D*katA1*. Additionally, the transcription of key development related genes was repressed in both mutant strains.

Conclusion: The results suggest that the morphology impaired phenotypes observed in the *S. natalensis* mutant strains, D*katA1* and D*catR*, are the consequence of an inhibition or retardation of PCD associated with ROS homeostasis disruption. This study highlights the importance of intracellular ROS levels in the morphological development program of *S. natalensis*.

Keywords:*S. natalensis*; Ros; Morphological development; PCD

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THE SER/THR KINASE PKAI IS INVOLVED IN SPORE WALL SYNTHESIS OF *STREPTOMYCES COELICOLOR*

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The actinomycete *Streptomyces coelicolor* has a complex life cycle. It involves formation of multi-branched substrate mycelium and unbranched aerial hyphae which septate into chains of exospores. Proper sporulation is directed by the *mre* genes (*mreB*, *mreC*, *pbp2* and *sfr(rodA))* , which form a multi-protein complex, the **S**treptomyces **s**pore wall **s**ynthesizing **c**omplex (SSSC), resembling the lateral wall synthesizing complex (elongasome) of rod-shaped bacteria. Whereas the *mre* genes all are essential in *Escherichia coli* or *Bacillus subtilis,* they only effect spore maturation in *S. coelicolor*. Unicellular and non-sporulating actinobacteria lack these genes.

Bacterial two-hybrid analyses of protein-protein interactions identified the serine/threonine (ser/thr) kinase PkaI (SCO4778) as an interaction partner of MreC, MreD, Pbp2 and Sfr (*Kleinschnitz et al* 2011), suggesting control of the SSSC activity by protein-phosphorylation.

Ser/thr phosphorylation is a quite common but poorly understood regulation mechanism in bacteria. This is due to the fact that ser/thr kinases often show little specificity but several cross reactions.With approximately 36 kinases, *S. coelicolor* is one of the prokaryotic organisms with the highest number of ser/thr kinases.

RT-PCR experiments revealed that *pkaI* is expressed throughout the whole life cycle of *S. coelicolor*. Expression was strongest during vegetative growth and not during sporulation. The in frame deletion mutant Δ*pkaI* was not affected in vegetative growth, but showed a delay in sporulation. Moreover, spores of Δ*pkaI* were more irregularly shaped than wild type *S. coelicolor* M145 spores. However unlike the spores of Δ*mre* mutants, the Δ*pkaI* spores did not show an increased sensitivity to lysozyme or vancomycin.

To demonstrate phosphorylation by PkaI, we co-expressed affinity tagged MreC or Pbp2 with PkaI in *E. coli* BL21 (DE3). MreC-S-tag and PkaI-His showed a strong physical interaction and always co-purified. This could be demonstrated by Western blotting, as well as by MS/MS analysis. Expression of Pbp2 seemed to depend on the presence of PkaI, and could not be detected in the absence of *pkaI*. Following protein purification via affinity chromatography, we could show by Pro Q Diamond staining that MreC and Pbp2 were phosphorylated in the presence of PkaI. Phospho sites of MreC, Pbp2 and PkaI were determined by MS/MS analysis of the enriched phosphopeptides.

Although the exact role of PkaI in morphological differentiation of *S. coelicolor* is not yet fully understood, this is the first evidence that PkaI might control activity of MreC and Pbp2.

Keywords: Ser/thr phosphorylation, Spore maturation, Mre genes

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IDENTIFICATION OF PLAYERS DNA OF HOMOLOGOUS AND ILLEGITIMATE RECOMBINATION IN *STREPTOMYCES*

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Double strand breaks (DSB) constitute the most deleterious form of DNA damage that a bacterial cell can encounter. Two major pathways can carry out DSB repair in bacteria: homologous recombination (HR) and non-homologous end joining (NHEJ). *Streptomyces* is a model bacterium to explore the relative impact of these recombination mechanisms on genome structure and evolution; the chromosome is indeed typified by its linearity, its compartmentalized genetic organization and its remarkable genomic plasticity. The objective of this research is to identify actors involved in DSB repair mechanisms which remain mostly elusive in *Streptomyces* up to now.

The first step of DSB repair by HR is the resection of broken DNA ends by a multisubunit helicase-nuclease complex exemplified by *Escherichia coli* RecBCD, *Bacillus subtilis* AddAB and *Mycobacterium tuberculosis* AdnAB. *In silico* analysis of *Streptomyces* genomes allowed to identify homologues for *adnA* and *adnB* which constitute a highly conserved locus within the genus. Attempts to disrupt these two genes were unsuccessful in *Streptomyces ambofaciens* as well as in *Streptomyces coelicolor*, unless an extra copy of *adnAB* was inserted in the chromosome. This indicates that AdnA and AdnB are both essential for *Streptomyces* growth. Complementation of an *E. coli ΔrecB* mutant by *S. ambofaciens adnAB* locus restored (i) cell survival in the absence of DNA damaging agent (ii) nuclease activity of crude extract and (iii) ultra-violet radiation resistance, strongly suggesting that *Streptomyces adnAB* encodes a functional homologue of *E. coli* RecBCD [1]. The key role of *adnAB* in RH and DNA replication is discussed.

The NHEJ mechanism shows a sporadic distribution in bacteria and is known to involve the two proteins Ku and LigD. The Ku protein binds to the ends of the broken DNA and recruits the ATP-dependent ligase LigD which is a multifunctional protein carrying ligase, polymerase and sometimes nuclease activity. *In silico* analysis of *Streptomyces* genomes revealed a complex organization with a variable number of *ku* homologues (1 to 3) and of homologues encoding one of the three distinct LigD activities. These homologues define two conserved loci. *S. ambofaciens* possesses 3 *ku* (named *kuA*, *kuB* and *kuC*) and 2 ATP-dependent ligases (named *ligC* and *ligD*). Exposure to DNA damaging agents (mitomycin C, electron beam irradiation) of mutant strains got involved *kuA* and *ligC*, two conserved actors, but also variable genes such as *kuC* and *ligD* in DNA repair. These results open up new prospects to understand the role of NHEJ in the biology and genome evolution of *Streptomyces*.

Keywords: Homologous recombination, NHEJ

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A NOVEL PARA-LIKE PROTEIN IN *STREPTOMYCES*

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Growth and division is an essential part of the bacterial life cycle. The highly conserved, tubulin homologue, FtsZ, is a key component of the divisome. FtsZ polymerisation results in the formation of the FtsZ ring and marks the future division site. In those bacteria that undergo binary fission, placement of the FtsZ ring is controlled by negative regulators such as the Min system and nucleoid occlusion (NO), which inhibit ring formation at locations other than the middle of the cell. The Min system and NO operate together to ensure that the division site occurs away from the poles (Min) and that division does not occur over the chromosomes (NO).

Members of the MinD-ParA family of proteins show an emerging common function in controlling the localisation of their partner complexes. In *Escherichia coli*, MinD oscillates between the poles, directing FtsZ ring formation to the mid-cell while in *Bacillus subtilis*, MinD is recruited to the poles through DivIVA and MinJ. The ParA members of this family are involved in chromosome segregation as part of the ParABS system. ParB, a DNA binding protein encoded as part of an operon with *parA*, binds to *parS* sites found in the chromosome close to the *oriC*. Once bound, ParB is then moved by ParA, helping to separate chromosomes.

The multi-genomic bacterium, *Streptomyces coelicolor,* generates multiple division sites during sporulation. After cessation of growth of the aerial hyphae, chromosome segregation is driven by the polymerisation of ParA which aids the alignment of chromosomes by organising the *ori* bound ParB protein. At the same time, polymerisation of FtsZ into regular Z-rings along the aerial hyphae is positively regulated by SsgB, marking the sites of future sporulation septation. So far no direct link has been shown between the segregation of chromosomes and the placement of FtsZ rings in *Streptomyces*. Here we identify a novel ParA-like protein found in *S. coelicolor* and we discuss its potential role in cell division.

Keywords: Para/mind family; Cell division, Chromosome organization

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STREPTOMYCES COELICOLOR: DNA METHYLATION AND DIFFERENTIATION

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DNA cytosine methylation is an epigenetic modification regulating many biological processes in eukaryotes, including chromatin organization, genome maintenance and gene expression. The role of DNA cytosine methylation in prokaryotes has not been deeply investigated. In *Escherichia coli* it was recently demonstrated that cytosine methylation regulates gene expression during stationary phase [1] and that an induced state of cytosine hypermethylation leads to chromosomal DNA cleavage and cell death [2]. *Streptomycescoelicolor* is a mycelial soil microorganism, which exhibits a complex life cycle that includes three different cell types: unigenomic spores, a compartmentalized mycelium (MI) and a multinucleated mycelium (substrate and aerial mycelium, MII) [3]. The importance of DNA methylation was already described in Streptomycetes [4], but its biological role remains unknown.

The main objectives of this study are to analyze cytosine methylation pattern of *Streptomyces coelicolor* M145 during growth in liquid and on solid media, and to investigate the relationship between DNA cytosine methylation and morphological/physiological differentiation.

Cytosine methylation of total genomic DNA extracted from different developmental stages was investigated by dot-blot experiments using antibody anti-5-methylcytosine. Cytosine methylome was analyzed by BiSulphite sequencing. The biological effect of cytosine methylation was studied adding 5-aza-2'-deoxycytidine (5-AC), a hypomethylating agent, to the cultures.

Dot blot analysis revealed that the level of cytosine methylation changes during development (MI, MII and spores). Specifically, DNA methylation is higher at the MI stage than in the MII or spores. BiSulphite sequencing revealed that 30% of *S. coelicolor* genes contained a methylated motif in their upstream regions. Genes harbouring these motifs included genes related to differentiation (aerial mycelium formation and sporulation), genes involved in DNA repair/replication/condensation, as well as genes encoding proteins with unknown functions. Phenotypic analyses of cultures treated with 5-AC demonstrated that DNA methylation influences germination, aerial mycelium formation and sporulation on solid medium and antibiotic production both, on solid and in liquid medium.

Overall, our preliminary results suggest a role for DNA cytosine methylation in morphological and physiological differentiation of *S. coelicolor*. Further experiments are ongoing to demonstrate the molecular mechanisms and pathways behind the observed phenotypes.

Keywords: DNA methylation, streptomyces, morphological and physiological differentiation

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NATURAL PRODUCTS

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BIOACTIVE MOLECULES SECRETED BY NEW STRAIN OF *ACTINOMADURA* ISOLATED FROM EZZEMOUL SEBKHA OF AIN M'LILA (ALGERIA)

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Since the introduction of antimicrobials in the therapeutic arsenal of infectious diseases, microorganisms developed means of defense which confers them resistance to antibacterial. The antibiotic resistances at therapeutic doses appear more or less rapidly according to the chemical complexity of the antibiotics and genetic patrimony of the bacteria. Currently whatever the antibiotic used, there are strains of several bacterial species resistant to them. In front of such emerging of antimicrobial resistance, the discovery of new compounds is therefore a necessity. Analysis of fermentation products of new bacterial or fungal species isolated of few or no explored ecosystems is a path that can fill that need. Many organisms are able to develop molecules with antimicrobial activity can be exploited among which we mention: microorganisms, plants, lichens and insects … This work fits into these lines and regards the production of bioactive compounds by an actinomycete isolated from soil of the Ezemoul Sebkha of Ain M'lila and belonging to a rare genus *Actinomadura*. This strain is a new species in the light of the morphological, physiological, chemotaxonomic characteristics and molecular (16S rDNA sequencing). The production of antibiotic substances was investigated using six culture media. The best antibiotic production was obtained on Bennett medium. Hexane and isoamyl acetate were found to be the best solvents for the extraction of bioactive molecules produced by the strain. The largest zone of inhibition was obtained with the test-bacteria *Staphylococcusaureus* Mu 50 by discs impregnated with the hexanoic extract. The UV-Visible spectrum shows no peaks characteristic of polyenes, suggested a non-polyenic nature of the active molecules produced by the strain. The chromatographic profile (HPLC) corresponding to an injection of 25 µl gives for the active extract of the strain SS5 three peaks, revealed that the crude extract contained three actives compounds.

Keywords: Antibiotics, Actinomycetes, *Actinomadura*

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NEW ANTI-MALARIAL METABOLITES OF *STREPTOMYCES* SP., A NOVEL ENDOPHYTIC ACTINOMYCETE FROM THE ETHNOBOTANICAL MEDICINAL PLANT

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Unknown anti-malarial compound had been isolated from the *Streptomyces* sp. *BJSG4*, a novel endophyte of *Kalanchoe pinnata* (Lam.) Pers. The chemical structure of the compound was elucidated by spectral analysis. Provisionally, the identified compound has 99% similarity with 3- Hydroxy -1- (4- {13- [4- (3-hydroxy -3- phenylacryloyl) phenyl) tridecyl} -phenyl) -3- phenylprop -2-en -1- one. The purified compound showed anti-plasmodial activity with the logIC_{50} value of 3.47nM concentration and the statistical correlation coefficient (R^2) value of 0.9 indicates the high degree of correlation between the drug and *Plasmodium falciparum 3D7*. The compound also has anti-bacterial activity and poor cytotoxicity in *in-vitro* conditions. The compound producing endophyte was new to this host plant and the compound was isolated from Streptomycetes for the first time. The present study proved that the ethanobotanical medicinal plants are the source of potentially novel drugs producing endophytic actinomycetes.

Keywords: Endophytic Streptomycetes, Anti-malarial activity, Ethanobotanical medicinal plants

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ALOPHILIC ACTINOMYCEYTES: ISOLATION, MOLECULAR IDENTIFICATION AND ANTIMICROBIAL POTENTIAL AGAINST MULTI DRUG RESISTANT (MDR) VENTILATOR ASSOCIATED PNEUMONIA (VAP) CAUSING BACTERIAL PATHOGENS

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Introduction: Ventilator associated pneumonia (VAP) causing pathogens, especially multidrug resistant (MDR) bacteria are a growing concern in medical field and there is an immense need of new drugs against them. The actinomycetes have remained the prolific sources of the largest number of bioactive secondary metabolites, however the traditional approaches based entirely on the screening of common soil actinomycetes have been tired and seems to be totally ineffective due to the replication of known compounds in the screening programs. Presently more attention is being given to the rare ecological niches and untapped environments as a source of unique strains and consequently new compounds.

Objectives: The present study was designed to screen the halophilic actinomycetes present in the Kalar Khar Lake situated in the salt range, province Punjab, Pakistan for their antimicrobial potential against multi drug resistant (MDR) ventilator associated pneumonia (VAP) causing bacterial pathogens.

Methods: The water and mud samples were collected from the saline lake, Kalar Kahar, and were treated and enriched for the selective isolation of halophilic actinomycetes. The selected strains were identified by morphological, biochemical and physiological characterization and by 16S rRNA gene sequencing. The selected strains were cultivated on small scale and the resulting culture broth was freeze dried and the residue was extracted with ethyl acetate to obtain the crude extracts. The crude extracts were screened against methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsella pnuemoniae*, *E. coli*, *Enterobacter* sp. and *Acinetobacter* sp. The crude extracts were also analyzed on TLC and by HPLC-UV/RI in order to get the so called metabolic fingerprints in each case.

Results: A total of forty halophilic actinomycetes were isolated and were screened to investigate their antimicrobial potential against multi drug resistant ventilator associated pneumonia causing bacterial pathogens. The isolates showed strong pH tolerance and grow at pH 9-11. The results revealed that majority of the isolates (90%) belong to the genus *Streptomyces* as 99% genetic similarity was observed with different *Streptomyces* species in BLAST analysis of 16S rRNA gene sequence. The isolates exhibited remarkable antimicrobial activity against VAP multi drug resistant bacteria along with moderate cytotoxicity against larvae of *Artemia salina*. In most of the cases a zone of inhibition in the range of 16 to 24 mm against the test strains was recorded. The chemical screening of the extracts obtained from the isolates, by thin layer chromatography using various staining reagents and by high performance liquid chromatography, indicated an impressive diversity of the compounds produced by these strains.

Conclusion: The study reveals that these halophilic actinomycetes are a promising source of bioactive compounds. The fermentation, purification and structure elucidation of the compounds produced by these isolates may yield commercially useful antimicrobial agents.

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Keywords: Halophilic actinomycetes, Biological screening, Multi drug resistant bacteria, Secondary metabolites

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CHARACTERIZATION OF A LARGE LINEAR PLASMID PSA3239 CONTAINING SEVERAL SECONDARY METABOLITE CLUSTERS IN *STREPTOMYCES AUREOFACIENS* CCM 3239

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Introduction: Bacteria of the genus *Streptomyces* are one of the main producers of bioactive natural products. The biosynthetic genes of these secondary metabolites are physically clustered and are normally located in the large linear chromosomes characteristic of streptomycetes. However, in several cases, antibiotic biosynthesis clusters have been identified in large linear plasmids. This may ensure efficient spreading of these genes in the soil bacteria population via horizontal gene transfer. The terminal telomere regions of these plasmids contain long inverted repeats (TIR), which are capped by specific TIR-binding Tap/Tpg proteins. The distribution of linear plasmids in dividing cells usually involves a specific partitioning system encoded by *parAB* genes [1]. We previously identified a polyketide synthase gene cluster, *aur1*, responsible for production of the angucycline antibiotic auricin in *Streptomyces aureofaciens* CCM 3239. PFGE detected the single, 240-kb linear plasmid, pSA3239, in this strain and the presence of the auricin cluster in pSA3239 was confirmed by several approaches [2].

Objectives: Sequencing and characterization of a large linear plasmid pSA3239. Preparation of a *S. aureofaciens* CCM 3239 strain lacking pSA3239 and its phenotypic characterization.

Methods: Growth of *S. aureofaciens* CCM3239 and analysis of auricin production was done as described in [3]. Sequencing of the cosmid library was done as described in [2]. Disruption of the *parA* gene in *S. aureofaciens* CCM3239 was done by the same method as described in [3]. PFGE was done as described previously [2].

Results: A chromosome-walking strategy was used to identify the successive overlapping cosmids containing the pSA3239 plasmid, and their sequencing revealed the substantial part of the 240 kb sequence. TIR sequences were sequenced by a nested-PCR strategy. Interestingly, the whole sequence of pSA3239 (241 077 bp) contains unprecedented small TIR sequence (13 bp). Bioinformatic sequence analysis revealed genes encoding homologues of the segregation ParAB system, replication proteins, and TIR-binding Tap/Tpg proteins. pSA3239 is rather rich of secondary metabolites clusters. In addition to auricin cluster, it contains another aromatic type II PKS cluster, NRPS cluster for indigoidine, NRPS-PKS I cluster, and modular type I PKS cluster. Disruption of *parA* homologous gene resulted in decreased stability of pSA3239. A *S. aureofaciens* CCM 3239 strain lacking pSA3239 was prepared. Interestingly, the growth of the strain was affected, and the strain did not produce only auricin and indigoidine.

Conclusions:

1. Sequence analysis of the pSA3239 plasmid (241 077 bp) revealed several secondary metabolites clusters, including auricin cluster and indigoidine cluster.

2.pSA3239 contains genes essential for plasmid replication and segregation, and mutation in the *parA* homologous gene affected pSA3239 stability.

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3. The *S. aureofaciens* CCM 3239 strain lacking pSA3239 grew worse compared to the wild-type strain.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0203-11.

Keywords: Auricin, Angucyclines, Antibiotics, Linear plasmids

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THE AURICIN GENE CLUSTER CONTAINS GENES ESSENTIAL FOR BIOSYNTHESIS OF AMINODEOXYSUGAR FOROSAMINE IN *STREPTOMYCES AUREOFACIENS* CCM 3239

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Introduction: Streptomycetes are soil bacteria important for their ability to produce various secondary metabolites including many medicinally important antibiotics. Aromatic polyketides comprise a large group of structurally diverse secondary metabolites, which are produced by type II PKSs, which comprise three essential subunits (KS, CLF, ACP) and further subunits (KR, ARO, CYC) involved in modification of the nascent polyketide chain. Various tailoring reactions catalyzed by diverse oxygenases, reductases, methylases, and glycosyltranferases produce a final bioactive polyketide [1]. We previously identified polyketide cluster *aur1* in *Streptomyces aureofaciens* CCM3239 responsible for production of an antibiotic auricin which is a unique angucycline polyketide conjugated to an amino deoxyhexose d-forosamine. It is produced during a narrow time period following entry into stationary phase. This unusual phenomenon likely arises from a complex regulation of auricin biosynthesis [2].

Objectives: Characterization of genes involved in the biosynthesis of forosamine and their regulation.

Methods: Growth of *S. aureofaciens* CCM3239 and analysis of auricin production was done as described in[3]. Disruption of the proposed forosamine genes in *S. aureofaciens* CCM3239 was done as described in [3]. Transcription from promoters was determined by S1-nuclease mapping using RNA isolated from different growth phases as described in [3].

Results: Bioinformatic analysis of the *aur1* gene cluster and its neighbouring regions revealed genes that are likely involved in biosynthesis of forosamine and its attachment to the aglycone. We disrupted the genes *aur1T,Q,S,V, sa52, sa59, sa46, sa53* and analysed mutant for the production of secondary metabolites by TLC and HPLC followed electrospray ionization mass spectrometry. Mutants in *aur1TQSV* genes (encoding NDP-deoxyhexose biosynthetic enzymes) and *sa59* gene (encoding a homologue of NDP-hexose aminotransferase) produced only auricin deglycosylated aglycones, which were also antibiotically active. Mutant in the *sa52* gene (encoding a homologue of NDP-aminohexose N-dimethyltransferase) produced modified auricin with demethylated forosamine. Both mutants in *sa46* and *sa53* (encoding homologues of glycosyltransferase) partially affected auricin production and double mutant produced only deglycosylated auricin aglycones. In addition we identified promoters for the sugar genes and found their differential dependence upon two auricin-specific positive regulators Aur1PR3 and Aur1PR4.

Conclusions:

1. We described and characterized genes involved in sugar part forosamine biosynthesis of the antibiotic auricin in *S. aureofaciens* CCM3239. Based on the results we suggested a proposed pathway of forosamine biosynthesis.

2. We identified promoters of the sugar genes and found their differential dependence upon two auricin-specific positive regulators, Aur1PR3 and Aur1PR4.

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3. Based on the results we assume that key auricin-specific positive regulator Aur1P is mainly responsible for regulation of the core auricin genes, and two additional SARP-family regulators, Aur1PR3 and Aur1PR4, for the regulation of auricin tailoring genes.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the contract No APVV-0203-11.

Keywords: Auricin, Angucyclines, Antibiotics, Forosamine

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EXPLORING THE HIDDEN POTENTIAL OF INDIGENOUS ACTINOMYCETES ISOLATED FROM PAKISTAN AS A PRODUCER OF ANTITUMOR COMPOUNDS

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Actinomycetes are a group of Gram positive bacteria with high G+C Content. This group of bacteria is capable of producing different types of novel secondary metabolites. The aim of the present study was to screen the indigenous actinomycetes for cytotoxic and antitumor compounds. In our screening, several water and soil samples were collected from various habitats of Pakistan. More than 500 isolates were isolated, 120 of which were selected for initial identification and cytotoxic profiling. The strains were characterized on the basis of their Morphological, Biochemical and Physiological behavior. In a biological screening the crude extracts obtained from the culture broth showed good cytotoxic activity against Brine shrimp larvae with maximum of 80% inhibition. The isolates with high larvicidal activities were then tested for anti-tumor activity against various cell lines (Hela, MD-BK, Vero cell lines) through methyl thiazolyl tetrazolium (MTT) bioassay method. 20 isolates were selected with high inhibition rate against proliferating cell lines. These selected isolates were then genetically characterized through 16S-rRNA sequencing and was found that majority of these strains were belonging to a well known family of actinomycetes named as *Streptomyces*. After preparative and chemical screening we were able to isolate several antitumor compounds like Chromomycin, indolic derivatives and floustatins.

Keywords: Antitumor activity, Actinomycetes, *Streptomyces*, MTT

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IDENTIFYING SALINISPORA SECONDARY METABOLITES AND THEIR BIOSYNTHETIC PATHWAYS USING GNPS MOLECULAR NETWORKING

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Genome sequencing has revealed that actinomycetes contain many more biosynthetic pathways then would be predicted based on the numbers of secondary metabolites they produce. These “silent” gene clusters may in fact be expressed but the products missed using traditional natural product discovery tools. The generation of molecular networks based on MS-MS data provides a rapid and highly sensitive approach to visualize secondary metabolite profiles in extracts of cultured bacteria. This technique facilitates strain comparison and can be used to prioritize strains for more detailed discovery efforts. Here we have generated molecular networks for 35 *Salinispora* strains for which draft genome sequences are available. The strains include representatives from all three species and 11 global collection sites. Each strain was fermented under standardized conditions in triplicate and the extracts analyzed by HR-MS-MS. The three *Salinispora* species can be delineated in the molecular network, supporting prior observations of species-specific patterns of secondary metabolite production. The networks were populated with standards that aid in chemical identification. This has allowed analogues of previously identified *Salinispora* secondary metabolites and novel secondary metabolites to be discovered from this well-studied genus. Select nodes have been linked to genome sequencing data in an effort to identify the associated biosynthetic pathways and facilitate structure predictions. Combining new fermentation approaches with molecular networking provides opportunities to improve the efficiency with which natural products are discovered.

Keywords: Salinispora, Molecular networking, Comparative metabolomics

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DIVERSITY AND BIOACTIVE POTENTIAL OF ACTINOBACTERIA FROM MARINE SEDIMENTS OF TOSA BAY, JAPAN

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Recently the great attention has been attracted to marine actinobacteria, as a valuable source of biotechnologically important compounds. In comparison to tropical Pacific Ocean sediments, actinobacterial diversity of temperate sediments remains less explored. The aims of this study were isolation of actinobacteria from offshore sediments of Tosa Bay (Shikoku, Japan) and analysis of their potential to produce bioactive secondary metabolites. Sediments were collected at depths from 10 to 100 m. A metagenomic approach revealed that actinobacteria represent between 1.4 and 8.5% of total microbial community with the highest abundance at 10-m depth. For culture-dependent analysis, samples were pretreated, plated on selective agar media and 101 actinobacteria-like colonies were observed after incubation at 28 ^{o}C for up to four months. Based on 16S rRNA gene sequence analysis, isolates were classified into 12 genera of 8 families with the majority belonging to *Streptomycetaceae* and *Micromonosporaceae*. Interestingly, 16S rRNA gene sequence of 76% of isolates from *Micromonosporaceae* family was 100% identical to the type strain of *Salinispora arenicola* NBRC105043. However, sequence analysis of their 16S-23S rRNA intergenic spacer (ITS) revealed novel sequence variations and further phylogenetic analyses using gyrase B and rifamycin ketosynthase domain sequences supported the phylogenetic diversity of the novel *Salinispora* isolates.

Bioactivity tests using cultivation media of actinobacterial isolates showed that 60% of analyzed strains produced compound(s) inhibiting growth of bacteria and 1.4% had antifungal activity. Moreover, 94% of tested strains yielded fragments of expected size in PCR screening with nonribosomal peptide synthetase gene-targeting primers.

The results of this study have revealed a good bioactive potential of isolated marine actinomycetes from temperate sediments of Pacific Ocean. Moreover, our study provided new insights to geographical distribution and phylogenetic diversity of obligate marine actinomycete *S. arenicola*.

Keywords: Marine actinomycetes, Salinispora, Bioactive natural product, NRPS

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BLOCKAGE OF THE EARLY STEP OF LANKACIDIN BIOSYNTHESIS CAUSED A LARGE PRODUCTION OF PENTAMYCIN, CITREODIOL, AND EPI-CITREODIOL IN *STREPTOMYCES ROCHEI*

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Streptomycesrochei 7434AN4 produces two structurally unrelated polyketide antibiotics, lankacidin and lankamycin, and carries three linear plasmids, pSLA2-L, -M, and -S. In our effort to find the key intermediates of lankacidin biosynthesis in *Streptomycesrochei*, three UV-active compounds were isolated from mutant FS18, a gene disruptant of *lkcA* encoding a non-ribosomal peptide synthetase (NRPS)-polyketide synthase (PKS) hybrid enzyme. Their structures were elucidated on the basis of spectroscopic data of NMR and MS to be a 28-membered polyene macrolide pentamycin, and an epimeric mixture of citreodiol and *epi*-citreodiol. The yields of citreodiols and pentamycin in FS18 were 5- and 250-fold higher compared with the parent strain. Introduction of a second mutation of *srrX*, coding a biosynthetic gene of the signaling molecules SRBs, into mutant FS18 did not affect the production of three metabolites. Thus, their production was not regulated by the SRB signaling molecules in contrast to lankacidin or lankamycin.

Keywords: Antibiotics, Polyketide, Biosynthesis, Signaling molecule

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THE BIOSYNTHETIC MECHANISM FOR THE POLYETHER ANTIBIOTIC SALINOMYCIN

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Salinomycin, a polyether antibiotic, is widely used in animal husbandry as a food additive due to its antibacterial and anticoccidial activities. Recently, salinomycin has also been identified to kill cancer stem cells efficiently. However, the mechanism of salinomycin biosynthesis remains largely speculative until now.

Using degenerate primers based on polyether-specific epoxidase sequences, a putative salinomycin-specific epoxidase gene *slnC* was cloned from the producer *Streptomyces albus* XM211. The involvement of *slnC* in salinomycin biosynthesis was confirmed through targeted gene replacement and subsequent *trans*-complementation. A 127-kb region containing *slnC* was then sequenced and analyzed. It is revealed that genes encoding type I PKS, epoxidase, epoxide hydrolases, regulators and transporters are included in this region.

Intriguingly, an *O*-methyltransferase-like gene *slnM* is found clustered with genes involved in salinomycin biosynthesis. However, there is no methylated salinomycin detected in XM211 fermentation extract. In vivo inactivation of *slnM* abolished the production of salinomycin and led to the accumulation of pre-salinomycin with hydroxyl groups on C-13 and C-19. In vitro enzymatic assays and site-directed mutagenesis showed that SlnM, with SAM or sinefungin as a co-factor, catalyzed the introduction of $\Delta^{18,19}$ double bond and tricyclic spiroacetal in a highly atypical fashion to yield salinomycin. The biochemical characterization of SlnM reveals an unprecedented mechanism for polyketide double bond formation.

To elucidate the biosynthetic mechanism of salinomycin, all other genes in salinomycin gene cluster were systematically characterized. Among these genes, 5 genes were identified to be essential for salinomycin biosynthesis and possibly responsible for polyketide chain release, modification, oxidative cyclization and regulation. Moreover, another 5 genes were found to be relevant to salinomycin biosynthesis and putatively involved in removal of aberrant residues from unprocessed PKS proteins, supply of precursors and regulation. These findings expand the understanding of polyether biosynthesis and set the stage for targeted engineering of salinomycin activity and productivity.

Keywords: Polyether, Salinomycin, Biosynthesis

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ACTINOALLOLIDES A-E, NEW ANTITRYPANOSOMAL AGENTS ISOLATED FROM ENDOPHYTIC *ACTINOALLOMURUS*

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In course of our screening, some new bioactive compounds have been found from secondary metabolites of endophytic rare actinomycetes. Spoxazomicins, antitrypanosomal alkaloides, were isolated from *Streptosporangium oxazolinicum* K07-0460^T.[1,2] Trehangelins, photo-oxidative hemolysis inhibitors, were isolated from *Polymorphospora rubra* K07-0510.[3] Endophytic rare actinomycete might be one of attractive sources for bioactive compounds.

Actinoallomurus fulvus MK10-036 was isolated from roots of *Capsicum frutescens* collected in Thailand. As a result of physicochemical (PC) screening from metabolites of the strain MK10-036, 5 new macrolides, namely actinoallolides A-E, were discovered. The planner structure and absolute configuration of actinoallolide A were elucidated by NMR spectroscopy and X-ray crystallography. The structures of actinoallolide B-E were determined by conversion from actinoallolide A. Furthermore they exhibited potent antitrypanosomal activities against *Trypanosoma brucei brucei*.

Keywords: Actinoallolide, Actinoallomurus

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BREAKING TRANSCRIPTIONAL 'LOCKS' FOR NATURAL PRODUCT DISCOVERY IN *STREPTOMYCES VENEZUELAE*

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Introduction: *Streptomyces* bacteria are prolific producers of natural products with uses including antibiotics, pesticides, immunomodulatory agents and anticancer compounds. Sequencing of *Streptomyces* genomes over the last decade has led to discovery of hundreds of gene clusters encoding for natural product biosynthesis whose products are unknown, and many of these cryptic biosynthetic clusters are silent in laboratory culture conditions.[1]

Transcriptional repressor proteins such as ArpA play a vital role in regulation of secondary metabolite biosynthesis.[2] The deletion of genes *scbR2* and *mmyR* encoding for transcriptional repressor proteins has previously been shown to lead to overproduction of antibiotic compounds coelimycin and methylenomycin respectively, in *S. coelicolor*. However, there are currently very few examples of these strategies being employed to discover new natural products.[2,3]

Objectives: To identify and characterise the metabolites produced in an ArpA-like deletion mutant *gbnR* strain of *S. venezuelae* and to investigate the biosynthesis of these compounds and the role played by the adjacent, usually silent, *gbnABC* cluster.

Methods:Gene deletion combined with growing *S. venezuelae* strains in a variety of culture conditions, followed by liquid-chromatography mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy.

Results: Gaburedins – a novel class of ureido-linked GABA derivatives – have been purified from *Streptomyces venezuelaegbnR* deletion mutant strain by comparing the metabolic profiles of the *S. venezuelae* wild type and a number of mutant strains *via* LC-MS analysis. Creation of double-deletion mutants and subsequent analysis of the metabolites produced has led to identification of a key biosynthetic enzyme, whose role has been explored *in vivo* by feeding with a variety of precursor molecules.[4] These feeding experiments have been expanded upon further by addition of other amino acids and amines to produce a library of gaburedin analogues and drug-like molecules, indicating the potential utility of the GbnA and B enzymes as biocatalysts. Authentic standards have been synthesised in order to confirm the proposed structures and to begin biological testing.

Conclusion: A series of novel ureido-linked dipeptides has been discovered to be produced by the previously cryptic, normally silent *gbnABC* cluster, leading to the discovery of analogous systems across a range of bacterial genera. This is the first time that targeted deletion of an ArpA-like transcriptional repressor has led to discovery of a new natural product series in *Streptomyces*.

Keywords: Natural product biosynthesis, Genome mining, Transcriptional repressors

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ECOLOGY AND EVOLUTION OF HYBRID ISOPRENOID PRODUCTION IN A MARINE-DERIVED STREPTOMYCETE LINEAGE

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Terpenoids are among the most diverse and ubiquitous classes of metabolites observed in nature. Many terpenoids provide essential conserved roles in primary metabolism, but little is know about the evolution and function of terpenoids produced as a part of secondary metabolism. In addition to purely isoprene-derived structures, terpenoid moieties can be appended to non terpene-derived compounds, resulting in a structurally diverse group of molecules called hybrid isoprenoids (HIs). Although terpenoid biosynthesis is widespread, pathways for the production of HI structures are relatively rare in bacteria. Genomic analysis of HI pathway distribution in the genus *Streptomyces* reveals that the 'MAR4' lineage of largely marine-derived strains possesses an unprecedented potential for the production of molecules in this interesting class, and suggests that biosynthetic pathways for HI production are positively selected for and maintained in MAR4. Phylogenetic analyses of biosynthetic enzymes in these pathways reveal that a variety of evolutionary mechanisms including vertical transmission, gene duplication, and horizontal gene transfer have all contributed to the evolutionary history of HI pathways. These examples provide evidence for how evolution creates novel structural diversity in secondary metabolism.

Ecological functions have yet to be determined for any HI molecules. These molecules are frequently redox-active and resemble electron shuttling molecules of primary metabolism and could possibly serve this role. To test this hypothesis, a MAR4 strain was found to reduce insoluble Mn (IV) via a diffusible electron shuttle, making this the first member of the Phylum Actinobacteria known to participate in extracellular electron transfer. Growth of this strain in microaerophilic conditions was found to induce a switch from production of a HI molecule towards a pigmented, non-prenylated napthoquinone precursor. The pigmented precursor was found to be redox-active, and its specific role for the producing organism in low-oxygen conditions is currently under investigation.

Keywords: Marine Streptomycetes, Biosynthetic pathway evolution, Hybrid isoprenoids, Electron shuttling

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CYCLIZING 5-AMINOLEVULINATE SYNTHASES IN THE BIOSYNTHESIS OF ACTINOMYCETE SECONDARY METABOLITES: OUTCOMES FOR GENETIC SCREENING TECHNIQUES

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Cyclizing 5-aminolevulinate synthases represent a novel subclass of enzymes, able not only to synthesize 5-aminolevulinic acid (ALA) from glycine and succinyl-CoA, but also to catalyze its subsequent intramolecular cyclization, forming 2-amino-3-hydroxypent-2-enone, the C_5N unit. The enzyme seems to be specific for secondary metabolite production in actinomycetes and can be used as a genetic marker for production of certain metabolites.

The biosynthesis of the C_5N unit is encoded by a triplet of genes. Aminolevulinate synthase (ALS) forms ALA and the cyclic unit, aminolevulinate-CoA ligase (ALL) activates ALA, and a specific amide synthesis attaches (AMS) the unit to an antibiotic core. The relevant gene triplet can be found in all biosynthetic gene clusters encoding C_5N-containing secondary metabolites. These represent a diverse group of bioactive compounds, encompassing manumycins, moenomycins, bafilomycins, reductiomycin and others. Among these, many show pharmaceutically-attractive biological activities: cancerostatic, immunomodulatory, antifungal, antiviral, etc.

In our project based on the genetic screening of about 1200 natural actinomycete strains isolated world-wide for the presence of the cyclizing type of ALS, as a marker of possible novel producers of manumycin-type compounds, we have collected more than 150 positives, which were further genetically characterized. The data were compared with characterized producers of C_5N-carrying secondary metabolites. Also, information originated from the actinomycete genome sequencing projects was included. Here we report a direct correlation between a type of synthesized C_5N-containing metabolite and ALS amino acid sequence and spatial organization of the *als-all-ams* gene triplet.

Comparison of amino acid sequences of cyclizing ALS with representatives of classical ALS from animals, fungi and proteobacteria shows, that they form a distinct group of enzymes. During evolution they gained a novel, secondary metabolites-related function. The knowledge provides a useful tool in designing a genetic screening techniques specific to particular metabolite types.

The project has got a financial support from the Ministry of Education, Youth and Sports (LH12191) and Ministry of Health (NT/13012) of the Czech Republic.

Keywords: Actinomycetes, Secondary metabolites, Manumycin, C5n unit, 5-aminolevulinate synthase, Genetic screening

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SCREENING OF *STREPTOMYCES SCOPULIRIDIS* KR-001 AND ITS HERBICIDAL CHARACTERISTICS

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With increasing environmental issues from synthetic chemical herbicides, microbe-originated herbicides could be a fascinating alternative in current farming. In this study, we isolated a *Streptomyces* strain that produced herbicidally active metabolites against a grass weed Digitaria sanguinalis. According to the result from 16S rDNA sequence comparison with the close strains, the isolate was identified as Streptomyces scopuliridis KR-001. The closest type strain was *Streptomyces scopuliridis* RB72 which was previously reported as a bacteriocin producer. The optimal culture condition of *S. scopuliridis* KR-001 was 28°C, pH 7.0 and culture period 4 to7 days. Both soil and foliar application of the crude culture extract was effective on several troublesome or noxious weed species such as Sciyos angulatus, Humulus japonicus, Altemisia princeps, Equisetum arvense, Trifolium repens in greenhouse and field conditions at 1,000 to 250 ug/mL. Phytotoxic symptoms of the culture broth concentrate of *S. scopuliridis* KR-001 by foliar application were wilting and burn-down of leaves, and stems followed by discoloration and finally plant death. These results suggest that the new *S. scopuliridis* KR-001 strain or herbicidal metabolites is a new bio-herbicide candidate and/or may provide a new lead molecule for a more efficient herbicide.

Keywords: Bioherbicide, Sciyos angulatus, *Streptomyces scopuliridis*, Toublesome weeds

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BIOSYNTHESIS AND ISOLATION OF DD-PEPTIDASE 64-575 INHIBITOR FROM *STREPTOMYCES* SP. 19729

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National Institute of Public Health - National Institute of Hygiene

For many years microbial natural products have been one of the major sources of novel drugs for pharmaceutical companies. Even today all evidence suggests that novel molecules with potential therapeutic applications are still waiting to be discovered from these natural sources, especially from actinomycetes [1]. Secondary metabolites from *Streptomyces* spp., which are members of actinomycete genera have major importance in drug discovery process. They are diverse and unusual in their chemical structures and may be used as scaffolds for further modifications. These compounds are active against bacteria, viruses, fungi, parasites and cancer cells [2].

Streptomyces spp., are responsible for the production of the majority of bioactive compounds. In our study we used *Streptomyces badius* 19729. This strain proved to be a producer of DD-carboxypeptidase 64-575 inhibitors. DD-carboxypeptidase is involved in biosynthesis of peptidoglycan - bacterial cell wall component.

The first aim of the research was to increase the productivity of bioactive compounds, isolation and purification of DD-carboxypeptidase 64-575 inhibitors. The fermentation process was proceeded in flasks, and next in 5l fermenters. Isolation and purification of DD-carboxypeptidase 64-575 inhibitor scheduled using techniques such as liquid chromatography with cation change resin, solid phase extraction of selected fraction and high performance liquid chromatography on a C18 column.

The molecular formula of isolated metabolite in negative ion mode UHPLC-HR-MS is $C_9H_8NO_4$ $[M-H]^-$ m/z 194.0436. Positive ion mode gives protonated ion after loss of water molecule $[M-H_2O+H]^+$ m/z 178.0490.

Studies on its biological activity and structure elucidation are currently underway.

The second aim of the study was to evaluate the influence of carbon and nitrogen sources availability on the production of inhibitors of DD-peptidase 64-575 and biomass growth.

The production of the most potent DD-peptidase 64-575 inhibitors was obtained at 72h of biosynthesis (medium rich in nitrogen from tryptone, peptone, yeast extract and corn steep liquor and with moderate amount of lactose).

The elimination of all nitrogen sources from culture media resulted in the lack of DD-peptidase 64-575 inhibitors production.

Reduction of lactose content in culture media and doubling the amount of nitrogen sources like tryptone, peptone, yeast extract and corn steep liquor had a positive effect on the biosynthesis of DD-peptidase inhibitors.

High values of DD-peptidase inhibition were obtained in culture media rich in both carbon and nitrogen sources. A significant increase of biomass growth was achieved at 24 hours of cultivation.

This study was partially supported by the European Union, Ministry of Regional Economy, Grant UDA-POIG. 01.03.01-14-136/09.

Keywords: Biosynthesis, DD-peptidase inhibitors, Isolation

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SEQUENCE ANALYSIS OF POROTHRAMYCIN BIOSYNTHETIC GENE CLUSTER

Bojana Radojevic, Zdenek Kamenik, Magda Pavlikova, Marketa Koberska, Lucie Najmanova, Dana Ulanova, Marketa Jelinkova, Eliska Kettnerova, Radek Gazak, Jiri Janata

Institute of Microbiology AS CR

The biosynthetic gene cluster of porothramycin, a sequence-selective DNA alkylating compound, was identified in the genome of producing strain *Streptomyces albus* subsp. albus (ATCC 39897) and sequentially characterized. A 39.7 kb long DNA region contains 27 putative genes, 18 of them revealing high similarity with homologous genes from biosynthetic cluster of closely related pyrrolobenzodiazepine (PBD) compound anthramycin. However, considering the structures of both compounds, the number of differences in the gene composition of compared biosynthetic clusters was unexpectedly high, indicating participation of alternative enzymes in biosynthesis of both porothramycin precursors, anthranilate and branched L-proline derivatives. Based on the sequence analysis of putative NRPS modules Por20 and Por21 we suppose that in porothramycin biosynthesis the methylation of anthranilate unit occurs prior the condensation reaction, while modifications of branched proline derivative, oxidation and di-methylation of the side chain, occur on already condensed PBD core. Corresponding two specific methyltransferase encoding genes por26 and por25 were identified in the porothramycin gene cluster. Surprisingly, also methyltransferase gene por18 homologous to orf19 from anthramycin biosynthesis was detected in porothramycin cluster even though the appropriate biosynthetic step is missing, as confirmed by UHPLC-DAD-MS analysis of the product in the *S. albus* culture broth. In addition to that, porothramycin biosynthetic pathway will be discussed in the context of biosynthetic machineries of other PBDs, eg. limazepines and sibiromycin. This work was supported by project: CZ.1.07/2.3.00/20.0055

Keywords: *Streptomycesalbus* subsp. albus strain (ATCC 39897), Porothramycin, Pyrrolobenzodiazepines, Biosynthetic cluster

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TAILORING *STREPTOMYCES* ANTIBIOTIC BIOSYNTHESIS: PRODUCING NOVEL MINOR GROOVE BINDING ANTIBIOTICS

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The rise of antibiotic resistant strains and the decline of antibiotic discovery have resulted in an urgent need to develop and discover novel antimicrobials. Minor groove binders (MGB's) are compounds that shown antibiotic, anticancer and antiviral activity by binding to the minor groove of the DNA helix.

MGB's are naturally produced by *Streptomyces*, and recently some of the clusters encoding for those antibiotics have been characterised. In order to produce a new drug, we are using synthetic biology and combinatorial biosynthesis to modify a pathway of a *Streptomyces* strain, forcing the assembly of a new antibiotic.

For this we transformed several *Streptomyces* strains with an MGB producing biosynthetic cluster containing four independent non-ribosomal peptide synthetases. These strains were fermented and sampled at different time points and the organic extractions were analysed looking for the production of novel compounds. The HPLC, LCMS and HRMS data suggest the assembly of a novel compound, which exhibits antibiotic activity according to our bioassays. Further characterisation of the molecule will be done by NMR .We have also cloned one of the non-ribosomal peptide synthetases in order to engineer this enzyme to broaden its substrate specificity.

This research is carried out in the excellent SIPBS facilities at the Strahtclyde University, thanks to the financial support of SULSA and MSD.

Keywords: New antibiotics, Minor groove binders, Synthetic biology

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APDOS 2.0 – PHYLOGENETIC METHODS TO PREDICT SECONDARY METABOLITE DIVERSITY

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New bioinformatic tools are needed to effectively analyze the constantly growing volume of DNA sequence data generated with next generation sequencing methods. Here we introduce our newly revised web-tool NaPDoS 2.0 for the automatic assessment of secondary metabolite biosynthetic gene diversity and novelty of strains or environments. NaPDoS uses the phylogenetic relationships of ketosynthase (KS) and condensation (C) domainss to classify and predict biochemical function of the associated polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) gene clusters. NaPDoS 2.0 includes a major expansion of the reference domain sequences, which doubles the size of the current database to approximately 1400 domains derived from 130 diverse PKS and NRPS pathways. This expansion of the reference data set allowed for the refinement of PKS and NRPS classes and the identification of novel clades that represent distinct biochemical functions.

Keywords: Secondary metabolites, Phylogenomics, NRPS, PKS, Genome mining

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TANDEM REPEAT EXPRESSION OF AN ENTIRE TAUTOMYCETIN BIOSYNTHETIC GENE CLUSTER IN *STREPTOMYCES* HOSTS USING E. *COLI-STREPTOMYCES* SHUTTLE BACTERIAL ARTIFICIAL CHROMOSOME SYSTEM

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Developments of next-generation sequencing technologies have brought recognition of microbial genomes as a rich source of diverse natural products. Functional expression of an entire secondary metabolite biosynthetic pathway gene cluster is an attractive alternative to facilitate production improvement and biosynthetic modification of a potentially-valuable natural product derived from various genetically-recalcitrant *Streptomyces* species. Previously, a versatile *Escherichia coli-Streptomyces* shuttle Bacterial Artificial Chromosomal (BAC) conjugation vector, pSBAC was successfully used for heterologous expression of a meridamycin biosynthesis gene cluster in *S. lividans*. Using pSBAC-driven genome engineering techniques as well as PCR-targeted gene manipulation, here we show the both homologous and heterologous expressions of an entire biosynthetic gene cluster of tautomycetin (TMC), a protein phosphatase PP1/PP2A inhibitor and T-cell-specific immunosuppressant. Unique *Xba*I restriction sites were first inserted at the both border regions of the TMC biosynthetic gene cluster (approximately 100 kb) in the chromosome of *Streptomyces* sp. CK4412, followed by site-specific recombination of the modified pSBAC into the flanking region of the TMC gene cluster. The entire TMC gene cluster was then recovered as a giant single recombinant pSBAC by the *Xba*I digestion of the chromosomal DNA and the subsequent self-ligation. The recombinant pSBAC construct containing the entire TMC cluster in *E. coli* was conjugated into the model *Streptomyces* strains such as *S. lividans* or *S. coelicolor*, resulting in the fast and enhanced TMC production. Moreover, re-introduction of the TMC cluster-containing pSBAC into the wild-type *Streptomyces* sp. CK4412 as well as the TMC cluster-containing *S. coelicolor* resulted in a tandem repeat of an entire TMC cluster in the chromosome with increased TMC productivities. More detailed results will be discussed.

Keywords:*Streptomyces*, Tautomycetin, Homologous and heterologous expression, Tandem repeat, pSBAC

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COMBINED INDUCTION: A FERMENTATION METHOD FOR HETEROLOGOUS SECONDARY METABOLITE PRODUCTION IN *STREPTOMYCES*

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Introduction: The *Streptomyces* genome contains 30–40 secondary metabolite biosynthetic gene clusters, yet researchers usually identify only a few secondary metabolites from each strain under pure culture conditions. This suggests that while several biosynthetic gene clusters are expressed, the majority of gene clusters is silent or expressed below the level of detection. Development of new methods to activate these silent or cryptic secondary metabolites, will allow us to identify natural products containing novel chemical structures that may be used in therapeutic applications.

“Combined culture” is a co-culture method used to activate secondary metabolism.[1] When *Streptomyces* was co-cultured with mycolic acid-containing bacteria (MACB), MACB interacted with *Streptomyces*, changing its secondary metabolites profile. We developed this new co-culture method for antibiotic screening. Alchivemycins, novel antibiotics, were isolated by combined culture of *Streptomycesendus* S-522 with the MACB *Tsukamurella pulmonis* (Tp).

Objectives: In our presentation, we describe the "combined induction" method—application of the combined culture method to a heterologous expression host to increase heterologous antibiotics production. *Streptomyces lividans* is known to be a conditional producer of 2 types of red pigments, actinorhodin and undecylprodigiosin. We observed that *S. lividans* produced these red pigments in combined cultures with Tp, *Rhodococcus erythropolis* (Re), or *Corynebacterium glutamicum* (Cg). This result indicated that the secondary metabolism of *S. lividans* was stimulated by MACBs. We then evaluated whether combined culture was suitable for heterologous production of secondary metabolites.

Methods: The staurosporine, rebeccamycin, and goadsporin biosynthetic gene clusters from *Streptomyces* sp. TP-A0274, *Lechevarielia aerocolonigenes*, and *Streptomyces* sp. TP-A0584, respectively, were transformed into *S. lividans* to construct heterologous expression strains. Combined culture was then performed using these heterologous strains and 3 MACBs: Tp, Re, and Cg. The culture broth extracts were analyzed using HPLC and the amounts of each product was compared to that in the pure culture.

Results: In combined induction, the heterologous strains increased the production of each metabolite above that observed in the pure cultures. After 7 days of co-culture with Tp, 215.6 mg/L of goadsporin was produced—3.58-fold more goadsporin than in the pure culture on the same day. After 13 days of co-culture with Tp, 101.5 mg/L of staurosporine was produced—209-fold more staurosporine than in the pure culture. Lastly, after 6 days of co-culture with Tp 27.5 mg/L of rebeccamycin was produced—123.6-fold more rebeccamycin than in the pure culture.

Conclusion: In combined induction, *S. lividans* received MACB-stimulated signals to activate heterologous secondary metabolism as well as endogenous metabolism. These results suggest that the combined culture method is a useful tool not only for screening novel secondary metabolites, but also for improving heterologous expression productivity.

Keywords: Natural product discovery, Cryptic genes, Co-culture, Heterologous expression

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POST-PKS MECHANISM OF A DI-SUGAR CONTAINING NOVEL POLYENE, NPP IN *PSEUDONOCARDIA AUTOTROPHICA*

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Inha University

Polyene macrolides, a large family of polyketides with antifungal activities including nystatin A1 and amphotericin B, continue to be the most potent broad-spectrum antifungal agents available for clinical use, which mostly covers life-threatening fungal infections, particularly in patients who have undergone organ transplantation, received aggressive chemotherapy, or have AIDS. The disadvantages of polyene macrolide are its relatively high toxicity, especially toward kidney cells, and poor distributions in tissues due to low water solubility. Previously, the rare actinomycetes *Pseuconocardia autotrophica* was shown to produce a solubility-improved toxicity-reduced novel polyene compound named Nystatin-like Pseudonocardia Polyene (NPP) which contains an aglycone identical to nystatin and harbors a unique di-sugar moiety, mycosaminyl-(a1-4)-*N*-acetyl-glucosamine. To further maximize NPP potential, we analyzed *P. autotrophica* whole genome sequence to identify a unique glycosyltransferase present only in the NPP producing strain. As a result, NppY was predicted to be a putative NPP-unique glycosyltransferase gene which is responsible for transfer of second sugar, N-acetylglucosamine in the NPP biosynthesis. Also, we identified unique post-PKS mechanism for NPP biosynthesis in *P. autotrophica*. Targeted gene disruption-&-complementation have been utilized to confirm functions of NppY and NPP biosynthetic mechanism. Also, Site-directed mutagenesis and construction of hybrid glycosyltransferase were used. The more detailed results will be further discussed.

Keywords: Post-PKS mechanism, Polyene, *Pseudonocardia autotrophica*

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GENERATION OF AMINOCOUMARIN DERIVATIVES CONTAINING A 2,3-DIHYDROXYBENZOYL MOIETY

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The aminocoumarin antibiotics are highly effective inhibitors of the bacterial DNA gyrase. They are mainly active against Gram-positive pathogens, including MRSA strains. Their antimicrobial activity against Gram-negative pathogens is limited, due to the bacterial outer membrane acting as permeability barrier and due to active efflux systems in the bacterial cell envelope [1].

Siderophores are iron(III) chelators, secreted by bacteria to scavenge rare and essential iron(III) from the environment. The siderophore/iron(III) – complexes are transported across the cell envelope via siderophore transporters. Antibiotic-siderophore-conjugates mimic natural siderophores and thus the siderophore transporter system can be exploited to facilitate the import of antimicrobial active compounds into their target organism [2].

The aim of the present study is the introduction of a 2,3-dihydroxybenzoyl moiety into the aminocoumarin antibiotic clorobiocin. The most important natural siderophores contain such 2,3-dihydroxy-benzoyl moieties, which are recognized by siderophore transporters. Thus we want to overcome the limitations of aminocoumarin antibiotics in Gram-negative bacteria and improve their import into the cell across the bacterial cell envelope.

For the generation of a novel clorobiocin derivative with a 2,3-dihydroxybenzoyl moiety six different artificial gene operons were created covering all genes required for biosynthesis of the desired compound. Each artificial gene operon contains an inducible tetracycline controllable promotor (*tcp830*) followed by genes required for each structural moiety of clorobiocin, respectively. In addition, an artificial gene operon (*dhbACEBG*) for the biosynthesis of 2,3 – dihydroxybenzoic acid was generated. For the assembly of all six artificial gene operons λ-RED – mediated recombination was used.

The first artificial gene operon comprising the *dhbACEBG* – genes was expressed heterologously in *Streptomyces coelicolor* M1146 and formation of the expected product, 2,3 – dihydroxybenzoic acid was confirmed by HPLC – analysis.

Keywords: Aminocoumarin, Synthetic biology, λ-red – mediated recombination, Trojan horse strategy

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SECONDARY METABOLITES WITH ANTIMICROBIAL POTENCY PRODUCED BY NEWLY DESCRIBED *STREPTOMYCES* SPP. AND *LENTZEA* SP.

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Introduction: *Streptomyces* genus constitutes a major group of producers of antibiotics used in medicine. The alarming increase in frequency of isolation of antibiotic resistant "superbugs" constitutes a growing problem worldwide. Thus, novel and effective antimicrobial agents are urgently needed to be discovered.

Objectives: Isolation of soil *Streptomyces* spp. and determine the taxonomic position of the isolates with antimicrobial properties. Purification and characterization of antimicrobial secondary metabolites produced by selected strain *Streptomyces* sp. L1.

Methods: Selective agar was used to isolate *Streptomyces* spp. from soil samples. Lyophilized supernatants of 144th h fermentation of isolates were tested for antibacterial activity against *Staphylococcus aureus* subsp. *aureus* ATCC® 43300 and *Escherichia coli* ATCC® BAA-198. Phylogenetic and chemotaxonomic analysis were proceeded for strains with antimicrobial activity. Morphological observations of hyphae were conducted under scanning electron microscope. Antimicrobial activity of *Streptomyces* sp. L1 cultured with different carbon sources in the production medium was tested on several bacterial strains (reference and clinic). The process of antimicrobial secondary metabolites isolation from supernatant was conducted using extraction with ethyl acetate, anion exchange resin Amberlite® IRA-400 (AcO^-), Solid Phase Extraction (SPE) (CN column) and HPLC (C_{18} column).

Results: 196 *Streptomyces* cultures were isolated from soil samples from different parts of the world. 41% of strains produced compounds, which inhibit growth of *S. aureus* ATCC 43300, 0.5% inhibit growth of *E. coli* ATCC® BAA-198. Growth of both bacteria was inhibited by 11% of isolates. 1% of actinomycete strains showed higher activity against *E. coli* ATCC® BAA-198 than against *S. aureus* ATCC 43300. 20 actinomycete isolates with the highest antimicrobial activity were selected for genetic analysis. DNA of all isolated bacteria had G+C content between 67-69%. 16 of isolates are known *Streptomyces* strains, two are new *Streptomyces* strains, one is new *Lentzea* strain (firstly described *Lentzea* strain producing antibacterial metabolites). The genera of the new strains were confirmed by presence of LL - diaminopimelic acid in the cell wall extracts. The profiles of fatty acids, polar lipids and respiratory quinones were characteristic for the genus of new strains. Under scanning electron microscopy spores with sheaths were observed for *Streptomyces*, the broken fragments of mycelium were observed for *Lentzea.Streptomyces* sp. L1 was selected for the analysis of secondary metabolites produced in submerged culture. The selected strain produces deep purple dye during fermentation, which colored colonies of Gram – negative bacteria. Supernatant of this strain strongly inhibited growth of *E. coli* BAA-198, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC® 13882, *Salmonella enterica* ATCC® 10708™. The inhibition of *Pseudomonas aeruginosa* ATCC® 27853, *Proteus vulgaris* ATCC® 6896™, *Proteus mirabilis* ATCC® 12453, *Stenotrophomonas maltophilia* ATCC® 13637™ and *S. aureus* subsp. *aureus* ATCC® 29213™ was lower. Similar results were obtained against clinical strains. Two active compounds are under purification.

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Conclusions: Compounds derived from nature such as secondary metabolites from *Streptomyces* fermentations are still a rich source of biologically active substances. Searching for novel *Streptomyces* strains increases possibility for discovered new antimicrobial agents.

This study was supported by European Union, Ministry of Regional Economy, Grant UDA-POIG.01.03.01-14-136/09/09.

Keywords:*Streptomyces* , Secondary metabolites, Antimicrobial activity

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A METABOLOMIC APPROACH FOR IDENTIFYING NOVEL POLYKETIDES COMPOUNDS

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Actinomycetes are a rich source of secondary metabolites including polyketides. Recent genome analyses revealed that each actinomycete strain possesses a large number of polyketide synthase (PKS) gene clusters suggesting that the number of hitherto undiscovered polyketide compounds may be much higher than that of the currently recognized ones. Thus, the development of high throughput screening methods to identify novel polyketide compounds is highly anticipated.

Mass spectrometry (MS) is rapidly becoming a powerful tool for the comprehensive profiling of small molecules (metabolites). Especially, the FT-ICR-MS provides the high mass accuracy and resolution; and thousands of metabolites in a sample can be identified at one time. Still, a minor proportion of metabolites detected by FT-ICR-MS could reliably be assigned to individual molecular formulas. Thus, it would still be in infancy to identify novel polyketide compounds among non-targeted comprehensive metabolome data obtained by FT-ICR-MS analyses. In this study, we develop a FT-ICR-MS method to select polyketide molecules among the metabolomes of actinomycetes strains.

We cultivated tree actinomycetes strains, *Streptomyces violaceoruber* NBRC 15146 [formerly *Streptomyces coelicolor*A3(2)], *Saccharopolyspora erythraea* NBRC 13426^T and *Streptomyces eurocidicus* NBRC 13491^T, with or without the PKS inhibitor cerulenin. As cerulenin is unstable, 40 ug/ml of cerulenin was added to the cultures every 24 hours. In these strains, the production of polyketides such as purple pigments, erythromycin and eurocidin was remarkably suppressed by the addition of cerulenin. Cerulenin seemed to inhibit specifically the synthesis of polyketides, because the growth and the production of non-polyketide compounds in these strains were not affected by the addition of cerulenin.

Two types of cultures, one with and the other without cerulenin, were prepared for each strain, and the metabolites of cells grown in these cultures were analyzed by LC/FT-ICR-MS. By comparing the metabolome data from two types of cells, we identified mass signals which intensities remarkably decreased in cerulenin-supplemented cells. The possible chemical formulae based on their mass values were determined, and a database search suggested that some of them represent novel compounds.

In summary, by comparing two metabolome sets collected by LC/FT-ICR-MS, one from cells grown in the absence of cerulenin and the other from cells grown in the presence of cerulenin, we successfully identified mass signals which may correspond to polyketides. From the highly accurate mass values of these polyketide candidates, we could determine the molecular formulae of these candidates. Such techniques will be useful in the discovery of new polyketide compounds.

This research was supported by the New Energy and Industrial Technology Development Organization (NEDO).

Keywords: Polyketide, PKS inhibitor, Cerulenin, FT-ICR-MS, Metabolome

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NEW AMINOCOUMARINS FROM THE RARE ACTINOMYCETE *CATENULISPORA ACIDIPHILA*: IDENTIFICATION, STRUCTURE ELUCIDATION AND HETEROLOGOUS PRODUCTION

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Aminocoumarin antibiotics are produced mainly by *Streptomyces* strains and defined by their 3-amino-4,7-dihydroxycoumarin moiety. So far five aminocoumarins, novobiocin, clorobiocin, coumermycin A1, simocyclinon D8 and rubradirin, have been discovered and their biosynthetic gene clusters have been published and investigated during the last two decades. [1]

Genome mining led to the discovery of a novel aminocoumarin gene cluster in the rare actinomycete *Catenulispora acidiphila* DSM 44928. Sequence analysis revealed the presence of genes putatively involved in export/resistance, regulation, biosynthesis of the aminocoumarin moiety and halogenation, as well as several genes with so far unknown function. Two new aminocoumarins, cacibiocin A and B, matching the structural characteristics predicted from the gene cluster, could be identified in the culture broth of *C. acidiphila*. Heterologous expression of the putative gene cluster in *Streptomyces coelicolor* M1152 confirmed it to be responsible for cacibiocin biosynthesis. Furthermore total production levels of cacibiocins could be raised by heterologous expression and media screening from initially 4.9 mg/L in *C.acidiphila* to 60 mg/L in *S. coelicolor* M1152. By HR-MS and NMR analysis cacibiocin A was found to contain a 3-amino-4,7-dihydroxycoumarin moiety linked by an amid bond to a pyrrol-2,5-dicarboxylic acid. The latter structural motif has not been identified previously in any natural compound. Additionally, cacibiocin B contains two chlorine atoms at positions 6' and 8' of the aminocoumarin moiety.

Both features open new possibilities for combinatorial biosynthesis of aminocoumarins. Furthermore, investigations of the formation of the novel pyrrole-2,5-dicarboxylic acid moiety might reveal a new pathway to pyrroles in nature.

Keywords: Actinobacteria; Gene expression; Heterologous expression; Structure elucidation; Natural products

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LINK BETWEEN GLYCOPEPTIDE RESISTANCE AND PRODUCTION IN ACTINOMYCETES

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Glycopeptide resistance is intensively studied in enterococci and staphylococci because of their role in human infections. However little is known about immunity mechanisms in actinomycetes that produce glycopeptides [1]. These bacteria are resistant to the antibiotics they make, thus avoiding suicide during production phase. In previous work, we identified a D, D-carboxypeptidase encoded by *vanY* as the determinant of glycopeptide resistance in *Nonomuraea* sp. ATCC 39727, the producer of the glycopeptide antibiotic A40926[2]. Interestingly, homologues of *vanHAX* genes mediating glycopeptide resistance in many clinical isolates are not found in the A40926 biosynthetic cluster nor elsewhere in the *Nonomuraea* genome [2].

Subsequently, we have investigated in more detail the role of the β -lactam-sensitive D, Ddipeptidase/D, D-carboxypeptidase encoded by $vanY_n$ in conferring immunity to A40926 in *Nonomuraea* sp.Taking advantage of tools we developed recently to genetically manipulate this uncommon actinomycete, we varied $vanY_n$ gene dosage and expressed $vanH_{at}A_{at}X_{at}$ from the teicoplanin producer *Actinoplanes teichomyceticus* in *Nonomuraea* sp.

Deleting $vanY_n$, complementing a $vanY_n$ mutant or duplicating $vanY_n$ had no effect on growth, but affected antibiotic resistance and, in the cases of complementation and duplication, antibiotic production. *Nonomuraea* sp. was resistant to penicillins, but its glycopeptide-resistance was diminished in the presence of penicillin G, which inhibits $VanY_n$ activity. Heterologous expression of $vanH_{at}A_{at}X_{at}$ increased A40926 resistance in *Nonomuraea* sp., but did not increase antibiotic production, indicating that the level of antibiotic production is not limited by the level of resistance. The $vanY_n$-based self-resistance in *Nonomuraea* sp. resembles the glycopeptide resistance mechanism described recently in mutants of *Enterococcus faecium* selected *in vitro* for high level resistance to glycopeptides and penicillins [3].

Keywords: Glycopeptide antibiotics, Immunity, Production, A40926, Nonomuraea

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REVISION OF BIOSYNTHETIC PATHWAY FOR COMMON PRECURSOR OF PYRROLOBENZODIAZEPINES, LINCOMYCIN AND HORMAOMYCIN

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Biosynthesis of natural compound lincomycin shares with some pyrrolobenzodiazepines (anthramycin, sibiromycin, porothramycin, tomaymycin) and hormaomycin nearly identical precursor, branched L-proline derivative (BPD), particularly 4-propyl-L-proline (PPL) in lincomycin. All these BPDs are synthesized from L-tyrosine by specialized biosynthetic pathway. Accordingly, biosynthetic gene clusters of all mentioned compounds contain corresponding set of 5-6 homologous genes (in lincomycin cluster named *lmbA, lmbB1, lmbB2, lmbW, lmbX* and *lmbY*) encoding BPD biosynthesis. Lincomycin, anthramycin, sibiromycin and porothramycin biosynthetic gene clusters share full set, while tomaymycin and hormaomycin clusters lack homologues of *lmbW* or *lmbX*, respectively.

Overall concept of BPD biosynthetic pathway was proposed decades ago. First two proposed steps in lincomycin biosynthesis catalyzed by LmbB2 and LmbB1 have been biochemically proved: L-tyrosine is hydroxylated to L-2,3-dihydroxyphenylalanine (DOPA) and then aromatic ring of DOPA is opened. The molecule is subsequently rearranged resulting in a five membered ring with a substituted four carbon side chain. Logically, conversion of this compound to PPL should be catalyzed by remaining four proteins LmbA, LmbW, LmbX and LmbY. However, some recent findings indicate that following steps of accepted PPL biosynthesis concept should be revised: Protein LmbX was proposed to continue with the biosynthesis by cleavage of a two carbon fragment from the four carbon side chain of the LmbB1 product. However, the hormaomycin gene cluster does not contain *lmbX* homolog but the final product structure suggests that this cleavage step is present in the biosynthesis. Moreover, the *lmbX* deletion mutant of *Streptomyces lincolnensis* resulted in production of lincomycin derivative with BPD moiety corresponding to that of hormaomycin. These observations indicate that LmbX is not a hydrolase but probably an isomerase.

We prepared also deletion mutant strains Δ*lmbA*, Δ*lmbW*, Δ*lmbY*, Δ(*lmbWX*) to find out the functions of respective proteins. Protein LmbA was proposed to have some supporting but essential function in a reduction catalyzed by LmbY. Therefore, it is logical that inactivation mutants Δ*lmbA* and Δ*lmbY* should have the same production profile. The LC-MS analysis however showed that Δ*lmbA* has produced different lincomycin derivatives than Δ*lmbY*. Probably, LmbA has not the supporting function but it is rather directly involved in the PPL biosynthetic pathway. The predicted functions of LmbW (methyltransferase) and LmbY (F420 dependent reductase) were in accordance with the production profiles of respective deletion mutant strains.

Our experiments show that functions of two proteins (LmbA, LmbX) have to be reconsidered which encouraged us to come up with a new hypothesis of the biosynthetic pathway of PPL corresponding to all known and obtained data.

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Keywords: Propylproline biosynthesis, Lincomycin, Pyrrolobenzodiazepines, Hormaomycin

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A TWO-STEP SULFATION IN ANTIBIOTIC BIOSYNTHESIS REQUIRES A TYPE III POLYKETIDE SYNTHASE

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Caprazamycins (CPZ) were first isolated from culture broth of the producer strain *Streptomyces* sp. MK730-62F2 in 2003[1] and revealed a promising anti-mycobacterial activity by targeting MraY translocase, an essential enzyme involved in peptidoglycane biosynthesis. They belong to the group of liponucleoside antibiotics and are closely related to the liposidomycins, A-90289 and the muraminomicins.

Identification and heterologous expression of the caprazamycin gene cluster [2] resulted in the detection of caprazamycin analogues that are decorated with a sulfate group[3]. A set of genes (*cpz5* – *cpz8*) were likely to be responsible for the formation of the sulfate-donor. Manipulations of the caprazamycin producer strain and biochemical investigation of the involved enzymes led to the discovery of an unprecedented two-step sulfation mechanism during the biosynthesis of CPZs [4]. A type III polyketide synthase (PKS) known as Cpz6 is used in the biosynthesis of a group of new triketide pyrones that are subsequently sulfated by an unusual 3'-phosphoadenosine-5'-phosphosulfate (PAPS)-dependent sulfotransferase (Cpz8) to yield phenolic sulfate esters, which then serve as sulfate donors for a PAPS-independent arylsulfate sulfotransferase (Cpz4) to generate sulfated CPZs. This finding is the first demonstration of genuine sulfate donors for an arylsulfate sulfotransferase and the first report of a type III PKS to generate a chemical reagent in bacterial sulfate metabolism.

Keywords: Nucleoside antibiotics, Sulfation

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A NOVEL BROAD SPECTRUM ANTIBIOTIC FROM THE *STREPTOMYCES*ISOLATE DEM 30543

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Screening of the Goodfellow Actinomycete Collection has led to the identification and characterisation of a putatively novel antibiotic with broad spectrum activity produced by the *Streptomyces* isolate *DEM 30543*. Preliminary 1H NMR and 13C NMR studies have been conducted, revealing the core structure of the molecule. The compound has been shown to have antibiotic activity against a wide range of both Gram positive and Gram negative bacteria, including several pathogenic strains such as *Methicillin-resistant Staphylococcus aureus* (MRSA)and *Klebsiella pneumoniae.* The biological and chemical characteristics of the molecule have been investigated further.

The producing strain *DEM 30543* has been cultivated at both laboratory (5 litres) and pilot scale (500 litres) where growth and antibiotic production profiles have been characterised. Semi-continuous cultivations have been carried out in order to gain sufficient antibiotic material for further study. Using whole cell bioassays it has been indicated that the potential mechanisms of action of the molecule are inhibition of cell wall synthesis or disruption of cell wall metabolism. To understand this further, the molecule has been screened against a gene knock out collection of *E. coli* mutants. Several single gene knock outs exhibit a marked increase in susceptibility to the antimicrobial in comparison to the wild type parent strain. Analysis of the genes causing sensitivity and the pathways affected will offer further insight into the specific mechanism of action of the antibiotic. Several genes knock out strains identified have been shown in previous studies to increase susceptibility to cell wall focused antibiotic action. The Minimum Inhibitory Concentration (MIC) values of the molecule against both Gram positive and Gram negative bacteria have been determined (ranging from 4 to 16 µ g/ml), and although the activity spectrum has been shown to be widespread across bacteria, early toxicity studies have shown little cytotoxic effect against mammalian cells. Stability studies have shown the antibiotic molecule is stable at ambient temperatures as well as elevated temperatures (37 and 55 degrees Celsius) for prolonged periods.

Keywords: Antibiotics, *Streptomyces*

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ISOLATION AND BIOLOGICAL ACTIVITY OF *STREPTOMYCESGOUGEROTTI* FROM THE RHIZOSPHERE OF THE PLANT TERMINALIA FAGIFOLIA

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The increasing antimicrobial resistance in recent years has led interest in new bioactive metabolites as an alternative to control them. Once the actinobacteria always show as producers of antibiotics and isolation of extreme regions are always of great importance, due to the production of new bioactive compounds with biotechnological potential. The aim of this study was to determine the antifungal potential of actinomycetes isolated from the rhizosphere of the plant Terminalia fagifolia, the Caatinga biome. 16 Actinobacteria strains were isolated at temperatures of 37° and 45° C and tested against six clinical isolates of collection-URM Department of Mycology-UFPE: *Candida albicans* (URM 6401), *C. guilliermondii* (URM 6403), *C. parapsilosis* (URM 6431), *pelliculosaC*. (URM 6281), *C. glabrata* (URM 6392) and *C. albicans* (URM 6395). In the first screening, the actinomycetes were cultivated in different culture media: ALA and ISP4 and selected those that showed greater inhibition zone. These strains were inoculated in media MPE, M1, ISP4 and ALA for the secondary assay in order to select the best culture medium and fermentation time to produce bio-composite. Then, extraction with different solvents of bioactive product from biomass and metabolic liquid at different pH was done. Subsequently, the minimum inhibitory concentration (MIC) and cytotoxicity by MTT method the crude extract was carried out. The actinobacteria which show as the best production of of bioactive metabolite was identified by molecular techniques based on 16S rDNA. From the sixteen isolates tested in the first assay, four (25%) were used in the secondary assay, with emphasis on strain TUR 711 that showed the best results among MPE for 48 h at 37 ºC, with inhibition zones ranging between 20 and 28 mm for all yeasts tested. The best solvent in the extraction of biocompound was ethanol at neutral pH, which showed MIC of 62.5 mg / mL for *C. albicans* (URM 6401), *C. guilliermondii* (URM 6281) and *C. albicans* (URM 6395) and of 125 µg/mL to *C. pelliculosa*.The cytotoxic activity of the crude ethanol extract showed IC 50 values for the human cell lineages Hep-2 (laryngeal carcinoma), HT-29 (colon carcinoma), always smaller or equal to doxorubicin, used as standard, with the highest activity observed for HEp-2 with 87.52% inhibition. The Actinobacteria TUR 711 was identified with high similarity (99%) to *Streptomyces gougerotti*. The results are promising and are currently being purification for chemical characterization of the structure.

Keywords:*Streptomycesgougerotti*; Caatinga biome; Antimicrobial activity; *Candida* spp.

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ANTIMICROBIAL PROPERTIES OF ACTINORHODIN, AN ANTIBIOTIC PRODUCED BY *STREPTOMYCES COELICOLOR*

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The soil-dwelling streptomycetes are known for being a valuable source for novel antibiotic discovery. The secondary metabolites that these Gram-positive bacteria synthesize can be divided into many classes, of which one is the polyketide natural products. This major class of molecules is structurally and functionally diverse; not only are some of these compounds being used as antibacterials, but others have clinically relevant activities such as anticancer, antifungal, antiparasitic, and immunosuppressive properties. One of the best model compounds for studying polyketide synthesis is the pigmented antibiotic actinorhodin (ACT), which is produced by *Streptomyces coelicolor.* Despite great efforts at understanding the biosynthesis of ACT, there are several areas of the ACT system that remain elusive. Here, we aim to examine the antimicrobial properties of ACT, elucidate its mode of action, and characterize the self-resistance mechanism that *S. coelicolor* confers for ACT. Our work has shown that ACT exhibits potent antibacterial activity against Gram-positive cells, including many streptomycetes. Data from this study will provide insight into why *S. coelicolor* produces ACT in abundance and what advantage, if any, ACT confers to this antibiotic producer.

Keywords: Secondary metabolites, Antibiotics, Mechanism of action

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A NEW FATTY ACID SYNTHESIS INHIBITOR WITH POTENT ANTIBACTERIAL ACTIVITY FROM *STREPTOMYCES CHARTREUSIS*

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Introduction:Bacterial fatty acid synthesis (FAS II) has been as an attractive antibacterial target since FAS is organized differently in bacteria and mammals. Bacterial enoyl-ACP reductase (FabI) catalyzes the final and rate-limiting step in bacterial fatty acid synthesis. Therefore, FabI inhibitors could be interesting lead compounds for treatment of multidrug-resistant bacteria.

Objectives: This study isolates a new FabI inhibitor from *Streptomyces* that have been rich in antibiotics with unknown mechanisms.

Methods: The screening program led to the selection of *Streptomyces chartreusis* producing a strong FabI-inhibitory metabolite. Its chemical structure of the isolated FabI inhibitor was elucidated by mass (MS) and nuclear magnetic resonance (NMR) spectral data. Antibacterial target of the inhibitor was examined using FabI inhibition, macromolecular biosynthesis, and supplementation assay.

Results: The isolated FabI inhibitor was elucidated to be a tricyclic peptide. It strongly inhibited *Staphylococcus aureus* FabI with an IC_{50} (uM) of 0.2. The compound also potently prevented the growth of *S. aureus* as well as methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus* (QRSA) with MICs (ug/mL) of 1. Time-kill study showed its bacteriostatic activity. Consistent with its FabI inhibition, the inhibitor selectively prevented intracellular fatty acid synthesis; wherase did not affect the biosynthesis of DNA, RNA, protein, and cell wall. Furthermore, the supplementation with exogenous fatty acids reversed the antibacterial effect of the compound, which showing that it target fatty acid synthesis. Our data demonstrate that the antibacterial action of the compound is mediated by the inhibition of fatty acid synthesis.

Conclusion: The compound is a new fatty acid synthesis inhibitor that could have potential for further development of a new anti-MRSA agent.

Keywords: Enoyl-acp reductase, FabI, Inhibitor, Fatty acid synthesis, *Streptomyces chartreusis*, Antibacterial

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ACTINOBACTERIAL DIVERSITY IN HIGH ALTITUDE ATACAMA DESERT SOILS AS SOURCES NOVEL SPECIALISED METABOLITES

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Preliminary studies have shown that taxonomically diverse actinobacteria present in arid Atacama Desert soils have the capacity to produce novel antibiotics[1,2]. In the present study, two filamentous actinobacteria, H9 and H45, isolated from a subsurface soil sample collected from the Atacama Large Milimeter Array (ALMA) Observatory site (23°04'39''S 67°57'43''W) at 4000m and 5000m height were found to produce novel bioactive compounds. Primary screening using standard plug assays showed that the isolates produced inhibition zones when tested against a panel of wild type microorganisms. The isolates were cultivated in starch casein broth and 410 broth and the resultant extracts examined by liquid chromatography mass spectrometry (LCMS) and found to produce novel bioactive compounds. Analysis of 16S rRNA gene sequences showed that the isolates formed distinct branches within the genera *Streptomyces* and *Lechevalieria*. Taxonomic studies were done to classify this isolates and describe them as new species. These results thereby provide yet further evidence that novel filamentous actinobacteria isolated from Atacama Desert soils are a promising source of novel antibiotics.

Keywords: Filamentous actinobacteria, Bioactive compounds, Novel antibiotics

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DEVELOPMENT OF *STREPTOMYCES VENEZUELAE* AS A HOST FOR THE HETEROLOGOUS EXPRESSION OF NATURAL PRODUCT GENE CLUSTERS

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Heterologous hosts are proving to be extremely useful tools for the discovery and exploitation of natural products (e.g. Gomez-Escribano, and Bibb, 2012). *Streptomyces venezuelae* ATCC® 10712 was first isolated from soil from Caracas, Venezuela and, unusually for an actinomycete, is capable of growing rapidly in a dispersed fashion to high cell density. *S. venezuelae* is the original source of the antibiotic chloramphenicol and in an industrial setting was capable of high levels of chloramphenicol production. The genome sequence for *S. venezuelae* was published in 2011 (http://streptomyces.org.uk/), enabling the strain to be developed as a model for morphological differentiation with an expanding tool kit for genetic manipulation. As well as the chloramphenicol biosynthetic gene cluster, *S. venezuelae* also contains the biosynthetic gene cluster for the anglucycline polyketide jadomycin. We set out to develop *S. venezuelae* as an attractive host for the expression of heterologous secondary metabolite gene clusters.

The objectives were to delete the endogenous gene clusters for the production of chloramphenicol and jadomycin, and to introduce mutations that would increase the level of secondary metabolite production. The former was to be achieved by using flanking regions of the chloramphenicol and jadomycin gene clusters to promote marker-less deletions by homologous recombination. Enhanced production would be obtained by isolating rifampicin and streptomycin resistant mutants that were known to increase the production of secondary metabolites in *Streptomyces* strains (Hu, and Ochi, 2001; Xu, J., *et al.,* 2002; Okamoto-Hosoya, Y., *et al.,* 2003). Heterologous gene clusters would then be introduced into *S. venezuelae* to assess the effect of such mutations on metabolite production.

Our results demonstrate that the native gene clusters of *S. venezuelae* can be deleted from the chromosome in a sequential manner, removing unwanted sinks for carbon and nitrogen, and markedly simplifying the metabolite profile of the organism and thus facilitating the identification of compounds produced by heterologous expression. Introduced pleiotropic mutations significantly increased expression of the heterologous actinorhodin biosynthetic gene cluster of *Streptomyces coelicolor*, the blue pigmented polyketide providing a useful visual screen for increased production.

In conclusion *S. venezuelae* has been developed as a host for the expression of heterologous gene clusters for secondary metabolite production.

Keywords:*Streptomyces venezuelae*, Antibiotics, Heterologous expression, Natural products, Secondary metabolites

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KIL-KOR SYSTEM FROM AN ACTINOMYCETE INTEGRATIVE AND CONJUGATIVE ELEMENT INFLUENCES SECONDARY METABOLITES PRODUCTION IN *STREPTOMYCES COELICOLOR*A3(2)

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*Streptomyces coelicolor*A3(2), a model organism for genus *Streptomyces*, is a well-known producer of many secondary metabolites, among others coloured polyketide antibiotics: red undecylprodigiosin, blue actinorhodin and recently reported yellow coelimycin. It was shown that the production of coelimycin is dependent on a number of factors: medium composition - influence of glucose and glutamate, the quorum-sensing regulatory system and others.

A large quantity of complex factors needed for the coelimycyn synthesis and the fact that despite 50 years of intensive research, coelimycyn has not been previously discovered, has prompted us to seek for direct factors regulating the synthesis of coelimycyn.

Here we present the results of searching for direct regulators of coelimycin producing gene cluster. Using the DNA region between genes *cpkA* i *cpkD* in affinity chromatography we caught the protein KorSC. That protein is a KorSA homolog and it is also homological to GntR protein family containing wHTH DNA binding motif and UTRA effector domain. In *Streptomyces ambofaciens* KorSA is a central transcriptional repressor for integrative element pSAM2. It is a part of Kil-Kor system maintaining the plasmid integrated into the chromosome.

We have shown that protein KorSC binds the promoter region of structural genes required for coelimycin synthesis *cpkA/cpkD*, and promoter region of *actII*ORF4, the central activator of actinorhodin synthesis. By combination of chromatin precipitation experiments and mutants with regulated KorSC protein level we have shown that elevated level of protein KorSC, inhibits the synthesis of actinorhodin and coelimycin. Sequences recognized by KorSC protein were also identified.

At the same time KorSC protein is active in the system Kil-Kor of an integrated pSAM-like element in *S. coelicolor*A3(2). The *korSC* gene deletion is lethal for *S. coelicolor*A3(2). KorSC binds to the intergenic region covering its own promoter region and the promoter of the neighboring gene coding a homologue of Pra regulatory protein.

Presented findings indicate a specific relationship between the integrated mobile elements and secondary metabolism of *Streptomyces coelicolor*A3(2). These findings demonstrate a new aspect of the regulation of secondary metabolites synthesis and should be taken into account in new synthetic biology projects based on Streptomyces, especially on *S. coelicolor* super host.

Keywords:*Streptomyces coelicolor*, Coelimycin, Actinorhodin, Actinomycete Integrative and conjugative element, Kil-kor

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STRUCTURAL ELUCIDATION AND BIOSYNTHETIC ORIGIN OF AZOXYALKENE COMPOUND PRODUCED BY A MULTIPLE GENE DISRUPTANT OF *STREPTOMYCES ROCHEI*

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Streptomyces strains have a genetic potential to produce >20 secondary metabolites. However, many secondary metabolite gene clusters are expressed poorly, or not at all, under laboratory growth condition. *Streptomyces rochei* 7434AN4 produces two polyketides lankacidin (LC) and lankamycin (LM) as major metabolites under normal culture conditions, suggesting that the expression of other secondary metabolites biosynthetic clusters appears to be silent. In this respect, we constructed a disruptant of three genes, including *srrB* (a repressor gene that has a negative function on antibiotic production), *lkcF-KR1* (a ketoreductase domain 1 of *lkcF* involved in lankacidin biosynthesis), and *lkmE* (a type-II thioesterase gene *lkmE* involved in lankamycin biosynthesis). This mutant accumulated several compounds with a strong UV absorption at 235 nm. The major compound (KA57-A; Rf = 0.5 in $CHCl_3$-MeOH = 15:1) was purified and subjected to structural analysis on the basis of spectroscopic data of ESI-MS and NMR. This compound was determined to be an azoxyalkene compound with a molecular formula of $C_{10}H_{20}N_2O_3$. The biosynthetic pathway leading to KA57-A has also been studied using ^{13}C-labeled acetate and amino acids, which will be presented.

Keywords: Antibiotics, Biosynthesis, Genome mining, Repressor

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FUNCTIONAL ANALYSIS OF A PQQ-DEPENDENT ENZYME IN LANKACIDIN BIOSYNTHESIS

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Lankacidin is a unique 17-membered carbocyclic polyketide antibiotic, whose biosynthetic gene (*lkc*) cluster is located on the giant linear plasmid pSLA2-L in *Streptomyces rochei* 7434AN4. It is noteworthy that the *lkc* cluster contains the biosynthetic genes for coenzyme pyrroloquinoline quinone (PQQ), which was found to be essential for lankacidin biosynthesis by gene disruption experiment. To reveal the function of PQQ in lankacidin production, we extensively analyzed the metabolites of mutant of a PQQ-dependent dehydrogenase gene *orf*23. This mutant produced not lankacidin C but lankacidinol A and lankacidinol, suggesting that Orf23 is responsible for the oxidation at a C-24 hydroxyl in the lankacidin skeleton. The recombinant Orf23 protein specifically converted lankacidinol to lankacidin C in the presence of PQQ. Furthermore, we analyzed its biochemical characteristics including substrate specificity, metal requirement and optimum pH, which will be presented in this meeting.

Keywords: Antibiotics, Polyketide, Biosynthesis, Pyrroloquinoline quinine

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A NEW STRATEGY FOR THE DISCOVERY OF NOVEL SECONDARY METABOLITES FROM ACTINOMYCETES

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Microbial natural products, particularly those produced by actinomycetes are a valuable source of various clinically important compounds. Genome sequencing has shown that the streptomycete chromosome encodes far more secondary metabolites than can be detected under standard laboratory conditions. Despite continued efforts on tapping their biosynthetic potential, there is still a steady decline in finding novel chemical structures. This warrants the need for developing new strategies to overcome the challenges of dereplication. Our group has recently identified a new and effective approach to natural products discovery that involves applying small molecules that perturb secondary metabolism. Here, we present the use of these small molecules called the ARCs (Antibiotic Remodeling Compounds) to isolate novel secondary metabolites from a broad diversity of actinomycete strains. In each case, the production of the compounds of interest was significantly increased, most of which would have been otherwise undetected by traditional isolation techniques. We have also found that these compounds exhibit antibacterial as well as antifungal properties. Our chemical activation approach provides an effective alternative strategy to the discovery of novel secondary metabolites and hopefully new therapeutic agents.

Keywords: Novel secondary metabolites, Small molecules, Chemical activation

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SELECTION OF ACTINOMYCETES FROM RHIZOSPHERE OF CAATINGA BIOME - BRAZIL, WITH THE POTENTIAL FOR PROMOTION OF PLANT GROWTH

Milca Rachel Ribeiro Lins[1], Graciely Gomes Correa[1], Jessica Martins Fontes[1], Nataliane Marques Vasconcelos[1], Ozias Elias Ferreira[1], Itamar Soares Melo[2], Tiago Domingues Zucchi[2], Raphael Carlos Ferrer De Santana[1], Janete Magali De Araújo[1], Gláucia Manoella De Souza Lima[1]

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The Caatinga is unique biome of Brazil and it is unexplored in relation to the isolation of microrganisms producers of substances for agribusiness. The actinobacteria are filamentous bacteria Gram positive distributed in nature and are capable of producing auxin and antimicrobial substances, besides providing nutrients such as nitrogen and phosphate favoring plant development. This study aimed to analyze 80 actinobacteria isolated from plant's rhizosphere of Caatinga producing bioactive metabolites which assists in plant growth promotion. The auxin production was assessed and found that 86.25% (69) of isolates produced auxin at concentrations ranging from 1.75 to 76.48 µg.mL-1. It was also determined Posphate Solubilization Index (PSI) in the media VERMA and NBRIP and found that 93.75% (75) of the strains solubilized phosphate in both media with IS ranging from 1.15 to 2.25, and VERMA statistically better medium for phosphate solubilization. The production of phosphatases was observed in 97.5% (78) of actinobacteria. The antimicrobial activity against bacterial and fungal phythopathogens showed that 15.0% (12) produced antimicrobial substances against fungi (*Fusarium oxysporum* UFPEDA 2455 and *Colletotrichum* sp. UFPEDA 2419) and 6.25 % (5) had activity for *Xanthomonas campestris* UFPEDA 407. The ammonia production was observed in 80% (64) of the strains at concentrations ranging from 3.59 to 292.31 µg.mL-1. Analysis of variance and comparison of means by Tukey test (5%) showed that the actinobacteria PR 34 and TUR 704 are the strains that stood out in relation to the test performed. Through chromatographic analysis it was possible to identify and quantify the 3-indoleacetic acid produced by PR 34. The strains belong to the genus *Streptomyces* by microculture technique, confirmed by sequencing of the 16S rRNA gene, which indicated that the PR 34 and TUR 704 strains are similar to actinobacteria *Streptomyces fimbriatus* NBRC15411 and *Streptomyces xanthocidicus* NBRC 13469, respectively. The results suggest that the actinobacteria of Caatinga biomed have biotechnological potential as they include mechanisms that assist in plant development, and it is necessary to evaluate the feasibility of these microrganisms in plants aiming to reduce the use of pesticides in the agricultural industry.

Keywords: Antimicrobial activity; Caatinga biome; Indoleacetic acid; Plant growth; Phosphatases; Rhizobacteria; Phosphate solubilization.

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PRODUCTION OF BIOACTIVE COMPOUNDS BY ACTINOBACTERIA G74 ISOLATED FROM CAESALPINIA PYRAMIDALIS TUL RHIZOSPHERE OF THE BIOME CAATINGA

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The Caatinga biome is the main ecosystems of the Northeast which has peculiar characteristics due to its hot and dry climate typical of semi-arid. The actinomycetes of regions of extreme conditions are important due to the adaptation mechanisms that stimulate the production of new compounds with biotechnological potential. The aim of this work was to select the best means and time of production of the antimicrobial compound produced by actinobacteria G74 isolated from the rhizosphere of Caatinga. The assay was performed in blocks of agar against Gram positive, Gram negative, yeast and filamentous fungus, then performed secondary assay (fermentation), using the means MPE, M1, ISP3 and Ciclamycin. From the fermentation of actinobacteria, on the best means of production and fermentation time, experiments of extracting bioactive principle of biomass and metabolic fluid, at different pHs, were performed. Then, the minimum inhibitory concentration (MIC) of crude extract of biomass and metabolic liquid were determined. In the test blocks of agar, the actinobacteria G74 was selected for secondary testing by presenting excellent inhibition zones ahead to Gram positive bacteria: *Bacillus subtilis* UFPEDA-86, *Staphylococcus aureus* UFPEDA-02 and methicillin resistant *Staphylococcus aureus* UFPEDA-705 (MRSA). The strain G74 was identified as *Sreptomyces* sp. via molecular tests and it was found that the MPE medium was the best for the production of biocompound with 72 hours of fermentation, the best solvents were acetone and hexane, both at pH 7.0 for the extraction of biomass and metabolic liquid, respectively. The acetone crude extract of biomass showed MIC of 61.5 µg/mL for *B. subtilis* UFPEDA-86 and 245 µg/mL for both *S. aureus* UFPEDA-02 and MRSA UFPEDA-705Already the hexane crude extract of metabolic liquid, showed MIC of 50 µg/mL for *B. subtilis* UFPEDA-86 and 200 µg/mL for *S. aureus* UFPEDA-02 and MRSA UFPEDA-705. The CMB (minimum bactericidal concentration) of *B. subtilis* was 122.5 µg/mL for the crude extract of biomass and 100 µg/mL for the crude extract of metabolic liquid. Isolate G74 showed promising results in the production of bioactive compounds and more detailed studies should be conducted to better characterization.

Keywords: Caatinga, *Streptomyces* sp.,Bioactive metabolites, Methicillin resistant *Staphylococcus aureus.*

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A COLLECTION OF ACTINOMYCETES ISOLATED FROM MOUNTAIN SOILS AND ITS EXPLOITATION TO SEARCH FOR NOVEL ANTIBIOTICS

Hua Zhu[1], Mariana Avalos[1], Jasper Swierstra[2], Changsheng Wu[1], Geneviève Girard[1], Young Hae Choi[1], Willem Van Wamel[2], Stephanie Sandiford[1], Gilles P. Van Wezel[1]

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The rapid emergence of multiple drug resistant (MDR) bacterial pathogens poses a major threat for human health. In recent years, genome sequencing unveiled many poorly expressed antibiotic clusters in actinomycetes, which form a potential source of new drugs. We isolated a collection of over 800 actinomycetes from sites in the Himalaya and Qinling mountains, and exploited them for the identification of antibiotics. Using 40 different growth conditions, some of which had not previously been assessed, 96 actinomycetes were identified that produced antibiotics under specific growth conditions. Of these, pH 10 appeared to be particularly effective in triggering antibiotics. The antibiotics were shown to have efficacy against one or more of the MDR clinical isolates referred to as ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and/or *Enterobacter cloacae*. Antimicrobial activities that fluctuated strongly with growth conditions were correlated to specific antibiotics, by the use of NMR-based metabolomics in combination with high resolution mass spectrometry (FT-MS). This work provides insights into the potential of actinomycetes as producers of drugs with efficacy against recently emerged clinical isolates and also underlines the importance of targeting a specific pathogen. The latest advances and examples of the antibiotics that were identified will be presented.

Keywords: Actinomycete; Clinic; Elicitor; Metabolomics; Strain collection

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ISOLATION AND IDENTIFICATION OF BROAD SPECTRUM ANTIMICROBIAL COMPOUNDS FROM NOVEL STREPTOMYCETES

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Newcastle UniversityDemuris Ltd

New secondary metabolites, especially antibiotics are needed to control the spread of multi-drug resistant pathogens. Filamentous members of the phylum Actinobacteria, notably *Streptomyces* strains, remain a unique source of natural products, unlike those of nearly all other prokaryotes, have whole genomes rich in biosynthetic gene clusters that code for known or predicted novel secondary metabolites. There is evidence that novel streptomycetes from extreme habitats are a promising source of new natural products, an approach that rests on the premise that harsh environmental conditions give rise to unique taxa which will have a novel chemistry.

A new Demuris habitat screen was performed, from which several hundred putative streptomycetes were isolated, using a range of selective media supplemented with antifungal antibiotics. Over 100 representative isolates were assigned to 15 multi-membered and 15 single-membered colour-groups based on aerial spore, substrate mycelial and diffusible pigment and melanin pigments.

Following 16S rRNA gene analysis, the majority of strains were found to be *Streptomyces* sp., ten of which form a distinct phyletic line in 16S streptomycetes gene tree. One isolate was found to be a putatively novel *Nocardiopsis* sp. The taxonomic integrity of these strains was supported by corresponding phenotypic data.

Isolates were screened against a panel of known pathogens, and twelve showed inhibitory activity against both gram-positive and gram-negative organisms. The cytotoxicity of the putatively novel compounds was tested in whole cell assays, and several were found to be non-toxic. Activity of several of the compounds against a series of multidrug resistant organisms suggests that at least some of the compounds represent novel antibiotic classes. Media optimisations were carried out to find the most suitable medium for antimicrobial production, and lab scale fermentations were performed. The extracts are in various stages of purification, based on use of a range of chromatographic techniques, and the active molecules will be assessed by mass spectrometry to ascertain novelty.

Preliminary investigations into activating the production of antimicrobial compounds by co-culture gave promising results, and the next step is to investigate strains not previously showing activity in bioassays. *Streptomyces* strains isolated from different areas and climates will be used, to examine how species unknown to one another behave when introduced as neighbours. Additionally, the use of chemical elicitors and mutagenesis will be investigated to induce & increase the yield of antimicrobial compounds produced from strains previously proven to produce antimicrobials, and strains which have not.

Keywords: Streptomycetes, Antibiotics

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RAPID IDENTIFICATION OF NEW DRUG-LIKE NATURAL PRODUCTS FROM TERMITE-ASSOCIATED ACTINOMYCETES USING NMR METABOLIC FINGERPRINTS

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For decades microbial natural products have represented an important route to the discovery of novel bioactive small molecules with unusual chemical structures.Discovering novel chemical scaffolds from traditional microbial sources is difficult as they appear to be largely exhausted. Thus, microbial drug discovery programs have started focusing on the exploration of unique and under-explored habitats as they represent rich sources of novel strains with the potential to synthesize new biologically active natural products.For this study 50 actinomycete strains isolated from the intestinal tract of the wood-feeding termite *Coptotermes lacteous,*were used to carry out chemical fingerprinting investigations. The strains were initially fermented on four solid media in order to determine the influence of the media components on the production of natural products. A dereplication strategy based on HPLC-LC-MS-NMR was optimized for the rapid identification of new chemical structures from the fermented products. The methodology consisted of the generation of lead-like enhanced (LLE) fractions by selecting favorable physicochemical properties which permit to examine the complete drug-like-natural product microbial metabolome. ^{1}H NMR fingerprinting provided comprehensive information about the chemical structures of the different classes of compounds present in the fractions. Five strains showing unique chemical profiles were selected for large-scale fermentation. We demonstrate the effectiveness of the NMR-guided approach with the isolation and structure elucidation of two new 5-hydroxy-1,4-naphthoquinone analogues from one of the selected strains (USC-594). One of these analogues (ESK-112) showed a methyl-vinyl-ketone functional group attached to the naphthoquinone ring which, to the best of our knowledge, is the first report of a microbial natural product containing such functionality.

Keywords: Termite-associated actinomycetes, Natural products, LLE, NMR fingerprinting, Methyl-vinyl-ketone.

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UPREGULATION OF SECONDARY METABOLITE GENE CLUSTER BY EXOGENOUS SMALL MOLECULE

Shunji Takahashi, Suresh Panthee, & Hiroyuki Osada

RIKEN CSRS

An understanding of mechanisms regulating the production of secondary metabolite (SM) by small molecule is an important issue. Autoregulators that are effective on producing strain at nano molar concentrations have been extensively studied for *Streptomyces* spp. In addition, exogenous small molecules linked with the SM production have also been studied. Here, we define such exogenous compounds as biomediator.

In general, we culture microorganisms in various media to improve the production of SMs. We found that the production of reveromycin A (RM-A) was enhanced by an addition of tomato juice to culture medium of *Streptomyces reveromyceticus.* To address the exogenous small molecule 'biomediator', which enhanced the production of RMs, we tried to isolate active compounds. However, we could not obtain target compounds because of low abundance. Therefore, we explored the core structure of biomediator through screening of chemical library (RIKEN NPDepo). We set up two screening methods using *Magnaporthe oryzae* and src^{ts}-NRK cells because RM-A has antifungal activity and reverses the transformed morphology of src^{ts}-NRK cells to the normal.

We identified a β-carboline compound as biomediator from our library. To enhance its activity we performed structure-activity relationship (SAR) study and produced synthetic biomediator molecule. We will discuss how the molecule enhances the production of RM-A.

Keywords: Small molecule, Reveromycin

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DIVERSITY OF SIDEROPHORE BIOSYNTHESIS GENES IN *NOCARDIA*

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National Institute of Infectious Diseases

Iron acquisition is mediated by siderophore-dependent pathways in microorganisms and siderophores are important for some pathogenic microorganisms for their acquisition of iron in the host cell. Pathogenic bacteria *Nocardia* are known to produce and utilize siderophore to acquire iron. Despite that siderophore biosynthesis genes and gene clusters have been identified in *Nocardia* genomes, few of them have been characterized further. Nocobactin NA is produced by *Nocardia farcinica*, and we have recently revealed that the *nbt* clusters are responsible for the biosynthesis of nocobactin NA.

Therefore, we investigated siderophores in other *Nocardia* genomes (*N. cyriacigeorgica*, *N. brasiliensis* and *N. nova*) by genomic mining. In these three species, biosynthesis genes of mycobactin-type siderophore were predicted to locate at two genomic regions. In order to analyze the siderophore productivity, these strains were cultured under an iron limited condition. LC-MS analyses revealed that all strainsproduced metabolites which were not observed under high iron condition. In further analysis, *N. cyriacigeorgica* turned out to produce four metabolites (m/z of 745.4, 773.5, 801.5 and 829.5). Structure of these metabolites included salicylate, N-formyl-N-hydroxy lysine, carboxylic acid and caprolactam being in good agreement with predicted gene functions. These facts suggest that this clusters are responsible for the biosynthesis of siderophore of *N. cyriacigeorgica.*

Keywords:*Nocardia*, Siderophore, Siderophore biosynthesis genes

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PHAGE PHI BT1 INTEGRASE-MEDIATED SITE-SPECIFIC RECOMBINATION FOR DIRECT CLONING OF ANTIBIOTICS BIOSYNTHETIC GENE CLUSTERS FROM *STREPTOMYCES* GENOME

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Genes responsible for the biosynthesis of a specific secondary metabolite in *Streptomyces* are usually situated in clusters that vary in size from a few to over 100 kb. To gain insight into the biosynthesis and regulation of antibiotics in *Streptomyces*, it is of great importance to clone their gene clusters. Recently, several strategies have been developed to clone gene clusters directly from bacterial genomic DNA. These methods include RecET-mediated linear-plus-linear homologous recombination (LLHR), *oriT*-directed capture system and transformation-associated recombination (TAR). In this study, we developed a novel strategy based on phage phi BT1 integrase-mediated site-specific recombination. Our strategy requires both homologous and site-specific recombination. The homologous recombination was used for targeted integration of phi BT1 integrase recognition sites (*attB* and *attP*) into *Streptomyces* chromosome, while site-specific recombination is employed to excise targeted region of interest from the chromosome. This approach has been successful for cloning actinorhodin gene cluster from *Streptomyces coelicolor*, and several medium and large sizes of gene clusters from *Streptomyces roseosporus*. Furthermore, this strategy could be used readily to increase antibiotic titers in many *Streptomyces* and possible other *Actinomycetes*.

Keywords:*Streptomyces*, Gene cluster cloning, Strategy, Site-specific Recombination, phi BT1 integrase

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FUNCTIONAL CHARACTERIZATION OF GOUC, D, E AND I IN GOUGEROTIN BIOSYNTHESIS

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Gougerotin is a peptidyl nucleoside antibiotic and displays antitumor, antiviral, antibacterial, antimycoplasma, anthelmintic and acaricidal activities. The gene cluster responsible for gougerotin biosynthesis has been cloned. Our previous studies suggested that GouR modulates gougerotin production by coordinating its biosynthesis and export in *Streptomyces graminearus* (*ApplEnviron Microb*, 2014, 80: 714-722). The roles of several structural genes were also assigned in the proposed biosynthetic pathway based on the analyses of intermediates accumulated in inactivation mutants (*ChemBiol*, 2013, 20: 34-44). However, the functions of many other genes remain unclear. To examine the roles of *gouC*, *D*, *E* and *I* in gougerotin biosynthesis, we generated in-frame deletion mutant of these genes by PCR targeting. HPLC analysis shows that mutation in *gouC*, *D* and I lost the ability to produce gougerotin while *gouE* mutant can produce a compound with the same mass as gougerotin, though at a level significantly lower than that of the WT strain. New intermediate accumulated in *gouI* mutant was identified as compound **3.** Identification of intermediates accumulated in *gouC* and *D* mutants is still under work. Discrimination of gougerotin or its stereoisomeric ningnanmycin in *gouE* mutant will shed light on the formation of D-serine in gougerotin. D-serine is presented in the structure of gougerotin, whereas L-serine is found in the stereoisomeric ningnanmycin. Our result may reveal that *gouE* is responsible for conversion of L-serine to D-serine. These studies will substantially promote our understanding of gougerotin biosynthesis, especially the mechanism for conversion of L-amino acid to D-amino.

Keywords:*Streptomyces*, Gougerotion, Biosynthesis, Gene function

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ISOLATION OF INDOLEAMINE 2,3-DIOXYGENASE INHIBITORS FROM *STREPTOMYCES* SP. 11A057

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Indoleamine 2,3-dioxygenase (IDO) is the key metabolic enzyme implicated in tryptophan catabolism. IDO effects on immune suppression are due to decreased tryptophan availability and the generation of tryptophan metabolites, culminating in multipronged negative effects on T lymphocytes in proximity to IDO-expressing cells, notably on proliferation, function, and survival. IDO seems to act as one of the multiple immunoregulatory mechanisms modulated by the tumor to evade immune surveillance, in addition to the downregulation of MHC class I, the expression of other immunosuppressive molecules, and the recruitment of regulatory immune cells. For example, the blockade of IDO activity by 1-methyltryptophan (1-MT) results in decreased tumor proliferation dependent on specific T-lymphocyte response[1]. Modulation of IDO functions is, accordingly, one of targets for large-scale cancer treatment. In the course of screening for IDO inhibitors from microbial metabolites, we have found a strain, *Streptomyces* sp. 11A057 to produce some IDO inhibitory activity. In this presentation, we show the isolation, structure determination, and biological activities of IDO inhibitors from the strain.

Recombinant His-tagged human IDO1 protein was expressed in *E. coli,* and purified through Ni-NTA affinity chromatography. Using this recombinant IDO enzyme, we performed the screening of IDO inhibitors from microbial metabolites. We found a strain *Streptomyces* sp. 11A057, isolated in a green tea field in Boseong, Korea, to produce potent inhibitory activity. This strain 11A057 was already found to produce new pyrrolobenzodiazepine derivatives, boseongazepines A-C, which showed some cytotoxic activities, but not any IDO inhibitory activity[2]. From 16 L of culture broth, we purified active principles with silica gel column chromatography, followed by reversed-phase preparative HPLC. Finally, we obtained 2.9 mg of compound **1**, 6.3 mg of compound **2**, and 3.2 mg of compound **3**. Based on the instrumental analyses, compound **1** was a derivative of nybomycin, and compound **2** was an *N*-methylated analog of **1**[3,4]. Compound **3**, an *N*-demethyl deoxynybomycin[5], was identified as a new member of nybomycin. IC_{50} value of **1** against human IDO1 was 20 nM, whereas **2** was weaker than **1**, and **3** did not showed inhibitory activity at up to 1 mM. This result revealed that NH and phenolic OH groups are thought to be essential for binding to IDO protein to inhibit its activity.

Keywords: Indoleamine 2,3-dioxygenase, IDO inhibitor, Nybomycin, *Streptomyces*

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OPTIMIZATION OF THE PRECURSOR SUPPLY FOR THE IMPROVED PRODUCTION OF TACROLIMUS IN *STREPTOMYCES TSUKUBAENSIS*

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Tacrolimus, also known as FK-506, is a potent immunosuppressive compound with growing pharmaceutical interest that has firstly been isolated from *Streptomyces tsukubaensis* in the late 1980s[1]. It is a 23-membered macrolide lactone with the non-proteinogenic aminoacid pipecolate as sole peptide constituent. Unfortunately the levels of FK-506, produced by the wildtype strain, are very low. In order to enhance the productivity of *S. tsukubaensis,* the precursor supply for the biosynthesis of this secondary metabolite has been studied in detail and optimized by means of genetic engineering.

For this purpose primarily the genes involved in the pipecolic acid biogenesis in *S. tsukubaensis*, *fkbL* (encoding the lysine cyclodeaminase) and *fkbP* (encoding the pipecolate activating non-ribosomal synthase) have been overexpressed in the wildtype producer. In general pipecolic acid is a widely distributed component among biologically active compounds like pristinamycin, friulimicin and tacrolimus. To ensure the supply of this building block, the biosynthetic gene clusters of pristinamycin from *Streptomyces pristinaspiralis* and friulimycin from *Actinoplanes friuliensis* include a gene for a lysine cyclodeaminase (*pipA* resp. *pip*). To increase the yield of tacrolimus these two genes, *pipA* and *pip*, have been heterologously expressed in *S. tsukubaensis*. The obtained results show that an additional copy of a lysine cyclodeaminase gene in the wildtype producer exerts a positive effect on FK-506 production. Furthermore it could be shown that supplementation of the production medium with L-lysine (precursor of pipecolic acid) strongly enhances the tacrolimus production. Based on this information one of the key enzymes of the lysine biosynthesis pathway, the aspartate kinase (Ask), that is usually regulated via a feedback-inhibition of its endproducts lysine and threonine, has been replaced by a deregulated aspartate kinase (LysC*) derived from a high-level lysine producing *Corynebacterium glutamicum* strain. The expression of this deregulated LysC* in *S. tsukubaensis* resulted in a significant increase of the FK-506 production.

Keywords: Tacrolimus, Metabolic engineering

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THIS DOCUMENT IS ONLY THE ABSTRACT OF THE SYMPOSIUM BOOK WHICH AIMS TO CONTROL OF THE ABSTRACTS

MANAGEMENT OF *V. HARVEYI* IN AQUACULTURE EMPLOYING *NOCARDIOPSIS ALBA* AND ITS EXTRACELLULAR BIOACTIVE PRODUCT

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Vibriosis has been identified as a serious disease problem in shrimp culture all over the world. Mass mortalities in hatcheries and grow-out ponds of shrimp attributed to outbreaks of vibriosis have been recorded from many regions. Over a dozen species of *Vibrio* have been identified as having implication in the disease process. The severity of infection depends on the species and strain of *Vibrio* involved the stage of development and age of shrimp, and the ambient environmental conditions. In an attempt to screen antagonistic microorganisms from marine environment for the management of *Vibrio* in aquaculture, an isolate of actinomycete MCCB 110 was segregated based on its comparatively higher inhibitory property on *Vibrio harveyi* (MCCB 111) and profound luminescent inhibition using modular luminometer. Based on the culture characteristics, cell wall fatty acid profile by gas chromatography and the nucleotide sequence of the 16S rRNA gene (1495 bp), the isolate was identified as *Nocardiopsis alba.* Antibacterial activity was tested following Kirby Bauer disc diffusion method. The culture filtrate was extracted with ethyl acetate (3:1) and this fraction was concentrated by evaporation in a water bath at 80ºC.The crude extract was purified by preparative thin layer chromatography using silica gel plates (silica gel G 60 Merck) and developed with ethyl acetate: n-hexane (60:40) solvent system. The plates were then exposed to iodine vapour and bands with similar Rf values were scrapped off, pooled and dissolved in distilled water and centrifuged at 10,000 *g* at 4º C for 15 minutes. Supernatant, after passing through 0.45mm cellulose acetate filter (Sartorius), was lyophilized and dried over silica in a vacuum desiccator. A portion of it was re-dissolved in sterile distilled water and checked for its anti *V. harveyi* activity. The purified active fraction was analyzed by HPLC to check its purity which resulted in the isolation of a major fraction active against luminescent *Vibrio harveyi*. Partial characterization of this bioactive fraction based on spectroscopic data obtained from FT-IR, UV, MS-MS and ^{1}H NMR analyses, the compound was recognized to differ from those reportedly produced by the same genus. Cytotoxicity of the active fraction was evaluated against African green monkey kidney cell line (Vero cell) and shrimp haemocyte by the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide) assay and found not toxic to Vero cell line and shrimp haemocytes up to 1000 ppm tested. The study demonstrated the potential of the novel probiotic *Nocardiopsis alba* and its extra-cellular bioactive product for the management of *V. harveyi* in aquaculture.

Keywords: Nocardiopsis,*Vibrio harveyi*, Antibacterial activities, Purification, Structural elucidation

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MARINE ACTINOMYCETE ISOLATES AS BIOREMEDIATORS IN SHRIMP CULTURE SYSTEM

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Actinomycetes are potential source of bioactive compounds and highly versatile agents of biodegradation. Eventhough considerable work has been carried out on terrestrial actinomycetes, their marine counterparts remain underexplored. The present study was focused on segregation of potential actinomycete isolates for bioremediation in shrimp culture systems. For this purpose, actinomycetes were isolated from the marine environment (Arabian Sea) and culture ponds in Cochin (Kerala, India) and they were screened for hydrolytic enzyme production (protease, amylase, lipase, pectinase, ligninase and chitinase), antibacterial potential (against prawn pathogens), denitrification and biogranulation property. Of the 270 actinomycete isolates 99.1% were proteolytic, 74.2% amylolytic, 92.9% lipolytic, 12.3% pectinolytic, 15.5% ligninolytic and 47.1% chitinolytic. Strains with these properties (58 Nos) were segregated for further study. The cultures were screened for organic matter degradation in terms of Biochemical Oxygen Demand (BOD) of shrimp rearing water. Significant reduction in BOD could be observed in case of 10 cultures proving its potential for use as bioremediators in aquaculture. A few isolates were (*Streptomyces* spp.) were found to exhibit significant reduction in vibrios in penaeid prawn culture systems and therefore these isolates were segregated for the control of vibrios in culture systems. Different media were tested for the storage of the actinomycete granules for application in aquaculture. Of the five media tested (1.nutrient broth 2.spent nutrient broth medium 3. sea water 4.sea water with 0.01 % phytagel and 5.sea water with 20% glycerol), sea water was found to be the best storage medium for the actinomycete biogranules estimated in terms of its microbial biomass as ATP. When stored in sea water, the biogranules were found to be stable at both room temperature (28 ±2°C) and at 4°C for a period of 3 months.

Keywords: Marine actinomycete, Biogranule, Vibrio, Shrimp, Bioremediation, Water quality, Aquaculture

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MANUMYCIN-TYPE SECONDARY METABOLITES: FROM UNIQUE BIOSYNTHESIS TO ATTRACTIVE BIOLOGICAL FEATURES

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Manumycin-type compounds display potent inhibitory activities against numerous eukaryotic enzymes: Ras farnesyltransferase, IKK kinase ß, caspase I, and neutral sphingomyelinase. They are considered as drug candidates to treat cancers, inflammation, and Alzheimer disease. The compounds are characterized by the central 2-amino-4-hydroxycyclohex-2-enone ring (*m*-C_7N unit) to which, two short polyketide chains are connected. Typically, the 'lower' chain is terminated by the 2-amino-3-hydroxycyclopent-2-enone moiety (C_5N unit). The family consists of more than 30 compounds.

Here we report the comparison of biosynthetic routes of several manumycin antibiotics. Information from novel producers acquired from a specific genetic screening of natural actinomycete isolates and genome scanning of actinomycete genomes is also included. The biosynthetic gene clusters involved in the biosynthesis are described and the novel genes responsible for the control of carbon chains are identified.

The screened novel actinomycete isolates (about 1200 strains) originate from diverse soil and marine environments, human and animal bodies, etc., were isolated worldwide and are deposited in the CCACB collection (www.actinomycetes.cz). Producer of a new manumycin-type compound, colabomycin E, was identified by the screening, and the structures of it and of accompanying congeners, were determined with the help of MS and NMR techniques. Its immunomodulatory activities were assayed using the human THP-1 monocyte/macrophage cell line. Compared to manumycin A, it has similar anti-inflammatory features, but its pro-apoptotic characteristics are reduced.

The genome scanning of the GeneBank genomic information revealed another putative producer, *Saccharothrix espanaensis* DSM44229, however, the biosynthetic gene cluster is silent under all applied laboratory fermentation conditions. The gene cluster was transferred to a heterologous expression host *S. lividans* K4-114 where three novel putative manumycin-type metabolites were identified.

Summing up, the gene clusters encoding the biosynthetic routes of the manumycins from several characterized or novel producers were identified and cloned in heterologous *Streptomyces* hosts, where the production of manumycin-type compounds was achieved. The mutants with deleted genes for 'lower' and for 'upper' polyene chains were constructed and the missing genes were complemented by different combinations of PKS genes from original and heterologous strains. A novel type of the chain length factor (CLF) controlling the length of 'lower' carbon chains was identified in the strains. Deletion of the gene coding for the protein of the DSBA/HCCI family in *Streptomyces aureus* SOK1/5-04 resulted in the abolishment of colabomycin E production, with pentaene in place of its upper chain, while the synthesis of tetraene-containing colabomycin A and other triene-containing congeners remained unaffected. This effect points to the role of the gene in the biosynthesis of the upper chain starter unit. Other constructed mutant strains were affected in genes influencing the chain saturation and starter unit biosynthesis. The aim of the work was to achieve a collection of structurally related compounds and assess their biological activities for the selection of candidates with the best anti-inflammatory activity profiles.

Acknowledgements: This work was supported by the Czech Ministry of Education, Youth, and Sports grant LH12191 and by the Czech Ministry of Health Care grant NT/13012.

Keywords: Manumycin, Polyketide, *Streptomyces*, Biosynthesis of secondary metabolites, Genetic screening

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SCO7613 AFFECTS ANTIBIOTIC PRODUCTION IN *STREPTOMYCESCOELICOLOR*

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BLAST searches of the GeneBank database argued strongly that SCO7613 specifies a putative PHA synthase responsible for polyhydroxybutyrate (PHB) biosynthesis in *Streptomyces coelicolor*. Computer-aided analysis indicated that the product of SCO7613 is a 818 residue protein with a calculated molecular mass of 90.97 kDa. We showed that SCO7613 is not an essential gene in *S. coelicolor* and its deletion caused an important decrease in PHB biosynthesis. At 96th hour of fermentation, actinorhodin and undecylprodigiosin production by mutant strain was 3.5 times and 2.5 times less than the wild type, respectively.

Keywords: SCO7613, *Streptomyces coelicolor*, PHB, Antibiotic

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THE ROLE OF RESISTANCE GENES LMR(A), LMR(B) AND LMR(C) OF *STREPTOMYCES LINCOLNENSIS* IN THE LINCOMYCIN PRODUCTION AND RESISTANCE

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The three predicted resistance genes, *lmr(A)*, *lmr(B)* and *lmr(C)* are present in the lincomycin biosynthetic gene cluster of *Streptomyces lincolnensis*. Gene *lmr(B)* encodes 23S rRNA methyltransferase protecting ribosomes from lincomycin binding. Gene *lmr(A)* encodes a transporter from the major facilitator superfamily (MFS) and its main function is probably secretion of synthesized lincomycin. The function of the third resistance gene *lmr(C)* is not clear. Lmr(C) is an ABC protein from ARE (antibiotic resistance) protein subfamily of class 2 ABC transporters. The mechanism of resistance conferred by ARE proteins, which encompass clinically important resistance proteins is not well understood. The similarity of ARE proteins with translational factors and the fact that these proteins confer resistance only to antibiotics inhibiting translation suggests that they might interact with ribosome and thus affect the binding of antibiotics to its target site. However, ARE proteins which do not have any transmembrane domain, associate with a membrane. Therefore, it is presumed, that they may interact with a membrane partner to constitute a functional transporter. Indeed, evidence for such cooperation has been shown recently for ARE protein Msr(D) and for Mef(E) MFS transporter from *Streptococcus pneumoniae*.

Streptomyces lincolnensis provides attractive model for study the function of ARE proteins. It posses Lmr(C) together with MFS transporter Lmr(A) enabling us to test whether the cooperation of ARE proteins with MFS transporters is a general functional feature of this protein subfamily. Moreover, Lmr(C) more than ARE proteins from pathogens resembles recently characterized *E. coli* translation factor EttA. This suggests that Lmr(C) may retain regulatory function of the ancestor and could be involved in the control of antibiotic production and resistance.

We have genetically examined the role of each of the three resistance genes *lmr(A)*, *lmr(B)* and *lmr(C)* in the resistance and antibiotic production. We have constructed single, double and triple deletion mutants of *lmr(A), lmr(B)* and *lmr(C)* genes and we examined the effect of genes deletion on the resistance to lincomycin, clindamycin and erythromycin. We also determined the level of lincomycin production in mutant strains.

Keywords: Antibiotic resistance, ARE proteins, Class 2 ABC proteins, *Streptomyceslincolnensis*, Lincomycin, *lmr(A), lmr(B), lmr(C)*

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NEW INSIGHTS INTO THE PLASTICITY OF THE MEMBRANE COMPOSITION FROM *STREPTOMYCES COELICOLOR*

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Streptomyces coelicolor is a soil dwelling actinomycete that has been extensively studied as a model for morphological differentiation in bacteria. Recent research suggests that the membrane composition of this organism could have a role in its appropriate development [1,2].

We are interested in obtaining a deeper understanding of the lipid composition, synthesis and turnover in *S. coelicolor*. Our first goal was the identification of the lipid species present in cultures grown throughout different developmental stages, a task that can be challenging using standard lipid extraction and analysis methodologies. Therefore, we took advantage of a recently developed approach of mass spectrometry data analysis, called Mass Spectral Molecular Networking, to study the membrane composition of the different tissues of *S. coelicolor*. These experiments were complemented with traditional lipid analysis techniques. Phenotypic and metabolic analyses of several mutant strains were also performed to gain further insight into the process of lipid biosynthesis in *Streptomyces*, and its relationship with morphological differentiation and adaptation to the environment.

Our results indicate that the complexity of the lipid composition of *S. coelicolor* increases during later developmental stages. Phosphorous-free polar lipids seem to be accumulated mainly in the aerial hyphae and sporulation stages. In contrast, some of the main lipids found in liquid cultures and in vegetative mycelium, like cardiolipin and phosphatidylethanolamine, seem to be replaced by other lipids in several of the growth conditions tested. Additionally, we found a much greater variability between the metabolic profiles from our samples cultured in different growth media than expected, indicating that the plasticity of the membrane composition could also be strongly influenced by nutritional and environmental factors.

Another contribution from our work is that several of the lipid species that we found to be conditionally accumulated have not been previously described in *S. coelicolor*, thus increasing our understanding of the metabolic complexity of this species. These results will widen the scope of our comprehension regarding the function of individual lipids in actinomycetes and the role of membrane composition dynamics in development and stress response.

Keywords:*Streptomyces*, Lipids, Cell envelope, Development, Metabolomics

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THIS DOCUMENT IS ONLY THE ABSTRACT OF THE SYMPOSIUM BOOK WHICH AIMS TO CONTROL OF THE ABSTRACTS

FROM GENES TO MOLECULES: ACCESSING THE GENETIC POTENTIAL FOR SECONDARY METABOLITE PRODUCTION IN RARE MARINE ACTINOMYCETES

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The prospect of leaping from genomic code to molecule is fast approaching, with technological advances in sequencing, bioinformatics and heterologous gene expression paving the way to discover and manipulate unknown molecules from proposed biosynthetic pathways. Marine actinomycetes have recently come into the spotlight as fruitful producers of structurally diverse secondary metabolites, and remain relatively untapped. We have sequenced fifteen rare marine actinomycetes using Ion Torrent technology, and will present their secondary metabolite potential diversity and efforts to discover novel chemical entities for use in human medicine. Access to bacterial genome sequence data provides a precise blue print of the biosynthetic potential of each strain. One tool for interrogating secondary metabolite gene clusters is heterologous expression, specifically Transformation Associated Recombination (TAR) capture, where a targeted gene cluster is captured and introduced to a host bacterium, eliminating the need for cumbersome fosmid libraries.

Aside from *Streptomyces*, *Salinispora* is arguably the most prolific marine actinomycete genus discovered to date, producing a suite of potent bioactive chemicals. Until its discovery in 2002, this lineage of marine actinobacteria was completely unknown. The past decade of research on *Salinispora* has produced over a dozen bioactive compounds, from this rare marine genus, indicating that lesser known marine actinomycetes, not traditionally associated with secondary metabolite production, represent an untapped source for new bioactive molecules.

The genomes analyzed represent diverse genera within the Actinomycetales order, including *Kocuria*, *Nocardiopsis*, *Marmoricola*, *Saccharomonospora*, and other genera not normally explored for natural product production. We have found that many of these genera contain a wealth of secondary metabolite pathways, rivaling the big producers in *Streptomyces* and *Salinispora* genera. With such a diverse data set, correlations can be drawn between secondary metabolite potential and various traits. For example, we see a general correlation between genome size and canonical PKS/NRPS pathway numbers. Other comparative genomic correlations can be drawn from this data set to determine those genera representing untapped potential in novel secondary metabolite pathways. Heterologous expression of rare marine actinomycete pathways demonstrates the bioactive potential from these often overlooked genera and we will illustrate efficient pathway capture using TAR. Additionally, genome assembly from high GC organisms can be challenging; results using the SPAdes algorithm for improved genome assembly will be presented.

Rare marine actinomycetes are the perfect candidates for exploration, as their relatives are historically rich in secondary metabolites and are relatively unstudied. Our varied data set provides a glimpse into these marine actinomycetes previously overlooked in favor of more traditional secondary metabolite producers. We propose that these rare genera represent a worthy avenue for novel bioactive metabolite discovery, and we present an efficient and economical way to go about discovery of such compounds.

Keywords: Genome mining, Heterologous expression, Natural product biosynthesis

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FOXICINS: ORTHO-QUINONE DERIVATES PRODUCED BY POLYKETOMYCIN PRODUCER *STREPTOMYCESDIASTATOCHROMOGENES* TÜ6028

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During fermentation conditions the strain *Streptomyces diastatochromogenes* Tü6028 synthesizes various ortho-quinones derivates, named Foxicin, before producing big amounts of the antimicrobial compound Polyketomycin[1]. The structures of different Foxicins were elucidated by 1D-, 2D-NMR and MS analysis. Bioinformatic analysis of 7.9 Mb draft genome sequence of *S. diastatochromogenes* Tü6028 revealed 23 putative secondary metabolite gene clusters. The identity of *fox* biosynthesis gene cluster was confirmed by gene disruption. It spans 57 kb and encodes three modules of PKSI, one NRPS module and 40 other enzymes probably necessary for the secondary metabolite production. Foxicin A inhibits bacterial respiratory chain.

Keywords: Ortho-quinone, Streptomyces, Bacterial respiratory chain

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GENOME MINING OF *STREPTOMYCES VENEZUELAE* REVEALS A NOVEL POLYKETIDE-DERIVED NATURAL PRODUCT

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The recent application of genome mining to actinomycetes has led to the discovery of numerous novel natural products [1]. Analysis of the genome sequence of *Streptomyces venezuelae*[2], combined with synteny and micro-array analyses, led to the discovery of a new cryptic polyketide synthase gene cluster potentially encoding a novel natural product. The 30 kb biosynthetic gene cluster was predicted to possess 17 genes, including two encoding halogenases with the potential to chlorinate the corresponding product. Our aim was to analyse the biosynthetic gene cluster and to identify its product. A cosmid containing the cryptic gene cluster was introduced into the heterologous expression hosts *Streptomyces coelicolor* M1152 and M1316, and the metabolite profiles of the recombinant strains compared with their parents and *S. venezuelae*. LC-MS analyses revealed the production of new metabolites in the recombinant strains with masses $[M+H]^+$of 221 *m/*z (non-chlorinated) and $[M+H]^+$ 255/257 *m/*z (chlorinated). In an attempt to increase the level of production of these compounds for structural analysis, we expressed constitutively a putative pathway-associated regulatory gene in both the recombinant strains and in *S. venezuelae* resulting in significantly increased productivity of the non-chlorinated metabolite. NMR spectroscopy subsequently revealed a novel structure, further illustrating the value of genome mining and the activation of transcriptionally silent gene clusters in identifying new chemical entities. Further work is focused on studying the function of individual genes within the cluster and determining its transcriptional organization.

Keywords:*Streptomyces venezuelae*, Genome mining, Natural product

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IDENTIFICATION OF THE BIOSYNTHETIC GENE CLUSTER FOR THE HERBICIDE PHOSPHONOTHRIXIN IN *SACCHAROTHRIX* SP. ST-888

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Phosphonothrixin is a natural phosphonate that has been isolated from the fermentation broth of *Saccharothrix* sp. ST-888. This compound exhibits herbicidal activity by suppressing the germination of gramineous and broadleaf weeds, which induces chlorosis in leaves. To date, although the enantioselective chemical synthesis of phosphonothrixin has been reported, neither the biosynthesis nor the mode of action has been elucidated. In this presentation, we describe the identification of the biosynthetic gene cluster of phosphonothrixin in *Saccharothrix* sp. ST-888.

We first *de novo* sequenced the genome of *Saccharothrix* sp. ST-888. An assembly of the sequence reads yielded 2,822 contigs with 8,605,814 total base pairs. In the biosynthesis of fosfomycin, bialaphos, and FR-900098, the initial reactions begin with a conversion of phosphoenolpyruvate (PEP) to phosphonopyruvate (PnPy). This conversion is catalyzed by PEP phosphomutase (PEPPM), which is encoded by the *fom1*, *bcpB*, or *frbD* genes, for the biosynthesis of fosfomycin, bialaphos, or FR-900098, respectively. Therefore, we hypothesized that the initial reaction in phosphonothrixin biosynthesis is also catalyzed by PEPPM. Thus, we retrieved a PEPPM homolog from the genomic DNA sequence of *Saccharothrix* sp. ST-888 and identified a homologous open reading frame with 34, 40, and 49 % identities to Fom1, BcpB, and FrbD, respectively. Moreover, an analysis of the regions flanking the PEPPM homologous gene revealed 14 additional genes were located in these regions in the same direction as the PEPPM homologous gene, suggesting that these 15 genes form a biosynthetic gene cluster for phosphonothrixin.

We constructed a cosmid library for the ST-888 genome and screened the library for cosmids that contain the regions including PEPPM using the PEPPM homologous gene as a probe. Subsequently, three positive cosmids (cos-1, cos-11, and cos-12) were introduced into *Streptomyces albus* J1074. The resultant transformants were cultured for heterologous expression, and the metabolites were analyzed using ^{31}P NMR spectroscopy. The heterologous production of phosphonothrixin was confirmed in the transformants harboring cos-11 or cos-12.

We next sequenced and analyzed the insert DNA of the cos-12 cosmid. Cos-12 contains 35 ORFs, including a PEPPM homolog, and the length of the insert DNA spans approximately 43 kb. To identify the ORFs required for phosphonothrixin biosynthesis, we constructed a series of cosmids in which several genes were deleted from the parental cos-12 using the lambda-Red recombination system and individually introduced the deletion cosmids into *S. albus*. Each resultant transformant was cultured, and the metabolites in the culture broth were analyzed using ^{31}P NMR spectroscopy and LC/MS spectrometry. These analyses revealed that, at most, the region from ORF21 to ORF34 is sufficient to confer phosphonothrixin biosynthesis in *S. albus*.

Thus, we identified the *ptxB* genes required for phosphonothrixin biosynthesis using genome sequencing and heterologous expression. Future studies including the identification of the biosynthetic intermediates and in vitro assays of the *ptxB* gene products will enable us to clarify the entire phosphonothrixin biosynthetic pathway, leading to the elucidation of the novel biosynthetic machinery for natural phosphonates.

Keywords: Biosynthesis; Phosphonate; C-p bond

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WHOLE GENOME SEQUENCE OF EXTREMOPHILIC ENDOPHYTIC STREPTOMYCES SUK 42

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Endophytic streptomycetes are valuable sources for bioactive compounds.SUK 42 has become an organism of interest due to its morphology uniqueness, ability of producing diffusible brown pigment on media and had an active antibacterial activity against *A.baumannii*. With wrinkled brown aerial mycelia, diffusible brown pigment and Retinaculiaperti type of spore with smooth surface, phylogenetic tree of the isolate formed distinct taxon based on complete 16S rRNA gene analysis with 99.17 % similarities to *Streptomyces xantocidicus* NBRC 13469^T. Phenotypic analyses showed SUK 42 is extremophilic, able to grow at pH 6-11, temperature between 25-45°C and salinity up to 10%, suggesting this isolate is represents a novel species. The complete genome sequence of SUK 42 produced by next generation sequencing platform (Ilumina HiSeq2000) and analysed using *de novo* approach consists of 7,800,900 bp with 72.6% G+C content, 6,813 protein-coding genes, 86 tRNAs and one rRNA. 41 genes cluster postulated involved in secondary metabolite production (1-Thiopeptide lantipeptide, 5-Terpene, 8-Nrps, 4-Butyrolactone, 1-Siderophore, 2-Lantipeptide, 2-Bacteriocin, 1-Amyglyccycl, 1-Melanin , 3-Hglks, 4-PKS type 1, 1-Nrps-PKS type 1, 1-PKS type 2, 1-Oligosaccharide-PKS type 2 , 1-Amglyccycl-PKS type 3, 1-PKS type 3). It was evident from the genotypic and phenotypic data that SUK 42 belonged to a novel species of *Streptomyces*, a first reported whole genome sequence of endophytic actinobacteria from tropical plant that active against *A.baumannii*.

Keywords: Whole genome, *Streptomyces*, endophytic, extremophilic, secondary metabolites

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BIOACTIVE COMPOUNDS FRACTIONATED FROM ENDOPHYTE *STREPTOMYCES* SUK 08 WITH PROMISING EX-VIVO ANTIMALARIAL ACTIVITY

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Since *Plasmodium falciparum* has shown resistance to nearly all antimalarial drugs, malaria remained as one of the devastating parasitic disease throughout the country of human civilization. This study was carried out to determine the *ex-vivo* antimalarial activity and cytotoxicity of endophytic actinobacteria namely *Streptomyces* SUK 08 as well as to determine the main core structure fractionated from its crude extract. The crude extracts obtained analyzed by TLC using four solvents and gas GC-MS. *Plasmodium* lactate dehydrogenase assay (pLDH) used to determine the sensitivity of the crude extract against *P. berghei* as a replacement of *P. falciparum* where ethyl acetate-crude extract shows very promising antimalarial activity with IC_{50} of 1.25 mg/ml. The MTT assay on Chang liver cells showed the IC_{50} value for ethyl acetate and ethanol was > 1.0 mg/ml and the IC_{50} of parasitemia at 5 and 30 % for ethyl acetate-crude extract lower compared to chloroquine. The synchronization tests showed that ethyl acetate extract could inhibit at all stages of the *Plasmodium* life cycle, but more effective at the ring stage. The TLC via 1:9 ratios of ethyl acetate and hexane showed 9 different compounds isolated. Three core structures based on gas chromatography-mass spectrometry (GC-MS) analysis were cyclohexane, butyl propyl ester and 2,3-heptanedione where structurally, have the similarity on its derivatives with antimalarial drugs that available nowadays. The results suggest that *Streptomyces* SUK 08 has good potential as an antimalarial drug which these promising finding may contribute to the medicinal and pharmaceutical field for malarial treatment.

Keywords: Butyl-propyl-ester, Cyclohexane, 2,3-heptanedione, Endophyte, *Streptomyces* and Antimalarial

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BIOTECHNOLOGICAL POTENTIAL OF ENDOPHYTIC ACTINOMYCETES FROM MEDICINAL PLANTS OF ASTERACEAE FAMILY

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Due to its diverse climate Pakistan is quite rich in medicinal plants which are spread over a large area. For centuries, these plants have been used by Hakims (traditional physicians) in folk medicine. Approximately 80% of the population especially in villages and rural areas are dependent on the Unani (traditional) system of medicine. The Asteraceae family includes one of the most essential, oldest medicinal plants and also being the largest plant family in Pakistan. Considering this and the fact that there are few reports on such a study, this situation encouraged us to investigate the biotechnological potential of endophytic actinomycetes mainly their antimicrobial, cytotoxic and antioxidant activities. The study revealed the isolates displaying significant broad spectrum antimicrobial activity while a few of the isolates showed strong activity against medically important pathogens. In the brine shrimp lethality test the LC_{50} for some of the strains was observed to be very potent. When compared to the EC_{50} value of the ascorbate standard in the free radical scavenging activity assay using diphenylpicrylhydrazyl (DPPH), all isolates gave impressive results with notable EC_{50} values. The study not only describes an unexploited environment but also describes the potential usage of such bioactive isolates in biotechnological mainly pharmaceutical applications.

Acknowledgments

This work was supported by the funds provided by the University of the Punjab.

Keywords: Actinomycetes, Asteraceae, Endophytes

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THE MECHANISM OF MACRODIOLIDE FORMATION IN ELAIOPHYLIN BIOSYNTHESIS

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Elaiophylin, a natural product of numerous streptomycete bacteria, has been shown to possess antibacterial, antitumour and immunosuppressant activities. It belongs to a small family of symmetrical macrodiolide polyketides that also includes the antibacterial marinomycin (Kwon et al., 2006), the antitumour compound SIA7248 (Zou et al., 2013), and the immunosuppressant conglobatin (Westley et al., 1979). Elaiophylin is produced by a canonical modular polyketide synthase (PKS) (Haydock et al., 2004) but the precise mechanism of formation of its 16-membered diolide macrocycle has remained obscure. We describe here in vitro macrocyclisation using as substrates truncated (C11 and C9) synthetic analogues of the elaiophylin monomer (as their N-acetylcysteaminyl (SNAC) thioesters), and purified recombinant elaiophylin thioesterase/cyclase (Ela-TE) derived from the C-terminus of the PKS. The C-terminal thioesterase/cyclase DEBS-TE (Tran et al., 2008) derived from the erythromycin PKS, which creates the 14-membered macrocycle of 6-deoxyerythronolide B, was studied in parallel.

Incubation of the C11- SNAC thioester with Ela-TE led to the appearance of a symmetrical diolide product, which was characterised by LC-MSn, HRMS, and 1H, 13C and 2D NMR. Analysis of the product mixture also showed the presence of the acid formed by hydrolysis of the C11- SNAC thioester as well as the SNAC thioester of a linear dimer. In contrast, the shorter C9- SNAC thioester was not a substrate for Ela-TE. DEBS-TE catalysed hydrolysis of the C11- SNAC thioester with no evidence for dimeric products. The following mechanism for elaiophylin diolide formation is proposed: first, a TE-bound monomer is attacked by the distal hydroxy group of a second monomer tethered to the adjacent acyl-carrier protein (ACP) domain, to yield a linear dimer on the ACP. The linear dimer is then transferred to the TE active site, cyclised and released.

Keywords: Thioesterase, Macrodiolide, Elaiophylin, Polyketide

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GENOME MINING TO DETECT NEW METABOLITES FROM STREPTOMYCES OLINDENSIS

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Characterization of new metabolic biosynthetic clusters has become a priority among researchers in actinobacteria because of their biotechnological potential to produce new molecules with possible use in the pharmaceutical and industrial field. Moreover, most of the biosynthetic pathways discovered on the genomes on *Streptomyces* have been described as cryptic or silenced under standard laboratory conditions, which leads to the development of different technical approaches to express these new clusters. One of the strategies is the overexpression of the specific regulatory genes within these pathways, such as Streptomyces Antibiotic Regulatory Protein (SARP) to detect these new products in different culture media. Our group has been working with *Streptomyces olindensis* DAUFPE 5622 the producer of the anthracycline Cosmomycin D, which has an important antitumoral activity and has attracted interest because of its distinctive glycosylation pattern. Using bioinformatics tools the genome was sequenced and annotated in the NCBI under the accession number JJOH00000000, and by further prediction were identified thirty-four secondary metabolite clusters using the antiSMASH server, among them seven polyketides, being one of them a polyether type. Such compounds have a wide range of action including the treatment of bacterial and viral infections, parasitic diseases, and application on antitumoral therapies. Due to its medical and veterinary importance and low cost of production, this type of molecules have been studied and considered as candidates for combinatorial biosynthesis. The polyether cluster was manually annotated, spreading over at least 100 kb and in between there are six polyketide synthases, one epoxidase gene that facilitates its detection, five transport-resistance related genes and four putative regulators, being one of them a specific SARP-like regulator gene and several tailoring enzymes such as glycosyltransferases, methyltransferases and acetyltransferases, that possibly perform modifications to the molecule with sugar moieties that arise from another clusters such as Cosmomycin. To express this biosynthetic pathway we amplified by PCR the regulatory gene SARP and cloned it into PEM4A plasmid, which will be used to transform *Streptomyces olindensis* protoplasts in order to express constitutively this activator. To detect the polyether on culture media before (OSMAC approach) and after the overexpression of the regulator, we used oatmeal agar, B2-1 and SGG liquid media, followed by ethyl-acetate extraction to carry out biological assays. Our approach will assist on understanding the polyether biosynthetic pathways regulation and provide future mechanisms for bioprospecting new molecules for combinatorial biosynthesis.

Keywords: Genome mining, Polyether, SARP, Overexpression, Bioprospecting, Combinatorial biosynthesis

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GENOMIC INSIGHTS INTO THE BIOSYNTHESIS OF CLASSICAL PROTEASE INHIBITORS LEUPEPTIN AND ANTIPAIN

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Antipain, leupeptin, and related compounds (MAPI; microbial alkaline protease inhibitor) are Small Peptide Aldehydes (SPA) with potent protease inhibition activities, which are widely used in industry, bioresearch and with potential as therapeutic agents. However, despite the importance of these compounds, other than classical studies from the late 1970s lead by Professor Umezawa [1-4], their biosynthetic pathways remain unknown. To identify and propose genes that direct the biosynthesis of leupeptin, antipain and MAPI in general, we sequenced the genomes of historical strains presumed to produce these compounds, namely, *Streptomyces roseus* ATCC31245*, S. mauvecolor* ATCC29835, *S. yokosukanensis* ATCC25520 and *S.nigrescens* ATCC23941. The genomes of these organisms were thoroughly mined to identify putative systems directing the biosynthesis of SPAs. In parallel, ad hoc fermentation conditions and HPLC-MS compound detection methods were develop for each strain. Genetic tools for each strain are also under development, which would allow to experimentally test the predictions obtained after genome mining. Overall, these analyses lead to the conclusion that SPAs, including leupeptin and antipain, are synthesized by highly divergent NRPS based biosynthetic pathways, which agree with previous biochemical data obtained more than 30 years ago. Interestingly, none of these proposed biosynthetic pathways are conserved, opening the possibility for investigating novel and specific synthetic logics behind the assembly of unusual peptides.

Keywords: Biosynthesis, Leupeptin, Antipain, Protease inhibitors

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OTHER

OXIDATIVE ENZYMES FROM ACTINOMYCETES: AN OVERLOOKED RESOURCE FOR BIOCATALYSIS

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Actinomycetes are a ubiquitous group of filamentous bacteria and are known to produce various primary and secondary metabolites. Over the past few years, our research group has isolated actinomycetes from diverse environments. A multi-faceted screening program revealed that the oxidative enzymes, laccase, tyrosinase and peroxidase are found abundantly in actinomycete strains regardless of their phylogenetic grouping. Various studies with a focus on these oxidative enzymes were initiated, with the main objectives being: 1) The discovery of novel oxidative enzymes; 2) the production of the enzymes; and 3) the application of the enzymes in biocatalysis reactions. Three examples from these studies will be the focus of this presentation.

Example 1: Laccase-catalysed biocatalysis reactions mainly focus on the use of fungal laccases despite the potential of bacterial laccases. The small laccase (SLAC) produced by *Streptomyces coelicolor*A3(2) was investigated for its ability to oxidize ferulic acid. Medium engineering allowed for the optimized production of three dimeric products and one trimeric product. The structures of the products were confirmed by LC-MS/MS and NMR analyses. The products showed increased antioxidant activity when compared to the starting material.

Example 2: Tyrosinase production from *Streptomyces pharetrae* CZA14^T and *Streptomyces polyantibioticus* SPR^T was optimized and the enzymes biochemically characterised. The tyrosinases exhibited unique biochemical properties that would make them ideal for application in biocatalysis (high organic solvent tolerance), bioremediation (resistance to inhibitors) and biosensor technology (wide substrate range). Their ability to cross-link certain target proteins also allow for their potential application in the production of novel biomaterials with application in the food industry and various medical fields.

Example 3: Aside from the basidiomycetous fungi, actinomycetes are the only other microorganism known to produce extracellular peroxidases. A method for inexpensive and reliable production of peroxidase from an actinomycete was investigated. *Streptomyces* sp. strain BSII#1 was grown in different culture volumes using different bioreactor systems. Highest peroxidase production was achieved with the airlift bioreactor (4.76±0.46 U/ml) employing a complex production medium (pH8.0, 22°C). Application of the enzyme in biocatalysis reactions revealed the ability of the enzyme to catalyse coupling reactions. Compounds were purified and analysed by LC-MS/MS, allowing for the prediction of the product structures.

From these studies, it is clear that the oxidative enzymes produced by these selected actinomycetes show great potential for application in bio-based processes and for the production of bio-based products. Current sequence information (genome sequences) further supports the fact that actinomycetes are a rich source of oxidative enzymes and should therefore be explored for the discovery of new and interesting oxidative enzymes.

Keywords: Oxidative enzymes, Biocatalysis, Actinomycetes

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INCREASED ANTIBIOTIC SUSCEPTIBILITY IN *STREPTOMYCES COELICOLOR* PROTEIN GLYCOSYLATION MUTANTS

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A protein O-glycosylation pathway in *Streptomyces coelicolor* A3(2) has previously been shown to be required for ϕC31 phage infection. Mutant strains defective in either O-mannosyltransferase (SCO3154, Pmt) or poly prenol phosphate mannose synthase (SCO1423, Ppm1) had slower growth rates compared to their parent, J1929. In this study we measured the sensitivity of the *pmt*$^-$ and *ppm1*$^-$ mutants, in *S. coelicolor* A3(2), to a range of antimicrobials. Antibiotic disc diffusion assays revealed that mutants were hypersensitive to rifampicin, tunicamycin, ramoplanin, nisin, vancomycin, teicoplanin, imipenem, meropenem, ampicillin, bacitracin and daptomycin. With the exception of rifampicin all of these antimicrobials act by inhibiting various stages of the cell wall biosynthesis pathway in *S. coelicolor*. Ppm1 mutants were found, overall, to be more sensitive to antimicrobials than Pmt mutants, perhaps because Ppm1 acts at an earlier stage of the O-glycosylation pathway than Pmt and so affects a greater number of processes within the cell. To gain further insight into the systems that are influenced by the lack of protein O-glycosylation we isolated suppressor mutants of a *ppm1*$^-$ strain from the zones of antimicrobial inhibition. Strains were split into groups according to their antimicrobial resistance profile. The vancomycin resistance gene cluster in the vancomycin suppressor strains was sequenced and suppressor strains with reduced susceptibility to vancomycin, ampicillin and teicoplanin were found to have acquired mutations in the *vanS* gene. Complementation of suppressor strains with wild type *vanS* returned strains to the highly sensitive, parent phenotype. Based on previous work that describes the activity of the VanRS two-component regulatory system in *S. coelicolor* (Hong et al, 2002, Mol Microbiol, 44:1199), it seems likely that the *vanS* alleles in the suppressor strains result in constitutive expression of the vancomycin resistance pathway. The reason for the vancomycin susceptibility in the original, protein glycosylation defective, *ppm1*$^-$ mutant might be that the vancomycin pathway is not induced, possibly because VanS does not detect the signal.

Keywords: Antimicrobial sensitivity *Streptomyces coelicolor*A3(2) O-glycosylation

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SUGAR ON THE SURFACE: A PROTEOMICS APPROACH TO IDENTIFYING TARGETS OF GLYCOSYLATION IN *STREPTOMYCES COELICOLOR*

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Previous work has shown that *Streptomyces coelicolor* has a general protein O-glycosylation pathway that is also present in other Actinobacteria, including the pathogenic *Mycobacterium tuberculosis*, yeasts and humans. Mutations in either of two enzymes required for protein glycosylation lead to greatly increased susceptibilities to some antibiotics and are resistant to infection by the phage fC31. To date, only a single glycoprotein in *S. coelicolor* has been identified, a periplasmic phosphate binding protein, PstS. The aim of this project is to better understand the physiological processes affected by protein O-glycosylation in *S. coelicolor* with a view to applying this knowledge to the use of antibiotics.

We have developed a reproducible and scalable method of enriching and detecting *S. coelicolor* glycoproteins from the culture filtrate and membrane proteomes. These methods will be incorporated into a glycoproteomics workflow to enable glycoprotein identification, modification site-analysis and glycan characterisation. Using a combination of cell fractionation, lectin affinity chromatography, lectin western-blot screening and mass spectrometry, six glycoprotein candidates, from the *S. coelicolor* culture filtrate and membrane fractions have been enriched and identified, including PstS. Among the new glycoprotein candidates identified is SCO4471, a secreted protein belonging to the same family as bacterial peptidoglycan deacetylases and possibly of some relevance given the sensitivity of glycosylation mutants to antibiotics that act on the cell wall. In addition SCO5776 is a putative lipoprotein that is also a glycoprotein candidate in *M. tuberculosis*. Future work will be directed towards further characterisation of these glycoprotein candidates, using a combination of biochemical and glycoproteomics approaches.

Keywords:*Streptomyces*, Glycoproteins, Glycosylation

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CRITICAL ROLE FOR *STREPTOMYCES COELICOLOR* CELL SURFACE POLYMERS IN CONTACT - MEDIATED INVASION OF *ASPERGILLUS NIGER*

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Streptomycetes and filamentous fungi are ubiquitous inhabitants of the soil, where they compete for nutrients. As a likely consequence, these microbes have evolved mechanisms to cope with their competitors, many of which remain largely unexplored. We have recently started to analyze such antagonistic interactions using the model organisms *Streptomyces coelicolor* and *Aspergillus niger*. Co-cultivations of these species indicated that microcolonies of *A. niger*are efficiently degraded by *S. coelicolor* after the formation of a mycelial *Streptomyces* network inside the fungal microcolony. In addition, *Streptomyces* pellets can adhere tightly to the outer surface of pre-existing fungal microcolonies of *A. niger*. Both mechanisms can lead to degradation of the fungal biomass. Notably, efficient adhesion of pellets to the fungal microcolonies requires extracellular matrix components produced by *S. coelicolor*. This is concluded from the observation that an *S. coelicolor* mutant strain lacking the *cslA* gene, encoding a cellulose synthase-like protein, is affected in adhering to the fungal microcolonies and is quickly overgrown by *A. niger*. Taken together, these data indicate that streptomycetes may employ different strategies to combat fungi.

Keywords:*Streptomyces, Aspergillus*, Cell surface, Interaction, Degradation

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VANYN, A NOVEL D,D-DIPEPTIDASE/D,D-CARBOXYPEPTIDASE INVOLVED IN GLYCOPEPTIDE ANTIBIOTIC RESISTANCE

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$VanY_n$ is a novel D,D-dipeptidase/D,D-carboxypeptidase involved in the mechanism of self-resistance of *Nonomuraea* sp. ATTC 39727, which produces the glycopeptide A40926, precursor of the recently FDA-approved second-generation antibiotic dalbavancin. $VanY_n$ is encoded by the only *van*-like resistance gene (named *dbv7)* found within the *dbv* biosynthetic cluster that is devoted to A40926 production. $VanY_n$ heterologous expression in *Escherichia coli* allowed its characterization as a bi-functional zinc-dependent enzyme, which hydrolyzes both the D-Ala-D-Ala dipeptide and the peptidoglycan precursor (UDP-Mur-NAc-pentapeptide) terminating in D-Ala-D-Ala [1]. $VanY_n$ is a membrane-bound enzyme containing conserved motifs (SxHxxGxAxD and ExxH) involved in the coordination of zinc and in the active site that are typical of VanY D,D-carboxypeptidases and VanX D,D-peptidases characterized in glycopeptide resistant enterococci. Despite of the high identity of the conserved sequences, the overall identity between $VanY_n$ and enterococcal enzymes is moderate [3]. Over-expression of $VanY_n$ in *Streptomyces venezuelae* and in *Nonomuraea* sp. ATTC 39727, increased their glycopeptide resistance, confirming the role of the protein [3,4]. Differently from enterococcal VanY enzymes that are typically insensitive to penicillins, $VanY_n$ activity is inhibited by penicillin G and ampicillin [1,4]. Since $VanY_n$ lacks the canonical Ser-X-X-Lys motif found in the active sites of penicillin binding proteins, how β-lactams inhibit $VanY_n$ activity is worthy of further investigation. This work provides new insights into evolution of resistant determinants from glycopeptides-producing actinomycetes to resistant pathogens. It can also contribute to an early warning system for emerging resistance mechanisms following introduction in clinics of the second–generation dalbavancin.

Keywords: Resistance, Glycopeptide, Dalbavancin, *Nonomuraea* sp.ATCC 39727, Vany

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ACQUISITION OF TRIMETHOPRIM-SULFAMETHOXAZOLE RESISTANCE BY *NOCARDIA FARCINICA* AND *NOCARDIA NOVA* AFTER PASSAGING IN SUB-MIC CONCENTRATIONS OF ANTIMICROBIALS

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As the incidence of nocardiosis continues to increase, so does the concern for the acquisition of antimicrobial resistance by *Nocardia* species. The combination of trimethoprim and sulfamethoxazole (TMP/SMX) is currently the treatment of choice for nocardiosis. Trimethoprim prevents the conversion of dihydrofolate to tetrahydrofolate and dTTP synthesis by inhibiting the dihydrofolate reductase encoded by *dfrA*. Sulfamethoxazole is a competitive inhibitor of dihydropteroate reductase, an enzyme encoded by *folP*, and blocks a key step in the biosynthesis of folate. While there have been reports of high levels of sulfa drug resistance *in vitro*, the mechanisms of resistance to TMP/SMX in nocardiae are not well understood. To determine if the genetic changes responsible for the resistance of nocardiae to TMP/SMX are similar to those previously described in other genera, we analyzed the gene sequences of *dfrA* and *folP* in five TMP/SMX-susceptible *Nocardia nova* and seven TMP/SMX-susceptible *Nocardia farcinica* clinical isolates obtained from the Special Bacteriology Reference Laboratory (SBRL) culture collection. After sequencing, the *dfrA* and *folP* sequences of these susceptible isolates were compared to the published sequence of *N. farcinica* IFM10152 as well as to those genes in previously characterized TMP/SMX-resistant clinical nocardial isolates selected from the SBRL culture collection to identify differences between susceptible and resistant isolates at these loci. Sequencing of *drfA* showed the presence of a lysine residue in the place of Glu151 in two *farcinica* isolates, W9150 and X0866, but revealed no other shared polymorphisms in susceptible isolates as compared to that of *dfrA* in sulfa-resistant nocardiae. No shared polymorphisms were observed in the *folP* sequences as well. As no single mutation appeared concomitant with drug susceptibility in these isolates, we tried to test if the susceptible isolates could acquire resistance by growing the isolates in Müeller-Hinton broth supplemented with cations and with 0.14 µg/ml of SMX. Before growth in media containing antibiotic, baseline antimicrobial susceptibilities for all 12 isolates were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative breakpoints. The isolates were then subsequently passaged in incrementally increasing concentrations of SMX. Regular assessment of antibiotic resistance profiles revealed decreasing susceptibility to TMP/SMX. Passaging was discontinued when the isolates were determined to be resistant to TMP/SMX under CLSI breakpoints (MIC ≥4/76 µg/ml). All of the initially susceptible isolates became drug resistant. Changes or genetic substitutions to the isolates over the course of passaging will be determined by Sanger sequencing of *dfrA* and *folP* and by next generation sequencing of the genomes of all the isolates obtained during passaging. These analyses will help elucidate the changes that occurred in the genome that lead to resistance and will also allow for the determination of the temporal sequence of the changes that result in resistance. Any genes of interest will then be cloned into susceptible isolates to ascertain if resistance is established by the introduction of a particular changed gene. While we have shown that *Nocardia* species can develop drug resistance through passaging, the cause of the resistance remains unknown.

Keywords: Drug resistance, *Nocardia*, TMP/SMX

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ANTIMICROBIAL ACTIVITY OF A NOVEL *STREPTOMYCES SP*. K430 ISOLATED FROM NORTHERN CYPRUS SOIL

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Objectives: The genus *Streptomyces,* first proposed by Waksman & Henrici (1943), consists of aerobic, gram-positive bacteria, which exhibit mycelial growth. *Streptomyces* genus are well known as a rich source of bioactive secondary metabolites, such as antibiotics and industrially and commercially important enzymes, therefore the continued interest in screening novel streptomycetes. The aims of the present investigation were to determine antimicrobial activity, cultural characteristics and isolation of strain K430 isolated from Northern Cyprus soil.

Materials and Methods: Strain K430 was isolated from soil sample collected from Karpaz National Park, Magusa, Northern Cyprus, after 4 weeks incubation at 28 °C on humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987), supplemented with cycloheximide (50 µg/ml) and nalidixic acid (10 µg/ml). Genomic DNA isolation was performed by Pitcher *et al*. (1989). The 16S rRNA gene was amplified by PCR using universal primers 27f and 1525r. Phylogenetic analysis was performed by using Neighbour Joining algorithm. Isomers of diaminopimelic acid in whole-cell hydrolysates and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed by thin layer chromatography (Staneck & Roberts, 1974). Antimicrobial activity of strain K430 to inhibit the growth of twenty one microorganisms like Gram (+) and Gram (-) bacteria, fungi was observed using an overlay technique described by Williams *et al*. (1983).

Results: K430 shared highest 16S rRNA gene sequence similarity with *Streptomycesnanshensis* SCSIO 01066^T (98.29 %), *S. abyssalis* YIM M 10400^T (98.03 %) and *S. javensis* NBRC 100777^T (97.81 %). Chemotaxonomic analyses showed that the cell wall contained ll-DAP. Whole-cell hydrolysates contained glucose and galactose. K430 isolate exhibited activity against a lot pathogenic microorganisms tested.

Acknowledgements: This study was supported by Ondokuz Mayis University (PYO. FEN.1901.12.014).

Key words: *Streptomyces*, Northern Cyprus, 16S rRNA gene, Antimicrobial activity

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ANTIMICROBIAL ACTIVITY OF *STREPTOMYCES* SPP. ISOLATED FROM HAZELNUT HUSK WASTE

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Objectives: The members of *Streptomyces* genus produced more than half of the 10100 (45%) documented bioactive compounds, such as antibiotics, enzymes, inhibitors and pharmacologically active agents (Berdy, 2005). The filamentous bacterial species included in the Streptomycetes genus (widespread in soil) are able to produce different enzymes, associated in enzymatic complexes with hydrolytic activity on celluloses and hemicelluloses substrates (Coman *et al*., 2008). The aims of the present investigation was to determine antimicrobial activity, phylogenetic analyses, 16S rRNA gene amplification and isolation of *Streptomyces* strains isolated from hazelnut husk waste, Samsun and Ordu, Turkey.

Materials and Methods: Actinomycetes test strains were isolated from hazelnut husk waste collected from Samsun and Ordu, Turkey, after three weeks incubation at 28 °C on humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987) and starch-casein agar (Küster & Williams, 1964), supplemented with rifampicin (0.5 µg/ml) and cycloheximide (50 µg/ml). Genomic DNA isolation was performed by Pitcher *et al*. (1989). The 16S rRNA gene was amplified by PCR using universal primers 27f and 1525r. Phylogenetic analyses were performed by using Neighbour Joining algorithm. Cultural characteristics were determined after incubation for 4 weeks according to Shirling & Gottlieb (1966). Cellular fatty acids were extracted, methylated and analysed by gas chromatography with the standard Sherlock Microbial Identification (MIDI) system (Sasser 1990; Kämpfer & Kroppenstedt, 1996). Antimicrobial activity of *Streptomyces* strains to inhibit the growth of twenty one microorganisms like Gram (+) and Gram (-) bacteria, fungi was observed using an overlay technique described by Williams *et al*. (1983).

Results: All thirty nine organisms were isolated on two selective media and according to the 16S rRNA sequences, twenty three actinomycetes were classified in the *Streptomyces*. The organisms grew well on ISP 2, 3, 4 and modified Bennett's agar. *Streptomyces* isolates exhibited activity against numerous pathogenic microorganisms.

Acknowledgements: This study was supported by TÜBİTAK-TOVAG (Project no: 111O698).

Keywords:*Streptomyces spp*., Hazelnut husk waste, 16S rRNA gene, Antimicrobial activity

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POLYPHASIC CHARACTERIZATION OF A NOVEL *STREPTOMYCES* SP. K427 ISOLATED FROM ARID SOIL

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Objectives: The genus *Streptomyces* is the largest genus in the family *Streptomycetacae* of the phylum *Actinobacteria*(Kämpfer, 2006). *Streptomyces* species are aerobic actinomycetes and most of them are able to form an extensively branched substrate mycelium and aerial hyphae that typically differentiate into chains of spores. The aim of the present polyphasic study was to clarify the taxonomic position of the novel strain K427 of the genus *Streptomyces,* isolated from arid soil, Northern Cyprus.

Materials and Methods: *Streptomyces* sp. K427 was picked after 4 weeks of incubation at 28°C on humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987) supplemented with nalidixic acid (10 µg/ml) and cycloheximide (50 µg/ml). Cultural characteristics were determined after incubation for 4 weeks according to Shirling & Gottlieb (1966). Genomic DNA isolation was performed by Pitcher *et al*. (1989). The 16S rRNA gene was amplified by PCR using universal primers 27f and 1525r. Phylogenetic analyses were performed by using Neighbour Joining algorithm. DNA-DNA hybridisation was carried out as described by De Ley *et al.* (1970). Isomers of diaminopimelic acid in whole-cell hydrolysates and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed by thin layer chromatography (Staneck & Roberts, 1974). Respiratory quinones were extracted from freeze dried cells based on the two stage method described by Tindall (1990a; 1990b). Cellular fatty acids were extracted, methylated and analysed by gas chromatography with the standard Sherlock Microbial Identification (MIDI) system (Sasser 1990; Kämpfer & Kroppenstedt, 1996).

Results: K427 shared highest 16S rRNA gene sequence similarity with *Streptomyces longwoodensis* LMG 20096^T (98.05 %). Strain K427 showed DNA relatedness values 45.4 % to *Streptomyces longwoodensis* LMG 20096^T. Chemotaxonomic analyses showed that the cell wall contained *ll*-DAP. Whole-cell hydrolysates contained predominantly glucose. MK-9 (H_6) (45 %) and MK-9(H_8) (29%) were the predominant menaquinone.

On the basis of morphological, chemotaxonomic and phylogenetic evidence and physiological and biochemical distinctiveness, it is suggested that strain K427 is a new member of the genus *Streptomyces.*

Acknowledgements: This study was supported by Ondokuz Mayis University (PYO. FEN.1901.12.014).

Keywords:*Streptomyces*, Northern Cyprus, 16S rRNA gene, Sequence

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PRELIMINARY SCREENING FOR BIOACTIVE COMPOUNDS FROM NATURAL RESOURCES AGAINST *STREPTOMYCES* STRAINS

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From the first reports of streptothricin in 1942 and streptomycin a year later, thousands of naturally occurring bioactive compounds, that play an important role in the development of new therapeutic agents, have been isolated from streptomycetes. Despite this, over past two decades, it has become increasingly difficult to obtain new clinically useful secondary metabolites. In contrast, recent genome sequencing projects have revealed that many biosynthetic gene clusters, producing unknown secondary metabolites, exist in *Streptomyces*genomes[1-3]. Understanding the regulatory mechanisms that activate such genetic potential in streptomycetes and elicit the yield of cryptic secondary metabolites is therefore important for the exploitation and isolation of new biologically active compounds.

Based on the abovementioned principles, the overall aim of our study is to hunt for bioactive compounds that perturb morphological differentiation and secondary metabolism in streptomycetes, to analyze their molecular mechanisms in details, and to eventually assess the possibility that approaches utilizing their bioactive could be used effectively to elicit the yield of cryptic secondary metabolites. In the present study, *Streptomyces coelicolor*A3(2) and its close relative *Streptomyces lividans* 66, the physiologically and genetically well-characterized strains of *Streptomyces*, were used as a test organism, because their morphological differentiation and the production of pigmented secondary metabolites (both actinorhodin and undecylprodigiosin) can be easily detected visually. We preliminary screened natural resources, metabolites produced by 122 different kinds of lake-derived actinomycetes and methanol extracts of plant materials (49 samples), for their ability to alter morphological differentiation and secondary metabolites producing of *S. coelicolor* and *S. lividans* during growth on modified R5 or R4 agar medium. The best results were obtained by metabolites, produced by *Streptomyces* sp. WGN-12 isolated from Lake Nojiri, against the productivity of pigmented secondary metabolites, and by methanol extracts, prepared from plant materials of *Lamium amplexicaule* L., against the formation of aerial mycelia and spores. Therefore, we are now working on isolating, purifying, and characterizing the bioactive molecules in these samples.

Keywords: Secondary metabolism, Morphological differentiation, Bioactive compounds

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ANALYSIS OF MOLECULAR MECHANISMS BY WHICH THE RIBOSOMAL RPSL MUTATION ACTIVATES SECONDARY METABOLISM IN *STREPTOMYCES COELICOLOR*A3(2)

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*Streptomyces coelicolor*A3(2) is the best characterized strain in the genus *Streptomyces* and has been used as a model for studying the mechanisms regulating antibiotic production. This strain produces several chemically diverse antibiotics, including the deep blue-pigmented polyketide actinorhodin (ACT). We previously showed that the K88E mutation [changing the lysine (K) at position 88 to a glutamic acid (E)] in the *rpsL* gene, which encodes the ribosomal protein S12, enhances the protein synthetic activity of *S. coelicolor* A3(2) during the late growth phase, resulting in ACT overproduction[1]. Moreover, recent studies with comparative expression analysis by RT-PCR and Western blotting analyses and subsequent genetic verification revealed that increased expression (at both transcriptional and translational levels) of several translation factors, such as ribosome recycling factor, EF-Tu1, and EF-G, could be responsible for the enhanced protein synthesis during the late growth phase in an ACT-overproducing *S. coelicolor* A3(2).[2] However, it has been mystery why the ribosomal mutation leads to the increased expression of the translation factors at transcription levels.

In the present study, to clarify the mechanisms that the increased levels of translation factors caused by the ribosomal mutation is responsible for antibiotic overproduction, we conducted a detailed investigation of the effects of the *rpsL*K88E mutation on transcriptional apparatus in *S. coelicolor* A3(2) by using DNA microarray analysis. Intriguingly, the results demonstrated that the expression profiles of almost all of 65 sigma factors were very different between the wild-type strain and the ACT-overproducing *rpsL*K88E mutant, especially during the late growth phases. For instance, the expression of *SCO5147* gene, encoding an ECF sigma factor, in the *rpsL*K88E mutant was three-fold higher than that in the wild-type strain, and the expression of the *sigR* gene indicated vice versa. It is therefore highly probable that the altered expression of sigma factors holds the key to increasing the expression of the translation factors at transcriptional levels. Although, the mechanisms in the altered expression profiles of sigma factors in the ACT-overproducing *rpsL*K88E mutant may be much more complicated than it appears, our results presented here provided a big clue to uncover the question “why a specific ribosomal mutation enhances antibiotic production in *Streptomyces* strains.”

Keywords: Ribosomal mutation, Translation factors, Sigma factors

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MULTIFUNCTIONAL GDSL ENZYMES IN ACTINOBACTERIA ISOLATED FROM DIVERSE ECOLOGICAL NICHES

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Actinobacteria are versatile microorganisms that occupy a wide range of ecological niches. These bacteria exhibit a variety of lifestyles - mostly they live as saprophytes in soil, but can be also found in deep sea sediments, in gut as commensals, as plant or animal and human pathogens. Adaptation to different environmental conditions depends mainly upon the flexibility and diversity of cellular enzymes which enable the microorganism to adjust to the changes in the environment. Therefore the actinobacteria isolated from a wide range of ecological niches could be a good source of diverse hydrolytic enzymes. One enzyme family that shows a high potential for pharmaceutical and biotechnological applications is GDSL lypolitic family. GDSL enzymes exhibit various catalytic activities and by utilizing a wide range of substrates often could be considered lipases, phospholipases, esterases and thioesterases. They are characterized by five distinct protein motifs (or Blocks), among which Block I, III and V exhibit higher conservation and carry important catalytic residues. The number of proteins assigned to the GDSL family has increased rapidly over the last few years and continues to expand. Due to low overall sequence similarity within the GDSL family correct annotation of these enzymes could be a challenge. We used motif scanning as a tool for identification of GDSL sequences in Actinobacteria. Altogether, we performed a comprehensive analysis of 77 actinobacterial proteomes and found 484 GDSL enzymes (up to 26 per proteome), majority coming from soil-inhabiting species. By using CLANS clustering and phylogenetic analysis we gained a new insight into evolution of actinobacterial GDSL enzymes. Enzymes were divided into eight well defined groups. Our results indicated that horizontal gene transfer had a significant impact in evolution of actinobacterial GDSL genes and it can be hypothesized that these enzymes facilitated adaptation to novel ecological niches, e.g. saprophytic lifestyle in soil. Moreover, we have found undescribed variations in GDSL motifs that possibly reflect novel enzyme properties of biotechnological interest.

Keywords: Actinobacteria, Motif scanning, GDSL lipolytic family

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ASSESSING UNDISCOVERED BIOSYNTHETIC POTENTIAL OF THE ACTINOBACTERIA PANCLUSTOME

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High biosynthetic potential of Actinobacteria is the premise and the basic assumption of many research projects. According to previously published theoretical estimates, hundreds of thousands of new biologically active substances (anti-bacterial, anti-tumour, etc) are still to be discovered. However, to the best of our knowledge, no empirical efforts were undertaken to analyze this potential based on available genomic data. We close this gap using medium-scale computational mining of 203 complete Actinobacteria genomes, in order to identify the percentage of unique clusters.

With cluster similarity threshold set to an evidence-based value 0.5 and no other selection criteria, 1242 clusters (38%) of 3247 were non-unique in the set of 203 genomes. Requiring at least 10 similar genes for the clusters to be considered similar, 884 clusters (27%) remained non-unique. An additional constraint of average protein identity to the range 65-90% resulted in 585 (18%) of non-unique clusters.

Our results provide an insight into the added value of sequencing new genomes in terms of unique/novel biosynthetic gene clusters. Obtained database of similar clusters may facilitate bioactive substance production-oriented research, and improve our understanding of the Actinobacteria secondary metabolome landscape.

Keywords: Actinobacteria, Panclustome, Cluster, Unique, Biosynthetic potential, Genomics

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ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIZED SILVER NANOPARTICLES

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The biosynthesis and the use in bioprocess of nanopariticles are evolving a new area of research interests in bionanotechnology. For investigation of antimicrobial activity of silver nanoparticles, usually the silver nanoparticles are synthesized by chemicals which are toxic and hazardous in nature. In this work we present a comparative study of antimicrobial activity of silver nanoparticles which are synthesized from silver nitrate through an environment benign and cost effective biosynthesis route where Vasak (*Adhatoda vasika)* and guava (*Psidium guajava)* leaves extract have been used. Formation and characterisation of silver nanoparticles were confirmed by UV-Vis spectroscopy and transmission electron microscope (TEM). To investigate the antimicrobial activities of biosynthesied silver nanoparticles, on *Pseudomonas aeruginosa* MTCC 741 as a model Gram negative bacteria, the agar cup assay and serial dilution assay are performed. The results show these biosynthesized silver nanoparticles could be used as effective antimicrobial agent.

Keywords: Antimicrobial activity, *Pseudomonas aeruginosa*

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DEVELOPMENT OF A NEW METHOD, CALLED THE GOUPED-MIXED CULTURE APPROACH, FOR EFFICIENTLY ACTIVATING THE POTENTIAL OF ACTINOMYCETES TO PRODUCE SECONDARY METABOLITES

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Whole genome sequencing projects of actinomycete strains have shown that each species has the ability to produce many more secondary metabolites than expected. For example, *Streptomyces coelicolor* A3(2), *Streptomyces avermitilis* MA-4680, and *Saccharopolyspora erythraea* NRRL23338 are each known or predicted biosynthetic pathway for secondary metabolites, indicating that the great majority is unexpressed or scarcely expressed under standard laboratory conditions[1-3]. Therefore, there is great interest in exploring pragmatic methods to activate such genetic potential in actinomycetes, leading to the isolation of new bioactive secondary metabolites. The recent development of new methodologies, including both physiological and genetic engineering approaches has opened the door for discovery of novel secondary metabolites by stimulating cryptic biosynthetic pathway, in actinomycetes. The improvement and modification of culture conditions, as represented by the OSMAC (one strain-many compounds) approach and co-culture (bacterial-bacterial or fungal-bacterial physical interaction) approach are simple and effective methods for activating cryptic gene clusters and increasing the production of secondary metabolites[4, 5].

While seeking more intensive method of development cryptic gene clusters for secondary metabolic pathway in actinomycete strains, we hit on the terrific idea, called the Grouped-Mixed Culture approach, that cultivating more than one strain with simultaneously on the same solid medium can potentiate the productivity of secondary metabolites. Seven different kinds of actinomycete strains (four strains of *Streptomyces*, one strain of *Kitasatospora*, and two strains of *Nocardia*) isolated from soil samples were used in the present study. Modified R4 (MR4) gellan gum medium was used for phenotypic characterization and secondary metabolites production. Spores of one strain of *Streptomyces* were inoculated onto the center of the MR4 gellan gum medium. The other six strains were inoculated on the top of hexagon at even intervals (28mm) and incubated at 30^0C for 25days. Of seven strains, five showed obvious phenotypic alterations in the colonial morphology under the grouped-mixed culture. Interestingly, HPLC analysis of ethyl acetate-extractable metabolites demonstrated the detectability of several compounds only in the grouped-mixed culture. Our findings indicate that grouped-mixed culture approach can be used effectively to elicit the yield of cryptic secondary metabolite and should facilitate discovery of new biologically active compounds.

Keywords: Secondary metabolites, Cryptic gene

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RECOGNITION AND CLEAVAGE OF 5-METHYLCYTOSINE DNA BY BACTERIAL SRA-HNH PROTEIN SCO5333

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SET and RING-finger-associated (SRA) protein is involved in establishment and/or maintenance of DNA methylation in eukaryotes. It recognizes and flips out 5-methylcytosine from DNA backbone via its SRA domain. Here we identified two SRA domain-containing proteins Sco5333 and Tbis1 in bacteria which are both associated with a distinct HNH motif. Either Sco5333 in dimer or Tbis1 in monomer specifically binds to 5mC in all DNA sequence contexts. Tbis1 has a bias of ~1000 folds in binding affinity for fully-methylated DNA over non-methylated one. Calorimetric measurement revealed that one molecule of DNA, either fully- or hemimethylated, was bound by two SRA domains (or one dimeric Sco5333) with a dissociation contant (KD) of 1.47~4.13 μM, very close to that of SUVH5. Structural modelling and superposition suggested that both proteins employ dual flip-out mechanism as eukaryotic SRA_{SUVH5}. *sco5333*and *tbis1* failed to establish in *Escherichia coli* cells hosting DNA methylation of $C^{m}CWGG$ by Dcm. Mutational studies of Sco5333 revealed that *in vivo* restriction of the 5mC DNA required both SRA and cognate HNH domain, and *in vitro* specific binding to 5mC DNA was charged by SRA domain only. Analysis of the HNH domain of Sco5333 revealed that a Zinc finger composed of $CX_2CX_{36}CX_2C$ overlaps with the conserved HNH motif. Nicking and double strand breakage of plasmid DNA (dcm+) by both enzymes were stimulated by Mn^{2+}, Mg^{2+}, Ni^{2+} and Co^{2+} but suppressed by Zn^{2+}. Induced expression of Tbis1 caused cell lysis of either Dcm^{+} or Dcm^{-}*E. coli* hosts whereas Sco5333 didn't. Moreover, suppression of DNA cleavage activity *in vitro* required 4-folds higher Zinc ion for Tbis1 than for Sco5333. These differences are attributed to destruction of zinc finger in Tbis1 in which last cysteine was mutated into aspartate. Efficient coordination to Zn^{2+} by SRA-HNH protein might increase DNA cleavage specificity, or in other word, suppress promiscous DNA cleavage activity. This might represent a regulation mechanism by which cell adjust enzyme activity via differential binding to metal ion in respond to envirenmental changes.

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DEVELOPMENT OF AN ASSEMBLY TOOL FOR THE GENERATION OF NOVEL AND BIOACTIVE ANTIBIOTIC DERIVATIVES BASED ON ARTIFICIAL GENE OPERONS

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Synthetic biology offers a new platform for the generation of novel and bioactive pharmaceuticals. Especially today where anti-infectives against multi-drug resistant bacteria are in increasingly short supply, synthetic biology could be an important catalyst in the development of new, diverse and effective antimicrobial agents.

In this study we are developing an assembly tool which is capable of generating novel and bioactive antibiotic derivatives based on the combination of artificial gene operons.

Aminocoumarin antibiotics like novobiocin, clorobiocin and coumerymcin A_1 are potent inhibitors of bacterial DNA-gyrase.[1] Novobiocin and some of its derivatives have also revealed therapeutic potential against tumor cells and tropical neglected diseases, such as trypanosomiasis.[2,3,4] Due to the fact that their chemical structures are closely related and their biosynthetic gene clusters are well-defined, they provide an excellent starting point for the reprogramming of their natural biosynthesis machineries by employing our assembly tool.

The core of the assembly tool is a novel SuperCos1 based vector in which different artificial gene operons can be assembled and subsequently expressed in a heterologous production strain. Each artificial gene operon carries the information of one building block of a structural moiety of an aminocoumarin antibiotic. Thus, by their combination new artificial aminocoumarin gene clusters encoding for novel aminocoumarin derivatives can be created.

With the development of this assembly tool, we want to provide a platform which can in future be used for the generation of novel, diverse and effective antibiotic derivatives.

Keywords:*Streptomycetes*, Aminocoumarin antibiotics, Synthetic biology, Artificial gene operons, Novel antibiotic derivatives

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THIS DOCUMENT IS ONLY THE ABSTRACT OF THE SYMPOSIUM BOOK WHICH AIMS TO CONTROL OF THE ABSTRACTS

DO GENOME STUDIES OF STREPTOPHAGES ENABLE NEW INSIGHTS INTO SOIL MICROBIOLOGY AND BIOTECHNOLOGICAL SOLUTIONS?

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The continual increase in heavy metal contaminated soils asks for new concepts of handling and remediation. In this framework, microbially assisted phytoremediation has been proposed to alter metal bioavailability which can be applied to reduce concentrations and toxic effects. A major group of bacteria involved in this processes are aerobic, gram-positive and filamentous *Streptomyces* species, a dominant group of soil bacteria specifically enriched at metal containing sites. Their growth, distribution and activity is controlled mainly by specific bacteriophages, making investigations of major ecological influences of phages on the diversity of soil microbial populations necessary to evaluate the impact of streptomycetes on bioremediation approaches. In addition, phages are the largest genetic pool on Earth, and therefore can be used as tools for genetic and biotechnological processes, especially since they are naturally involved in horizontal gene transfer. In consequence, streptophages (infecting species of *Streptomyces*) may be active in distributing important metal resistance genes such as methallothioneins, chelators (e.g. siderophores) or efflux transporters among *Streptomyces* species.

To better understand the processes active between members of *Streptomyces* and their specific phages, three lytic streptophages were investigated. The polyvalent phage S7, originally isolated from compost, the narrow host range phage S3 from garden soil, and phage L44 from forest soil were sequenced and genes useful for genetic manipulation of *Streptomyces*, e.g. with a streptophage-*Streptomyces* transduction system, were identified, opening up possibilities to investigate the ecological role in metal resistance of streptomycetes. In addition, morphological investigations of the life cycle and phage size measurements allowed a taxonomic classification of the streptophages.

Our investigations are adding to the understanding of the role of phages for soil microbiology in general, as well as with specific impact for metal contaminated site ecology and bioremediation. They also allow to evaluate future use of streptomycetes for biotechnological applications, enhancing the already high versatility of *Streptomyces* for natural product production.

Keywords: Actinophages, Bioremediation, Genome sequencing, Electron microscopy, *Streptomyces*

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NON PATHOGENIC *STREPTOMYCES* SP. OE7 INDUCE DEFENSE PATHWAYS IN BOTH ARABIDOPSIS THALIANA AND BY2 TOBACCO CELLS AGAINST *PECTOBACTERIUM CAROTOVORUM*

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Diverse *Streptomyces* may induce plant defense pathways against pathogens, but the early molecular events underlying their interactions with plants are not well understood. In plants, calcium and reactive oxygen species are commonly involved as cellular messengers of a wide range of biotic stimuli from pathogenic to symbiotic bacteria. In the present work, we checked if non-pathogenic *Streptomyces* sp. Strains could induce early signaling events leading to defense responses against *P. carotovorum* in both *Arabidopsis* plants and BY2 tobacco cell suspensions.

The results showed that the application of *Streptomyces* sp. OE7 filtrates on *Arabidopsis* plants (7 days old) has reduced the severity (76.5%) and incidence (50%) of the disease caused by *P. carotovorum*. In parallel, we demonstrate that culture filtrate of *Streptomyces* sp. OE7 strain induced a cytosolic Ca^{2+} increase and a biphasic oxidative burst in the upstream signaling events leading to defense responses in BY2 tobacco cell suspensions. They induced also the overexpression of the gene coding for PAL (the enzyme key of the phenylpropanoid pathway), and the synthesis of scopoletin which is a derivative of L-phenylalanine phytoalexin by this way. Similarly, the overexpression of other genes was induced in particular those involved in the ethylene pathway (ET) as EREBP1 and AOX. This finding suggests that metabolites secreted by *Streptomyces* sp. OE7 could elicit the induced systemic resistance pathway (ISR). Finally, we demonstrated that filtrates of OE7 induced programmed cell death (PCD) in BY2 marked by the plasmolysis of cells and the activation of the translation and transcription of DNA. This PCD depends on the Ca^{2+} and the ROS generation.

In conclusion, new insights are thus provided into the interaction mechanisms between *Streptomyces* sp. and plants; *Streptomyces* sp. could be sensed by plant cells and through cytosolic Ca^{2+} changes and the generation of reactive oxygen species, defense responses were induced.

Keywords:*Streptomyces*, Culture filtrate, Biological control, Plant defense, Signaling events, Pectobacterium, *Arabidopsis thaliana*, BY2 tobacco cell

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REWIRING THE *STREPTOMYCES* CELL FACTORY FOR COST-EFFECTIVE PRODUCTION OF BIOMOLECULES

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STREPSYNTH is a FP7 granted project (2013-2018) aiming to set-up a *Streptomyces*-based new industrial production platform (SNIP) for high value added biomolecules. The project focuses on *Streptomyces lividans* because this host has been already shown to be highly efficient for the extracellular production of several heterologous molecules that vary chemically, has a robust tradition of industrial fermentation and is fully accessible to genetic intervention. The consortium consists of 16 research teams (5 SMEs, 4 research organization and 7 from Academia) from 7 EU (BE, UK, DE, ES, SK, EL, CZ) and 3 associated countries (IL, IS, NO).

SNIP is being developed using two strategic components: (1) engineering of a collection of reduced-genome *S. lividans* strains. This will metabolically streamline the cell and rid it of agents (e.g. proteases) of potential harm to the heterologous polypeptides. (2) Synthetic parts and cassettes will be constructed, i.e. reshuffled, rewired and repurposed genetic elements either indigenous to *S. lividans* or heterologous genes organized in artificial operon clusters. These elements will serve three aims: transcriptional and translational optimization, sophisticated on-demand transcriptional regulation that will provide unique fermentation control and metabolic engineering of complete cellular pathways channeling biomolecules to profuse extracellular secretion. Synthetic parts and cassettes will be either directly incorporated into the genome or be hosted in the form of plasmids. Systems biology tools will guide fine-tuning rounds of cell engineering and fermentation optimization. To set up and validate SNIP two classes of biomolecules with obvious immediate industrial value and application are chosen: heterologous proteins (industrial enzymes, biopharmaceuticals, biofuel enzymes, and diagnostics) and small molecules (lantipeptides and indolocarbozoles) useful for multiple industrial purposes (biopharmaceuticals, additives, food technology, bioenergy).

The outcome of the project will result in:

a. Minimal genome strains; b. Transcriptional/translational regulatory circuits; c. Portable protein secretion cassettes and optimally matched substrates; d. Small molecule biosynthesis and tools for the incorporation of non-canonical amino acids; e. Systems biology know-how for optimization of biomolecule-producing strains; f. Predictive modelling of molecular cell outcomes

Furthermore, SNIP intends to be a modular platform that can be repurposed for diverse future applications. More details of the project and the partners involved can be viewed on http://www.strepsynth.eu/overview.php

The research leading to these results has received funding from the European Commission's Seventh Framework Programme (FP7/2007-2013) under the grant agreement STREPSYNTH (project no 613877)

Keywords: Industrial biotechnology, Synthetic biology, *Streptomyces lividans*, Protein secretion, Metabolomics, Fluxomics, Lantibiotics, Proteomics, Transcriptomics, Fermentation

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THE CONNECTION BETWEEN ENERGY METABOLISM AND DEVELOPMENT IN *STREPTOMYCES*

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The complex lifecycle of Streptomyces consists of vegetative and developmental growth phases. In the former, the organism grows as a multicucleoid substrate mycelim, and then develops aerial mycelium and spores in the latter. In general, the initiation of cellular development is genetaically linked with the production of secondary metabolites. To date, a number of regulators including those encoded by the bld genes of Streptomyces coelicolor A3(2) are known for their involvement in the developmental decision. Thus, the knowledge regarding the constituents of the developmental signaling network is accumulating; however, it is poorly known how the onset of morphological and physiological development is linked with the energy metabolism. It is reasonable to think that the developmental decision should be made by appropriately evaluating the energetic state of cells. The issue correlates with the efficiency in the production of useful metabolites in the fermentation process.

We study the stimulatory effect of copper on Streptomyces development. In many Streptomyces spp., supplying a few micro-molar of copper ion facilitates the erection of aerial mycelium from vegetative mycelium and production of antibiotics. Previously, we assessed for the knockout effect of the Sco1/SenC family copper chaperone (ScoC) on cell differentiation and antibiotic production in S*treptomyces coelicolor*A3(2) *and Streptomyces griseus* (Microb Biotech 5:477, 2012). The results demonstrated that the ScoC protein encoded in the conserved *sco* (*Streptomyces*copper utilization) operon serves as a global copper chaperone, assisting copper incorporation into diverged copper-dependent oxidases, including cytochrome *c* oxidase (cox) and lysyl oxidase HyaS. The marked elevation of cox activity due to the supply of copper made us speculate that the stimulatory effect of copper is exerted via the regulatory linkage of respiration activity with the onset of differentiation.

To study the actual occurrence of this linkage, we generated knockout mutants for terminal respiratory enzymes in *S. coelicolor*A3(2). The null mutant for *ctaCD* encoding the copper-containing central domain of cytochrome *c* oxidase was viable but defective in cell differentiation and antibiotic production on both complex and minimal media. A noteworthy observation was that the respiratory mutant exhibited a high intracellular ATP level, and that its ability to perform differentiation was dramatically recovered by the addition of carbonylcyanide *m*-chlorophenylhydrazone, an inhibitor of ATP synthesis. Suh and colleagues previously reported that intracellular ATP level is a critical factor for development by observing the effect of exogenous supply of ATP to colony surface (Biosci Biotechnol Biochem. 75:1576, 2011). The high intracellular ATP level could be fundamental to the developmental defect of the respiratory mutant of S. coelicolor A3(2). We also report the possible homeostatic transcriptional response of housekeeping genes along with the knockout of respiratory enzymes and discuss about the possible mechanism of developmental regulation that senses the energetic state of cells.

Keywords:*Streptomyces*, Energy metabolism, Respiration, Development, ATP level

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PSEUDOMONAS FLUORESCENS PIRATES BOTH FERRIOXAMINE AND FERRICOELICHELINE SIDEROPHORES FROM *STREPTOMYCES AMBOFACIENS*

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Iron is essential for life. It plays an essential role in many biological processes such as DNA synthesis, respiration or photosynthesis. However, in aerobic environments such as soil, its bioavailability is reduced. To overcome this limitation, microorganisms have developed different strategies to acquire this element, including iron chelation by siderophores. Some bacteria have even gained the ability to detect and utilize xenosiderophores, i.e. siderophores produced by other organisms. Such a competitive interaction have been identified between the two soil bacteria, *Pseudomonas fluorescens* BBc6R8, a mycorrhiza helper bacterium, and *Streptomyces ambofaciens* ATCC23877 which are known to produce respectively the siderophores pyoverdine and enantio-pyochelin and the siderophores desferrioxamines B, E and coelichelin. During pairwise co-cultures on iron-limiting medium, *S. ambofaciens* ATCC23877 prevented the induction of the synthesis of the fluorescent pyoverdine by *P. fluorescens* BBc6R8. Real-time PCR experiments showed that transcription of genes involved in the biosynthesis of pyoverdine and enantio-pyochelin were not induced in presence of *S. ambofaciens*. Co-cultures with a *Streptomyces* mutant strain that only produced either coelichelin or desferrioxamines also prevented pyoverdin production by *P. fluorescens* BBc6R8. Furthermore, addition of purified desferrioxamine B in the medium showed similar effect. All together, our data demonstrated that *P.fluorescens* BBc6R8 was able to utilize the ferrioxamines and ferricoelichelin produced by *S. ambofaciens*, as xenosiderophores and therefore did no longer activate the production of its own siderophores. We identified the *P. fluorescens* FoxA protein, a TonB-dependent receptor, as responsible for the detection of ferrioxamines and ferri-coelichelin. This ability to utilize ferrioxamines and probably ferri-coelichelin is widespread amongst soil fluorescent pseudomonads. In a competitive environment such as soil, siderophore piracy could well be one of the driving forces that determine the outcome of microbial competition.

Keywords: *Streptomycesambofaciens*, *Pseudomonasfluorescens*, Siderophore, Iron chelation, Tonb-dependent receptor

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IN VITRO RECONSTITUTION SYSTEM GUIDE FOR TARGETED ENGINEERING

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Metabolic Engineering has become a major research field in the past several years. Several milestone and representative successes have been achieved for bioproduction in bacteria. However, due to the lack of steady-state kinetic data as well as a dearth of information regarding the factors controlling the overall flux through many multistep biosynthetic pathways, even the successful cases provide little guidance as to how much room for improvement has been left, or directions for further engineering.

Our strategy is that we reconstitute the biosynthetic pathway *in vitro*, and we can test the contributions of cofactors, substrates and each components. After we know much better about this system, we transfered this information back in vivo and guided us to engineer this pathway in bacteria. So we call this strategy "Targeted Engineering". In our first case, we successfully *in vitro* reconstituted *E. coli* fatty acid biosynthesis system and these kinetic information are ont only help us to understand fatty acid biosynthesis better, but also guide us to improve fatty acids production. Furthermore, we reconstituted fatty alcohol synthetic pathway, and we used this strategy to identify the rate-limited step and select the suitable condidates. We prove that the propose candidates can directly take fatty acyl-ACPs as the substrates, and this pathway save more ATPs than current available pathways.

To prove our concept, we transfered this targeted engineering strategy to terpenoids overproduction. Here, we reconstituted the mevalonate pathway to produce farnesene (a precursor of new jet fuel) *in vitro* using purified protein components. The information from this *in vitro* reconstituted system guided us to rationally optimize farnesene production in *E. coli* by quantitatively overexpressing each component. Targeted proteomic assays and intermediate assays were used to determine the metabolic status of each mutant. Through targeted engineering, farnesene production could be increased predictably step by step, up to 1.1 g/L (~2,000 fold) 96 hrs after induction at the shake-flask scale. The strategy developed to release the potential of the mevalonate pathway for terpenoid overproduction should also work in other multistep synthetic pathways. In the future, this targeted engineering strategy will be applied into many pathways.

Keywords:*In vitro* reconstitution; Metabolic engineering; Targeted engineering

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EFFECTS OF ENDOPHYTIC *STREPTOMYCES* ON THE LUCERNE (*MEDICAGOSATIVA* L.) SYMBIOSIS AT DIFFERENT LEVELS OF NITROGEN

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Nitrogen has strong effects on nodulation and symbiosis of *Rhizobium* and host plants. The amount of nitrogen in soil generally has a negative effect on the nodulation process when it is over supplied to host plants. Two endophytic actinobacteria LuP30 and LuP47B isolated from healthy roots of *Medicago sativa* L. and EN23 isolated from healthy roots of *Triticum aestivum* were examined for their effects on growth of *Medicago sativa* L. with and without *Sinorhizobium meliloti* RRI128 at three levels of NH_4NO_3 supply: 2.66ppm, 25ppm and 50ppm, under greenhouse conditions. The actinobacteria did not increase the height and dry weight of shoot at low nitrogen supply (2.66ppm) while they significantly increased the height and dry weight of shoot at 25ppm and 50ppm nitrogen supply and only EN23 did not increased height of shoot at 50ppm nitrogen supply after 8 weeks sowing. This indicates that the actinobacteria help lucerne use NH_4NO_3 more efficiently to develop their shoots. The number of nodules strongly correlated with the amount of NH_4NO_3 supply in this experiment when *S. meliloti* RRI128 was applied alone: 27, 47 and 69 nodules per plant at 2.66ppm, 25ppm and 50ppm, respectively, after 7 weeks inoculation. Co-inoculation of each of the three actinobacteria with *S. meliloti* RRI128, separately, significantly increased dry weight of shoots at 2.66ppm and at 25ppm after 7 weeks inoculation with the *Sinorhizobium,* while LuP30 and EN23 increased the height and dry weight of the shoot at 50ppm NH_4NO_3 supply. In the presence of *S. meliloti* RRI128 after 4 weeks EN23 increased the number of nodules by up to 75%, while LuP30 and LuP47B increased them by more than 100% compared to the *S. meliloti* RRI128 alone at 25ppm NH_4NO_3 supply. However, only EN23 and LuP30 significantly increased the number of nodules at 50ppm NH_4NO_3 supply.

Keywords: Enhanced nodulation, N levels, Endophytic actinobacteria

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ENDOPHYTIC ACTINOBACTERIA THAT ENHANCE NODULATION AND CONTROL FUNGAL PATHOGENS IN LUCERNE (*MEDICAGOSATIVAL*.)

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Actinobacteria have been used as biocontrol agents against common diseases in plants. They can exist as endophytes inside host plants and protect plants against pathogenic microbes. One hundred and forty eight endophytic actinobacteria isolated from different legumes were screened *in vitro* for their antifungal ability against *Rhizoctonia solani* AG8 and *Pythium irregular* 89. Of the 62 cultures that were active against *R. solani*, 4 showed strong, and 8 moderate inhibitory activities. Twenty five cultures were active against *Pythium irregulare,* of which 13 were moderately active. Eight of them inhibited both the fungal pathogens from medium to strong levels such as LuP35, LuP49, CM7, CM23, PP1, PP3, PG7 and PP9 *in vitro*. The endophytic actinobacteria were checked for their biocontrol ability against *R. solani* AG8 in a high throughput *in planta* tube assay in a controlled growth chamber followed by a pot experiment in the greenhouse. In the tube assay, 47 cultures, including 31 lucerne isolates, and 16 from the other host legumes showed efficacy in controlling the *R. solani* AG8. In the pot experiment six of the top 21 cultures -LuP10, LuP30, LuP44, LuP46B, LuP47B and LuP73B – were effective in controlling disease symptoms due to *R. solani.*It is interesting to note that all six were isolated from Lucerne plants and some of them are able to enhance N fixation by stimulating increased nodulation and nodule size. There was no correlation, however, between cultures which showed strong antifungal activity *in vitro* and biocontrol ability in this study. These six cultures are potential biocontrol agents against *R.solani* and could reduce damage from other pathogenic fungi affecting legumes.

Keywords: Biocontrol, Lucerne, Enhanced nodulation, Rhizoctonia, Symbiosis

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ISOLATION AND CHARACTERISATION OF ENDOPHYTIC ACTINOBACTERIA AND THEIR EFFECT ON THE EARLY GROWTH AND NODULATION OF LUCERNE (*MEDICAGOSATIVAL*.)

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Endophytic actinobacteria are known to benefit their hosts by improving plant growth and by reducing the severity of soil borne diseases. In this study, their role in enhancing the growth of lucerne and their interaction with its rhizobial symbiosis is examined. Comparison is made between endophytic actinobacteria isolated from wheat roots and isolates from the roots and nodules of four different legume species; lucerne (*Medicago sativa* L.*)*, field pea (*Pisum sativum* L.), sub clover (*Trifolium subterraneum*) and annual medic (*Medicago* spp.). Five *Streptomyces* spp. that showed efficacy against fungal root pathogens of wheat in field trials, were evaluated to test the concept of the interaction with legume-rhizobial symbiosis. Later, 255 endophytic actinobacterial isolates were recovered from the legumes.*Streptomyces* spp. constituted 56% of the total followed by 24% *Microbispora* and 11% *Micromonospora* spp. As these studies were carried out with the aim of identifying isolates suitable for commercial production 148 well-sporulating isolates were chosen to test their effects on lucerne germination and early growth. Screening for plant growth promotion and nitrogen fixation was accomplished by co-inoculating the 5 wheat endophytes and the 148 well-sporulating strains and *Sinorhizobium meliloti* RRI128 with lucerne plants. Co-inoculation with either the wheat isolate EN23, or lucerne isolates LuP30 and LuP47B, increased lucerne shoot dry weight at seven weeks after inoculation by 25% to 35 %, and the level of fixed nitrogen by 23% to 54%, compared to plants treated with *S. meliloti* RRI128. This study shows that selected endophytic actinobacteria have the potential to enhance the lucerne symbiosis.

Keywords: Endophytic actinobacteria, Symbiosis, Legumes, Improved nodulation

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LOCALIZATION AND INTERACTION OF TRAA AND TRAB, THE ESSENTIAL FACTOR FOR THE CONJUGATIVE TRANSFER OF *STREPTOMYCES* PLASMID PSN22

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Conjugation is one of the most important mechanisms for bacterial genome evolution. In *Streptomyces*, conjugation mechanisms of small rolling circle replication plasmids have been studied. Different from other bacterial conjugation systems having a single stranded DNA transfer system called Type IV secretion system, conjugation of *Streptomyces* is accomplished through double stranded DNA transfer. We have been studied the molecular and genetic mechanisms of a *Streptomyces* plasmid pSN22. The pSN22 conjugation is depended on the three genes, *traR*, *traA*, and *traB*, and the *cis* element downstream of traB. In these gene products, TraR is the regulator for expression of the *tra* operon, and TraB is a DNA channel essential for the intermycelial DNA transfer. TraA was defined as an efficient factor during intramycelia plasmid transfer within the recipient mycelia, but the function and molecular property have been remained obscure. Initially we divided TraB into three functional domains. Second we visualized TraB to know the expression timing and subcellular localization. Finally, to know the TraA function and the molecular properties, we investigated the phenotype of *traA* mutation, subcellular TraA localization, and interaction between TraA and TraB.

TraB was consisted of three domains, the N-terminus membrane localization domain, the central FtsK/SpoIIIE domain, and the C-terminus DNA binding domain. TraB was expressed in aerial mycelium and localized in the membrane region. The *traA* null mutation caused the same phenotype as a frame-shift mutation as we reported previously. This low efficiency transfer phenotype could not rescue by the expression of TraA in recipient cells. The traA expression and subcellular localization were studied using TraA-EGFP fusion protein. TraA-EGFP was expressed from early growth phase and timing was earlier than TraB expression timing. TraA was localized as punctate appearance and not like to soluble protein. Western blotting showed TraA localized in the membrane fraction nevertheless the there is no specific membrane localization structure in the primary structure. The pull down experiment using recombinant TraA and TraB expressed in *Eschericia coli* showed direct binding of both proteins. These results strongly suggest that TraA functions in cooperatively with TraB on DNA channel complex where the TraB hexmer may be core structure.

Keywords: Conjugation, Tra proteins

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EVIDENCE FOR THE EXISTENCE OF A NON-HOMOLOGOUS END JOINING MECHANISM IN *STREPTOMYCESAMBOFACIENS*

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Double strand breaks (DSB) are probably the most deleterious DNA damage for all living cells and their repair is essential for viability. In bacterial cells, DSB repair occurs in most cases through homologous recombination (HR), an error-free repair system that needs an intact DNA duplex homologous to the broken site as template. However, some bacterial species such as *Mycobacterium tuberculosis* or *Bacillus subtilis* can use Non Homologous End Joining (NHEJ), an error-prone pathway to repair DSB. After DSB recognition by the Ku protein, the multifunctional DNA ligase LigD is recruited and the two DNA ends are rejoined together. Extension or resection of the DNA ends can facilitate the ligation step by generating microhomologies.

In silico analysis performed in the soil bacteria *Streptomyces* showed the presence of homologous genes putatively involved in a NHEJ pathway. This suggests that *Streptomyces* could be able to repair DSB through illegitimate recombination. In order to test this hypothesis, DSB were induced using the expression of a heterologous endonuclease at a targeted locus on the chromosome of *Streptomyces ambofaciens* and the sequence at the repaired site were analysed. This experiment was realised in wild-type strain as well as in mutant strain deleted for putative actors of the NHEJ. The results show that (i) *Streptomyces ambofaciens* can use NHEJ to repair DSB and (ii) that at least one of the *ku*-like genes is involved in this process.

Keywords: Homologous recombination, NHEJ, *Streptomyces*

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LINCOMYCIN AT SUBINHIBITORY CONCENTRATIONS ACTIVATES THE POTENTIAL OF *STREPTOMYCES* STRAINS TO PRODUCE SECONDARY METABOLITES

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Hormesis is a biphasic dose-response phenomenon characterized by a low-dose stimulation (activation) and high-dose inhibition [1]. It has been reported that antibiotics show the phenomenon of hormesis; antibiotics have bactericidal or bacteriostatic activity but provoke considerable activation of gene expression in bacteria at concentrations below minimum inhibitory concentration [2,3]. Indeed, many studies of antibiotics at subinhibitory concentrations have investigated the effects of various antibiotics, such as transcription and translation inhibitors, on the physiology and gene expression in bacteria.

In this present study, we have presented experimental evidence that secondary metabolites production by *S. coelicolor* A3(2) and *S. lividans* is profoundly activated by exposure to lincomycin at concentrations below MIC. In *S. coelicolor* A3(2), lincomycin at one-tenth of MIC caused a marked increase in the expression of the pathway-specific regulatory gene *act*II-ORF4, accounting for the overproduction of ACT. It should be noted that *S. lividans* grown in the presence of lincomycin at the subinhibitory concentration (one-twelfth or one-third of MIC) produced abundant antibacterial compounds, including new congeners of CDA, which were hardly produced in the absence of lincomycin. These results indicate that lincomycin at concentrations below MIC has a great influence on activating the potential of *Streptomyces* strains to produce secondary metabolites and suggest that an approach based on the dose-response effects of antibiotics will facilitate the study of secondary metabolite production in streptomycetes and the discovery of new bioactive compounds. Our next research goal is to understand the mechanism by which lincomycin at concentrations below MIC potentiates the production of secondary metabolites in *Streptomyces* strains.

Keywords: Antibiotic, Lincomycin, Secondary metabolites production, Hormesis.

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MOLECULAR DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* IN HUMAN REMAINS FROM SPAIN AND MEXICAN PRE-COLUMBIAN AND COLONIAL PERIODS

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Tuberculosis (TB) is an infectious disease mainly caused by *Mycobacterium tuberculosis* (Mt), but also by other bacteria members of the *Mycobacterium tuberculosis* Complex (MTC). By detecting biomolecules specific to this bacterium in archaeological remains it has been determined that the disease was present in humans at least since the Neolithic period in different parts of the Old World. In Mexico, there are not historical registers regarding TB until the Colonial time, so in this work we applied ancient DNA (aDNA) and Mass Spectrometry (MS) techniques with the aim of detecting molecular fingerprints in human remains from different archaeological sites, including Mexican pre-Columbian (Pajones and Tamtoc), Mexican Colonial (Hospital de San José) and Spanish sites.

Using PCR, we amplified, cloned and sequenced a fragment of the Insertion Element 1081, characteristic of the MTC, in three of the 11 samples analyzed. The sequence identity was confirmed by sequence similarity searches. By MS, we detected in another sample three masses (m/z) that correspond with specific Mt lipids. These results, together with previously reported archaeological data suggest that TB was present in Mexico, prior to the contact with Europeans.

Keywords:*Mycobacterium tuberculosis*, Ancient DNA

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REGULATORY NETWORKS

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GLOBAL LEVEL REGULATION OF THE ANGUCYLINE ANTIBIOTIC AURICIN BY A GAMMA-BUTYROLACTONE AUTOREGULATOR-RECEPTOR SYSTEM IN *STREPTOMYCES AUREOFACIENS* CCM 3239

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Introduction: The gamma-butyrolactone (GBL) systems play a role in controlling secondary metabolism in streptomycetes [1]. We previously identified the *aur1* gene cluster, located on a large linear plasmid pSA3239 of *Streptomycesaureofaciens* CCM3239, which is responsible for the production of angucycline antibiotic auricin [2]. Auricin is produced during a narrow time period following entry into stationary phase. This unusual phenomenon likely arises from a complex regulation of auricin biosynthesis, involving several cluster-situated regulators (CSR) [3]. Sequence analysis of the upstream region of the *aur1* cluster revealed two genes, *sagA* and *sagR*, encoding proteins homologous to the GBL regulatory system [2]. SagA was similar to GBL synthases, and SagR to GBL receptors.

Objectives: Role of two GBL genes, *sagA* and *sagR*, in auricin production. Role of SagR in regulation of two auricin-specific CSR genes, *aur1P* and *aur1R*.Characterization of *sagA* and *sagR* regulation.

Methods: Growth of *S. aureofaciens* CCM3239 and analysis of auricin production was done as described in [4]. Disruption of the *sagA* and *sagR* genes in *S. aureofaciens* CCM3239 was done as described in [4]. Transcription from promoters was determined by S1-nuclease mapping using RNA isolated from different growth phases as described in [4]. The binding studies were done using purified SagR and Aur1R as described in [4].

Results: Expression of both GBL genes was governed by a single promoter, *sagAp* and *sagRp*, active in the exponential phase and interdependent. Disruption of *sagA* abolished auricin production. Disruption of *sagR* resulted in a precocious, but substantially reduced auricin production. Transcription from the *aur1Pp* and *aur1Rp* promoters, directing expression of the auricin-specific CSRs, was precocious and increased in the *sagR* mutant strain. In addition, SagR was shown to bind both promoters. SagR does not bind its own promoter. However, the auricin-specific repressor Aur1R, which belongs to the family of pseudo GBL receptors, binds both *sagAp* and *sagRp* promoters. Moreover, the expression of both promoters was deregulated in the *aur1R* mutant.

Conclusions:

1. Disruption of both *sagA* and *sagR* genes affected auricin biosynthesis.

2. Both *aur1Pp* and *aur1Rp* promoters directing expression of auricin-specific CSR genes were deregulated in the *sagR*mutant, and SagR binds both promoters

3. Aur1R binds both *sagAp* and *sagRp* promoters and they were deregulated in the *aur1R* mutant

4. The results indicate a direct role of two GBL system proteins, SagA and SagR, in a global level of regulation of auricin biosynthesis through direct regulation of auricin-specific CSRs.

5. Aur1R negatively regulated both GBL genes, constituting a regulatory feedback control of the GBL system.

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Keywords: Auricin, Angucyclines, Antibiotics, Gamma-butyrolactone system, Regulation

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Herein, we report on the design of two novel inducible expression systems based on CymR-cumate and RolR-resorcinol for *Streptomyces* and other *Actinobacteria*. Cumate (*p*-isopropylbenzoic acid)-inducible gene switch is based on the CymR regulator, the operator sequence (*cmt*) from the *Pseudomonas putida* cumate degradation operon and P21 synthetic promoter[1]. Resorcinol inducible expression system is composed of the RolR regulator and PA3 promoter[1] fused with the operator (*rolO*) from the *Corynebacterium glutamicum* resorcinol catabolic operon. Using the *gusA* (β-glucuronidase) gene as a reporter [2], we showed that the newly generated expression systems are tightly regulated and hyper-inducible. The activity of the uninduced promoters is negligible in both cases. Whereas the induction factor reaches 45 for *Streptomyces albus* and more than 100 for *Kitazatospora* sp. in the case of cumate switch and 33 for *S. albus* in the case of resorcinol toggle. The systems are also dose dependent, which allows the modulation of gene expression even from a single promoter. In addition, the cumate system is versatile, given that it is functional in different actinomycetes. Finally, these systems are non-toxic and inexpensive, as these are characteristics of cumate and resorcinol, and they are easy to use because inducers are water soluble and easily penetrate cells. Therefore, the P21-*cmt*-CymR and PA3-*rolO*-RolR systems are powerful tools for engineering actinobacteria.

Keywords:*Actinobacteria*, Inducible expression system, Cumate, Resorcinol

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EXPLOITING SIGNALLING MOLECULE AND TRANSCRIPTIONAL REPRESSOR INTERACTIONS IN *STREPTOMYCES* TO ENGINEER NOVEL INDUCIBLE EXPRESSION SYSTEMS

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MmfR, is a TetR-family[1] homodimeric protein consisting of an N-terminal DNA-binding domain and a C-terminal ligand-binding domain. MmfR specifically binds to the small signalling molecules MMFs (methylenomycin furans)[2] and has been shown to control the expression of methylenomycin biosynthetic genes in *Streptomyces coelicolor* A3(2).

With the eventual aim of developing a novel inducible expression system, the project first investigates the molecular interactions between MmfR and the MMFs as well as different promoter regions. *In silico*, key residues in the ligand- and DNA-binding sites of MmfR have been identified through sequence and structural analysis and a comparison of motifs with orthologues such as GBL-binding (gamma-butyrolactone) proteins. Work has begun purifying MmfR for DNA-bound crystallographic analysis as well as surface plasmon resonance. A luciferase reporter gene assay is also being developed *in vivo*, employing the MMFs and other potential analogous inducer molecules. Reporter genes (*luxCDABE)* replace methylenomycin biosynthesis expression with the bio-luminescing luciferase genes.[3] These *lux* genes are under the control of either MmfR and inducible with the MMF signalling molecules.

Keywords: Tetr, *Streptomyces coelicolor*, Luciferase, Methylenomycin

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EFFECT OF PHOSPHOFRUCTOKINASE DELETION AND FRUCTOSE-1,6-BIPHOSPHATASE OVEREXPRESSION ON TACROLIMUS PRODUCTION AND CARBON CATABOLITE REPRESSION IN *STREPTOMYCES TSUKUBAENSIS*

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Introduction: *Streptomyces tsukubaensis* is one of the main producers of tacrolimus (or FK506), a macrolide polyketide with immunossupressant activity. It is used in the prevention of graft rejection and dermatitis. Recently, neuroprotective and regenerative properties have been suggested for this compound.

S. coelicolor presents three phosphofructokinase-coding genes named *pfkA1*, *pfkA2* and *pfkA3*. Phosphofructokinases (PfkAs) catalize phosphorilation of fructose-6-phosphate (F6P) to fructose-1,6-biphosphate (FBP) in the glycolitic pathway. Interestingly, deletion of one of these kinases (i.e. *pfkA2*) in *S. coelicolor* led to antibiotic overproduction (Borodina *et al*., 2008). The authors attributed this effect to an increasing metabolic flux through the pentose-phosphate pathway. Based on this result, an alternative approach to reduce metabolic flux through PfkAs could be overexpression of *glpX*, FBP-phosphatase, which catalyze the opposite reaction.

Antibiotic production is transcriptionally repressed by preferred carbon sources, a phenomenon known as carbon catabolite repression (CCR). Although CCR is poorly understood in *Streptomyces,* glucose kinase has been shown to play an important role, possibly through interaction with phosphorylated sugars from glycolisis, such as FBP or phosphoenolpyruvate (PEP). In the relative *Bacillus* species, FBP is a key molecule involved in CCR. Previously, we demonstrated that glucose and glycerol are able to arrest tacrolimus production in *S. tsukubaensis*; being the effect of glucose stronger than that of glycerol.

Objectives:

- To obtain *pfkAs* deletion mutants as well as a *glpX*-overexpressing strain in *S. tsukubaensis* to test if there is a similar effect on tacrolimus production than that obtained for pigmented antibiotics in *S. coelicolor A3(2)*.
- To analyze tacrolimus repression by glucose (i.e. CCR) in the above mentioned strains (and a possible interaction of carbon and phosphate regulation on tacrolimus production).

Methods: *pfkA1* mutant strain was generated by Redirect technology and *pfkA2* and *pfkA3* mutant strains, through the use of the pKC1139 vector.

glpX-overexpressing strain was obtained using the integrative vector pIB139, which contains the strong constitutive promoter *ermE*.

The strains were cultured in MGm 2.5 production media, in flasks at 28ºC and 220 rpm for 236 hours. This medium is limited in phosphate in order to allow monitoring tacrolimus production onset. MGm2.5 contains starch as main carbon source and glutamate as carbon and nitrogen source. In order to analyze glucose repression, additions of this sugar were

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done at the mid-exponential growth phase (70 hours) to a final concentration of 3% (same amount of maltose was added in control cultures).

Samples for dry weight and tacrolimus extraction were taken regularly from all the cultures. Tacrolimus was extracted with methanol, detected by bioassays against hypersensitive *Saccharomyces cerevisiae* TB23 and quantified by HPLC.

Results: Analyses of the *pfkAs* mutants showed that deletion of these genes do not produce a change on the tacrolimus production pattern, contrary to the *S. coelicolor* results. Nevertheless, Δ*pfkA1* mutant showed a slightly lower production rate (although not very significant from an industrial point of view). On the other hand, glucose addition arrested tacrolimus production in all mutants, as it did in the wild type strain.

The integration of vector pIB139, seems to have no effect on growth or tacrolimus production in comparison to the wild type strain. Growth of *glpX*-overexpressing strain was slightly higher with maltose addition than with glucose addition. Tacrolimus production remained at wild type levels and glucose addition also resulted in tacrolimus production repression.

Conclusions:

- *pfkA* deletion did not influence tacrolimus production in *S. tsukubaensis.* Probably, the effects of single deletions are counteracted by the remaining two paralogs.
- *glpX* overexpression did not affect tacrolimus production. However, a post-transcriptional regulation of this gene should not be dismissed and therefore, the overexpression of this gene could not have resulted in an increased fructose-1,6-biphosphatase activity.
- Carbon catabolite repression of tacrolimus production (i.e. by glucose) under phosphate-limited conditions in *S. tsukubaensis* is not affected by *pfkA1, pfkA2* or *pfkA3* deletions or by *glpX* overexpression.

Keywords: Tacrolimus, *Streptomyces tsukubaensis*, Carbon catabolite repression, Phosphofructokinase, Phosphate, Fructose-1,6-biphosphate

A 1.8 MB-REDUCED *STREPTOMYCES CLAVULIGERUS* GENOME: RELEVANCE FOR SECONDARY METABOLISM AND DIFFERENTIATION

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Streptomyces clavuligerus ATCC 27064 is the organism producer of two important metabolites used in clinic: the β-lactam antibiotic cephamycin C (basic molecule for semisynthetic cephalosporin) and the β-lactamase inhibitor clavulanic acid (CA). Genes for the biosynthesis of both compounds are located side by side in the 6.76 Mb *S. clavuligerus* chromosome and share a common SARP-type regulatory gene (*ccaR*). The chromosome contains also is the gene cluster for holomycin, a dithiolopyrrolone compound (Li and Wals, 2010; Robles-Reglero *et al.,* 2013) with antitumoral properties. In addition, *S. clavuligerus* has the potential to produce up to 48 putative secondary metabolites including polyketides (PK), non-ribosomal peptides (NRP), mixed PK-NRP compounds and terpenes. A large part (21%) of the wild type *S. clavuligerus* genome is located in a 1.8 Mb megaplasmid that greatly influences secondary metabolites biosynthesis even if the secondary metabolites are chromosomally encoded. The megaplasmid copy number may change depending on the nutritional and environmental conditions. The S. *clavuligerus oppA2::aph* mutant, deleted in the *oppA2* gene which encodes an oligopeptide permease does not form aerial mycelium, spores or clavulanic acid, but overproduces holomycin (Lorenzana *et al.,* 2004). Transcriptomic studies, PCR, qPCR and RT-qPCR analysis showed that *S. clavuligerus oppA2::aph* has a drastically reduced number of copies (about 25.000-fold lower than the parental strain) of plasmids pSCL1 (10.5 kb), pSCL2 (149.4 kb) and of the megaplasmid pSCL4 (1.8 Mb). To clarify the role of the linear plasmids and the function of OppA2 in *S. clavuligerus oppA2::aph* we constructed *oppA2* mutants which contained: i) a normal copy number of the linear plasmids, ii) completely lack of the linear plasmids, and iii) a *parA-parB*$_{pSCL4}$ mutant what resulted in lack of only the pSCL4 plasmid. In addition, a strain with a functional *oppA2* gene was constructed lacking the megaplasmid pSCL4. The results confirmed that the *oppA2* gene is essential for clavulanic acid production, independently of the presence or absence of linear plasmids, but *oppA2* has little relevance on differentiation. We demonstrated that the lack of sporulation of S. *clavuligerus oppA2::aph* is due to the absence of linear plasmids (particularly pSCL4) and the holomycin overproduction is largely due to the lack of pSCL4 and is stimulated by the *oppA2* mutation.

Keywords:*Streptomyces clavuligerus*, Clavulanic acid, Holomycin, Linear plasmids, pSCL4.

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EFFECT OF A SMALL MOLECULE ON THE REGULATORY NETWORK OF SECONDARY METABOLITE PRODUCTION IN *STREPTOMYCES COELICOLOR*

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The genus *Streptomyces* is known for the production of antibiotics and other biologically active secondary metabolites. The secondary metabolites are produced by biosynthetic pathways encoded in discrete gene clusters. The expression of these clusters is controlled by factors that include growth rate, signaling molecules, metabolism, and physiological and environmental stresses. These diverse signals and the signal transduction proteins and transcription factors that mediate their responses, constitute a complex regulatory network that set the level of secondary metabolism. Understanding this network is important for the discovery and exploitation of these compounds. One method of disturbing the cell's regulatory network is through the use of chemical elicitors. I am exploring the effects of the ARC2 elicitor, which we identified in a previous small molecule screen. In the presence of ARC2, the production of the blue-pigmented secondary metabolite actinorhodin and the germicidins are increased, while the red-pigmented secondary metabolite undecylprodigiosin and the daptomycin-like calcium-dependent antibiotic are decreased. Like the related compound triclosan, ARC2 targets the FabI enzyme. We have shown that this elevates the production of unsaturated fatty acids in cells, consistent with the fact that FabI is an enoyl reductase required for fatty acid saturation. Our most recent work indicates that this effect of ARC2 perturbs the membrane inducing a stress response. In response to the potential stress, the expression of at least one transcriptional activator in the secondary metabolic regulatory network is elevated, leading directly to effects on secondary metabolite output.

Keywords:*Streptomyces coelicolor*, Chemical elicitor, Actinorhodin, Regulatory network, Secondary metabolism

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BINDING OF A BIOSYNTHETIC INTERMEDIATE TO ATRA MODULATES THE PRODUCTION OF LIDAMYCIN BY *STREPTOMYCES GLOBISPORUS*

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Streptomycetes are prolific producers for numerous clinically used antibiotics and other therapeutics.The control of secondary production in streptomycetes involves the regulatory cascade from environmental and physiological signals to the cluster-situated (transcriptional) regulators (CSRs) of the biosynthetic genes. For some systems, the binding of biosynthetic products to the CSR has been shown to provide negative feedback. AtrA (actinorhodin-associated transcriptional regulator), previously reported in *S. coelicolor*, is a TetR-family pleiotropic activator for *actII-ORF4* and *negE2*, which encode the pathway-specific transcriptional activator of the actinorhodin biosynthesis and the major permease for N-acetylglucosamine, respectively. In this work, AtrA-gl, an AtrA homologue from enediyne antitumor antibiotic lidamycin (C-1027) producer strain *Streptomyces globisporus* C-1027 was identified to positively regulate the production of lidamycin. Expression of *sgcR1* and *sgcR2*, two of the positive CSRs of lidamycin production, was transactivated by AtrA-gl. Additional experiments showed that AtrA-gl binds *in vivo* as well as *in vitro* to the promoter region of 5' coding region of SgcR1. AtrA-gl binding activities to target sequence were found to be inhibited by heptaene, a biosynthetic intermediate of C-1027 enediyne core, as well as an end product antibiotic actinorhodin produced in *S. coelicolor*. Here we show that negative feedback can extend to a point higher in the regulatory cascade for the production of lidamycin. The feedback to the pleiotropic regulator AtrA is likely to provide a mechanism for coordinating the production of lidamycin with that of other secondary metabolites. As AtrA is evolutionarily conserved, negative feedback of the type described here may be widespread within the streptomycetes.

Keywords: Pleiotropic regulator, AtrA, Feedback, Lidamycin, Biosynthetic gene cluster

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IDENTIFYING ARPA-LIKE BINDING SITES IN CRYPTIC *STREPTOMYCES* SECONDARY METABOLITE GENE CLUSTERS

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Since the sequencing of the model *Streptomyces* bacteria, *S. coelicolor* A3(2) [1], and the recent development of high-throughput sequencing technologies, the number of sequenced *Streptomyces* genomes is rapidly increasing [2]. The analyses of these bacterial genomes have identified dozens of secondary metabolite gene clusters, many of which appear to be silent [3]. Some of these silent secondary metabolite gene clusters are under the control of ArpA-like repressors, which belong to the TetR transcriptional repressor family.

Within the ArpA class of repressors, we are interested in the MmyR and MmfR-like transcriptional repressors that are found in the methylenomycin and gaburedin biosynthetic gene cluster in *S. coelicolor* A3(2) and *S. venezuelae* respectively [4, 5]. Other homologous *mmyR* and *mmfR* genes can also be identified in several other *Streptomyces* bacteria and the regulation mechanisms in controlling secondary metabolite biosynthesis appear to be complex.

The prediction of the exact binding locations of these TetR transcriptional repressor proteins is extremely challenging and with the aid of motif enrichment tools, highly conserved 24bp palindromic sequences were identified upstream of key regulatory elements within the *Streptomyces* secondary metabolite gene clusters. The TetR repressors are proposed to interact with these sequences and control the expression of biosynthetic genes involved in the production of antibiotic-like natural products.

Putative motifs identified, through this approach, were consistent with previously predicted motifs and unstudied secondary metabolite gene clusters in *S. avermitilis* and *S. hygroscopicus* revealed potential activators or SARPS. Future works include analyzing the transcriptome of *Streptomyces* transcriptional repressor mutants to refine our model of DNA-binding model and the model of transcriptional regulation involved in secondary metabolite biosynthesis.

Keywords:*Streptomyces*, Transcriptional regulation, Bioinformatics, Natural product

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GENETIC, BIOCHEMICAL AND STRUCTURAL STUDIES OF A REGULATOR OF AROMATIC CATABOLISM IN *STREPTOMYCES* BACTERIA

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Streptomycetes, Gram-positive soil-dwelling bacteria, produce half of the 23,000 known antibiotics (i.e., microbial natural products). Many antibiotics produced by streptomycetes can be described as commodity chemicals based on their use in medicine as antimicrobial drugs, anti-cancer agents, and immunosuppressants. Another type of commodity chemical that can be derived from streptomycetes is triacylglycerols, the precursors of biodiesel. The commercial value of antibiotics and biodiesel has motivated much research into how these bacteria produce them and how they can be produced at lower costs.

The inspiration for our work is the possibility of producing commodity chemicals from cheap and highly abundant sources of carbon. It is our contention that aromatic compounds represent a low-cost and readily available carbon source. Accordingly, we seek to understand the structure and regulation of pathways for the catabolism of aromatic compounds. Using a battery of tools from genetic, biochemistry and structural biology, we have discovered and characterized a peculiar transcription factor in *Streptomyces* bacteria that regulates the expression of a gene encoding an enzyme that catalyzes a rate determining step in the catabolism of aromatic compounds.

Keywords:*Streptomyces*, Commodity chemicals, Aromatic catabolism, Para-hydroxybenzoic acid, Iclr regulator

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ANTIBIOTIC INDUCTION OF STABLE SIGR CONFERS ANTIBIOTIC RESISTANCE IN *STREPTOMYCES COELICOLOR*

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Antibiotics are secondary metabolites produced by microorganisms in nature. At sub-inhibitory concentrations they induce physiological responses in exposed bacteria to have antibiotic resistance. Streptomyces spp., which produce more than half of known natural antibiotics, are also equipped with a variety of resistance mechanisms toward both endogenous and exogenous antibiotics. Previously, a stress response system regulated by an ECF sigma factor SigR has been reported to sense thiol-reactive chemicals that oxidize or alkylate cysteine thiols, and induce more than 100 genes including its own gene in *Streptomyces coelicolor*. Transcription from the upstream *sigR*p2 promoter is induced by activated SigR, expressing the unstable isoform SigR', which is longer by 55 N-terminal aa than the stable SigR. Since SigR' is degraded by its target gene products, production of SigR' ensures both positive amplification and timely shut-off of the stress response. In addition to this mode of induction, we recently found that production of SigR is also induced by sub-minimal inhibitory concentrations of diverse antibiotics, especially by those targeting translation. In contrast to thiol-oxidative stresses, expression of stable SigR from the downstream *sigR*p1 promoter is induced upon antibiotic treatments, enhancing the induction of its target genes for a longer period of time. Consistent with the induction by antibiotics, the *sigR-rsrA* null mutant became more susceptible to antibiotics, suggesting that one of the primary functions of SigR regulon is to confer antibiotic resistance. The antibiotic induction of *sigR*p1 transcription was dependent on a WhiB-like protein WblC/WhiB7, which is known to confer antibiotic resistance in *Streptomyces* and *Mycobacterium*. How WblC is involved in inducing SigR by antibiotics is being investigated.

Keywords: Antibiotic, Intrinsic resistance, Sigma factor, Stress, Regulator

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GENOME-WIDE ANALYSIS OF GENES INDUCED BY METAL CHELATOR TPEN AND ZINC-RESPONSIVE REGULATOR ZUR IN *STREPTOMYCES COELICOLOR*

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Metals affect bacterial physiology in diverse ways, as catalytic cofactors, structural or regulatory components of proteins, etc. When in excess, they disrupt cell physiology as toxic agents, and hence its homeostasis needs be strictly achieved in all life forms. In *Streptomyces coelicolor*, several metalloregulators were identified, among which a zinc-responsive regulator of the Fur family has been reported to be a primary regulator for zinc homeostasis, which is important for differentiation and antibiotic production. A handful of its target genes that encode zinc-uptake and mobilization systems have been identified to be negatively regulated by zinc-bound Zur. In order to find genome-wide target genes of Zur, and more broadly an array of genes that respond to metal availability, we performed ChIP-chip and RNA deep sequencing analyses. Following treatment with TPEN (*N*,*N*,*N*_,*N*_-*tetrakis*(2-pyridylmethyl)ethylenediamine) that can chelate various divalent metals, about 800 genes were up-regulated and about 700 genes were down-regulated. The up-regulated genes contain all known Zur-target genes, and enriched in various transporter genes. Among them, the genes that exhibit strong Zur-binding (top 1% peak intensity; 172 peak sites) within 500 bp of their start codons were selected and further analyzed. About half of Zur binding sites were found within the internal region of ORF. A large number of genes that are induced by TPEN and have prominent Zur binding peaks were not affected by zur deletion mutation, suggesting involvement of additional metallo-regulators. Characterization of their regulation profile is under investigation.

Keywords: Metal, TPEN, Metalloregulator, Zur

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GENE-SPECIFIC REGULATION BY SMALL RNA IN STREPTOMYCETES; INHIBITION OF NI-CONTAINING SOD GENE EXPRESSION BY A SMALL RNA PROCESSED FROM 3'UTR OF THE SODF MRNA ENCODING FE-CONTAINING SOD

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Superoxide dismutases (SODs) are widely distributed enzymes that convert superoxides to hydrogen peroxide and molecular oxygen, using various metals as cofactors. Many actinobacteria contain genes for both Ni-containing (*sodN*) and Fe-containing (*sodF*) SODs. In *S. coelicolor,* expression of the *sodF* and *sodN* genes is inversely regulated by nickel-specific Nur, a Fur-family regulator. With sufficient nickel, Nur directly represses *sodF* transcription, while inducing *sodN* indirectly. Bioinformatic search revealed that a conserved 19 nt stretch upstream of *sodN* matches perfectly with the *sodF* downstream sequence. This feature is conserved in nearly all streptomycetes. We found that the *sodF* gene produced a stable small-sized RNA species (s-SodF) of ~90 nt that harbors the anti-*sodN* sequence complementary to *sodN* mRNA from the 5' end up to the ribosome binding site. The s-SodF RNA is most likely a processed product of *sodF* mRNA. The s-SodF RNA caused a significant decrease in the half-life of the *sodN* mRNA. Therefore, nickel-responsive Nur activates *sodN* expression through inhibiting the synthesis of *sodF* mRNA, from which inhibitory s-SodF RNA is generated. Mutagenesis analysis showed that s-SodF binds to 5' end of sodN *mRNA* through anti-*sodN* sequence. This reveals a novel mechanism by which antagonistic regulation of one gene is achieved by a small RNA processed from the 3'UTR of another gene's mRNA. Why nickel is preferentially utilized to produce Ni-SOD and inhibits Fe-SOD production during exponential growth phase, and why Fe-SOD replaces Ni-SOD in the stationary phase is an interesting question to tackle.

Keywords: Small RNA,Nickel, Superoxide dismutase, Nickel uptake regulator

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γ-BUTYROLACTONE AUTOREGULATOR SYSTEM OF SECONDARY METABOLISM IN *STREPTOMYCES LAVENDULAE* FRI-5

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Osaka University

The γ-butyrolactone autoregulator has been shown to control secondary metabolism and/or morphological differentiation across many *Streptomyces* species. Among the known γ-butyrolactones autoregulators, IM-2 from *Streptomyces lavendulae* FRI-5 has unique characteristics regarding the regulation of secondary metabolism. In contrast to the solely positive effects exerted by other γ-butyrolactone autoregulators, IM-2 has opposing effects on the regulation of secondary metabolism; namely, it not only switches on the production of a blue pigment (indigoidine) and nucleoside antibiotics, but also switches off the production of D-cycloserine. Genetic and biochemical analyses revealed FarA (the IM-2 receptor protein) negatively controls the biosynthesis of indigoidine and nucleoside antibiotics, and it has also been proposed that the formation of the IM-2-FarA complex would be necessary for the termination of D-cycloserine production. These findings suggest that the IM-2/FarA system may have a novel regulatory system to control secondary metabolism. Here, we report the function of multiple regulatory genes governed by the IM-2/FarA system for secondary metabolism.

The *farA*-flanking region has seven regulatory genes, including *farX* (an IM-2 biosynthetic gene), and comprises a *far* regulatory island. Two putative regulatory genes (*farR3* and *farR4*) encoding the SARP-family protein are present in the *far* regulatory island together with two more putative transcriptional regulatory genes (*farR1* and *farR2*). Gene expression analysis demonstrated that *farX*, *farR1*, *farR2*, *farR3*, and *farR4* are under the control of the IM-2/FarA system. Furthermore, gene disruption analysis verified that they have distinct contributions to the regulation of secondary metabolism: FarR2 and FarR3 control indigoidine biosynthesis negatively and positively, respectively, and FarR4 plays a negative regulatory role in the IM-2 biosynthesis. Interestingly, this is the first case to show that an SARP-family regulator is involved in the biosynthesis of a signaling molecule functioning at the most upstream region of the regulatory cascade for *Streptomyces* secondary metabolism. Taken together with the fact that transcription of *farX* is under the negative control of IM-2 and the positive control of FarA, our findings suggested that *S. lavendulae* FRI-5 has a fine-tuning system to control γ-butyrolactone biosynthesis as well as a complex regulation for secondary metabolism.

Keywords: γ-Butyrolactone autoregulator, SARP-family regulator, Indigoidine

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HETEROLOGOUS EXPRESSION OF *STREPTOMYCES CLAVULIGERUS* ATCC 27064 CEPHAMYCIN C GENE CLUSTER

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Introduction: Heterologous gene expression is an interesting approach to study the limits of already known gene clusters. This technique is also useful to know whether the expression of the gene clusters is dependent on a cascade of regulatory mechanism (Gomez-Escribano and Bibb, 2014).

The *ccaR*$_C$ gene, which is located internal to the cephamycin C gene cluster in *S. clavuligerus*, encodes an activator protein of the SARP type acting on the cephamycin C and the clavulanic acid gene clusters. *S. coelicolor* M1146, an optimized host strain designed to facilitate the metabolic flux to the heterologous expressed antibiotic, *S. albus* J1074, a strain defective in the Pal6 restriction-modification system and *S. flavogriseus* ATCC 33331, phylogenetically closer to *S. clavuligerus* than *S. albus* or *S. coelicolor* were used as hosts. *S. flavogriseus* ATCC 33331 genome contains a clavulanic acid gene cluster with a *ccaR*$_F$ gene encoding a regulatory activator 63 % similar to CcaR$_C$ (Alvarez-Alvarez et al., 2013).

Objetives: The aim of the present work is to understand the possible requirement of upper hierarchy strain-specific regulators for cephamycin C production, and the importance of phylogenetic distance between heterologous *Streptomyces* host for cephamycin C biosynthetic genes expression.

Methods: The cephamycin C gene cluster, in cosmid [SCos-CF], was integrated in the genome of the three *Streptomyces* hosts. An additional copy of *ccaR,* expressed from the constitutive promotor P$_{fur}$, in plasmid [P$_{fur}$-*ccaR*] was also incorporated by integration in the previous strains. To determine the effect of the internal *ccaR*$_F$gene of *S. flavogriseus,* the *ccaR*$_C$gene of *S. clavuligerus* was deleted in cosmid [SCos-CFΔ*ccaR*]. Cephamycin C production was tested in eight solid and liquid media. HPLC-MS was performed to confirm heterologous cephamycin C production. RT-qPCR for the cephamycin biosynthesis genes were performed in *S.coelicolor* [SCos-CF] and *S. flavogriseus*-derived strains. *S. flavogriseus* and *S.coelicolor* derived strains were grown in different concentration of cephalosporin and penicillin G to test their penicillin and cephalosporin levels of resistance.

Results: Cephamycin C production was observed in *S. flavogriseus*-derived strains, but not in *S. coelicolor*- or *S. albus*-derived strains. As analyzed by qRT-PCR the biosynthetic genes were expressed at very low level (3.4%) in *S. coelicolor* and to a moderate level (9.6%) in *S. flavogriseus* in relation to *S. clavuligerus*. *S. flavogriseus* and *S. coelicolor*-derived strains were more resistant to cephalosporin and penicillin G than the original strain, probably due to the high expression level of resistance genes like *pbpA* and *pcbR*. *ccaR*$_F$ was found to be not functional in the expression of heterologous cephamycin C genes. *ccaR*$_C$ expressed from the P$_{fur}$ promoter in *S. flavogriseus* improves the cephamycin C production, but only to 10% of that found in the *S. clavuligerus* ATCC 27064.

Conclusions: To achieve an efficient gene expression and antibiotic production the phylogenetic proximity of hosts *Streptomyces* strains should been considered when heterologous expression of gene clusters is approached.

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S. flavogriseusccaR activator gene, $ccaR_{F,}$ is not functional in the expression of the cephamycin C biosynthesis.

Cephamycin C production in *S. flavogriseus*::[SCos_CF], was 8 µg/ml, and in *S. flavogriseus*::[Scos-CF]::[Pfur-ccaR] the production was 40-fold higher, but still in the order of 8% of the production by *S. clavuligerus* ATCC27064.

Genes related to antibiotic resistance, are $ccaR_C$ independent, and consequently, were well expressed. This results in the growth of *S. flavogriseus* and *S. coelicolor* exconjugants at higher concentration of penicillin or cephalosporin.

Keywords: Cephamycin C, *Streptomyces clavuligerus*, Heterologous expression, *Streptomyces flavogriseus*

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INVESTIGATION OF LYSOLIPIN BIOSYNTHESIS REGULATION

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Streptomyces tendae Tü 4042 produces the aromatic polyketide antibiotic lysolipin [1]. Lysolipin is highly active against Gram-positive bacteria (MIC 1,7-50 nM) and targets probably a yet unidentified component in the bacterial cell envelope. It also shows strong cytotoxic effects on eucaryotic cells (MIC 50 µM) and antifungal activity (MIC 17-50 µM) [2]. Lysolipin might therefore be involved in active defense against bacteria as well as higher organisms.

The lysolipin biosynthetic gene cluster was identified, sequenced and annotated. It comprises 44 genes involved in biosynthesis, regulation and export of the antibiotic. All genes necessary for lysolipin production were cloned on a 42 kb cosmid [3]. Expression of the lysolipin biosynthetic gene cluster in the heterologous host *Streptomyces albus* resulted in similar or even higher amounts of the antibiotic compared to *S. tendae* Tü 4042 production levels.

We have identified five regulatory genes (*llpRI-V*) within the cluster which may be involved in transferring environmental signals to initiate lysolipin production. *In silico* analysis revealed similarities of these genes with already described regulatory genes: LlpRIV has high sequence similarity with *Streptomyces* antibiotic regulatory proteins (SARPs), which usually act as activators. LlpRII and LlpRIII are likely to be activators of the TenA family. LlpRI shows similarity to a putative PadR-type transcriptional repressor. The sequence of LlpRV resembles a putative DNA-binding protein of *Streptomyces coelicolor* A3 [3].

Heterologous expression of the regulator genes in *E. coli* and subsequent bandshift assays with possible promoter regions were used to characterize the regulators' binding properties. In parallel deletion mutants will give additional hints on the role of these putative regulators in the biosynthesis of lysolipin.

Keywords: Regulation, Polyketide antibiotic, Lysolipin

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SRNA SCR5239 CONTROLS THE EXPRESSION OF THE METHIONINE SYNTHASE METE

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We are interested in the identification and characterization of small non-coding RNAs (sRNAs) in *Streptomyces coelicolor*. To find such transcripts we performed deep sequencing of the *S. coelicolor* transcriptome during a complete developmental cycle on solid media.

One sRNA we identified this way – a 159 nt transcript called scr5239 – especially attracted our interest because of its high degree of sequence and structure conservation. It is constitutively expressed under most conditions tested and its 2D structure – as validated by enzymatic probing – consists of five stem-loops. Stem-loop IV has to melt open before an interaction with the target-mRNA can take place.

Overexpression or deletion of scr5239 results in distinct macroscopic phenotypes. The scr5239 overproduction strain does not express the agarase DagA and therefore cannot use agar as a carbon source [1]. DagA, however, is not the only target of scr5239. We identified a second target, the methionine syntase *metE*. The Expression of scr5239 is induced by high methionine levels and deletion of the sRNA leads to an increase in MetE expression. We can see a direct interaction of the sRNA with the *metE* mRNA leading to translational repression of the *metE* mRNA. According to our current model scr5239, induced by the presence of methionine, represses *metE* translation in a feedback loop.

Currently, we are characterizing the biochemical and genetic features of this interaction.

Keywords:sRNA, Methionine, Riboswitch

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DESIGNING TOOLS TO MANIPULATE ANTIBIOTIC PRODUCTION: REWIRING OF *STREPTOMYCES* SPP. TWO COMPONENT SYSTEMS VANRS AND AFSQ1/Q2

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Every moment bacteria are bombarded by external signals and it is crucial that bacteria are able to recognise and differentiate these and mount the appropriate response. The most important of these signal transduction mechanisms are two component systems (TCS), made up of a histidine sensor kinase (HK) and a cognate response regulator (RR). This interaction is highly specific between partners in order to avoid deleterious cross talk. The interface formed between the Dhp domain of the HK and the catalytic domain of the RR results in phosphotransfer from an SK His to an RR Asp residue. The Dhp domain determines the specificity between cognate HK and RR and recent studies have shown that as little as four residue changes within the Dhp domain is sufficient to rewire HK specificity. We predict that rewiring of TCS can be used as a synthetic biology tool to manipulate antibiotic production in *Streptomyces* bacteria. Here, the vancomycin sensing HK VanS has been rewired to activate the non cognate RR: AfsQ1, which regulates primary and secondary metabolism. This should result in the vancomycin-inducible production of the pigmented antibiotics actinorhodin (blue), undecylprodigiosin (red) and yCPK (yellow). Here, we report on the construction and expression of three different VanS chimeras in *S. coelicolor* and *S. lividans* and their effects on pigmented antibiotic production in these strains.

Keywords: Two component systems; Signalling transduction rewiring; Antibiotic production

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STUDY OF THE INFLUENCE OF A SARP REGULATOR CPKN ON CPK SYNTHASE GENES EXPRESSION IN *STREPTOMYCESCOELICOLOR*A3(2)

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Antibiotics and other bioactive compounds production by streptomycetes coincides with morphological differentiation. *Streptomyces coelicolor*A3(2) is a model organism for investigation of the regulatory network which coordinates these processes. One of the newest examined secondary metabolites of *S. coelicolor A3(2)* is coelimycin (CPK) – yellow polyketide synthesized by a type I modular polyketide synthase, which is coded by *cpk* gene cluster. Expression of *cpk* genes is tightly controlled by a butanolide signalling system. The *cpk* gene cluster contains five regulatory genes. One of them, *cpkN*, codes a protein which is homologous to SARPs (*Streptomyces Antibiotic Regulator Proteins*). The aim of the study was to investigate the role of CpkN protein in the control of coelimycin production and expression of *cpk* polyketide synthase gene cluster. The work involved construction of *S. coelicolor* mutant strain with an additional copy of *cpkN* gene in the attachment site on the chromosome and a deletion mutant with the gene replaced by the kanamycin resistance cassette. The phenotype of strains with changed level of CpkN protein was strongly medium dependent. CpkN protein plays an important role in control of coelimycin production on minimal medium, but it is not necessary on rich media. Results of the qRT-PCR experiment suggest indirect effects and involvement of additional factors in the regulation. Deletion of *cpkN* decreased *cpkC* (one of the main subunits of polyketide synthase) expression. Surprisingly, overproduction of CpkN did not stimulate transcription of the structural gene. We conclude that it is a non-essential activator of the polyketide synthase Cpk. We also found that its transcription is subject to feedback control.

Keywords: Coelimycin, *Streptomyces coelicolor*, Regulation

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FUNCTIONAL ANALYSIS OF THE RESPONSE REGULATOR TCRA INVOLVED IN THE FLAGELLAR SYNTHESIS AND SPORE DORMANCY IN *ACTINOPLANES MISSOURIENSIS*

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The rare actinomycete *Actinoplanes missouriensis* is characterized by its ability to produce spores within a terminal sporangium. The spores are released from sporangia upon contact with water and an unknown compound(s) through the process termed dehiscence. The released spores possess flagella and swim at very high speed. Although microscopic observations of mycelia and sporangia have been performed, little is known about molecular mechanisms of the morphological development. The purpose of this study is to analyze the function of TcrA (two-component system response regulator), a global regulator in the flagellar synthesis and spore dormancy in sporangia.

The complete genome sequence of *A. missouriensis* NBRC102363^T was determined in 2012 and the flagellar gene cluster was found next to one of the three chemotaxis gene clusters. *tcrA* locates at the end of this chemotaxis-flagella gene cluster. Because TcrA is the sole putative transcriptional regulator encoded by the gene cluster, TcrA was assumed to be a regulator of flagellar synthesis and *tcrA* was disrupted to analyze its function. First, to examine whether TcrA regulates flagellar genes, transcription levels of them were compared between the wild-type and Δ*tcrA* strains by qRT-PCR. As a result, transcription levels of all flagellar genes in theΔ*tcrA* strain were much lower than those in the wild-type strain. TcrA bound to four of the ten promoter regions in the flagellar gene cluster as determined by the gel mobility shift assay. In addition, the DNA-binding activity of TcrA was increased when the recombinant TcrA protein was phosphorylated with acetyl phosphate *in vitro*. These results indicate that TcrA is activated by phosphorylation, similarly to typical two-component system response regulators, and that it directly activates at least four transcriptional units in the flagellar gene cluster. The TcrA-binding consensus sequence, two 11-bp direct repeats, was also identified.

Interestingly, the Δ*tcrA* mutant formed apparently normal sporangia, but they could not dehisce and release spores under appropriate conditions. Transmission electron microscopic observation revealed that the spores inappropriately germinated in dormant sporangia in the Δ*tcrA* mutant. These results suggest that TcrA is a global regulator in the morphological development in *A. missouriensis*; TcrA seems to regulate spore dormancy, as well as spore maturation (including flagellar synthesis), in sporangia. To investigate TcrA-regulated genes comprehensively, the RNA sequencing analysis was performed using RNAs extracted from sporangia of the wild-type and Δ*tcrA* strains. As a result, 26 and 261 genes were significantly up- and down-regulated in the Δ*tcrA* mutant. This result was consistent with our hypothesis that TcrA is a global transcriptional activator. The functional analyses of several TcrA-dependent genes are in progress.

Keywords: Rare actinomycete, Response regulator, Morphological development

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NITRIC OXIDE - A NEW SPORULATION SIGNAL IN *STREPTOMYCETES*?

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Streptomyces species are filamentous multicellular bacteria with a complex life cycle that play an important role in the turnover of complex, insoluble organic materials in the soil. They must rapidly sense and respond to environmental stresses, including nitric oxide (NO), a lethal and highly diffusible gas produced by denitrifying microbes. *S. coelicolor*, and many other bacteria, use a transcription factor called NsrR to sense and respond to NO by switching on expression of the NO dioxygenase HmpA. Additionally, the NsrR regulon was predicted to be large and extensive with over 300 regulated genes including antibiotic synthesis and developmental regulators. We have shown that NO delays sporulation (cell division) in *Streptomyces* and may therefore play a signalling role during differentiation. To further investigate the role of NsrR in Streptomyces spp. we determined the NsrR regulon for both *S. coelicolor* and *S. venezuelae* utilising ChIP-seq. Additionally, to determine how NO inhibits *S. coelicolor* sporulation specifically, we are measuring the transcriptome in *S. coelicolor* and *S. venezuelae* carrying out RNA-seq in the presence and absence of NO. To facilitate ChIP-seq analysis, disruption mutants of *nsrR* and complementing 3xFlag tagged NsrR constructs were produced for each species and ChIP-seq was carried out using these strains. The *S. coelicolor* analysis indicated the NsrR regulon is far smaller than predicted, only containing 3 rather than 300 target genes. The ChIP of *S. venezuelae* NsrR and the RNA-seq results of NO treated *Streptomyces* are pending. The targets in *S. coelicolor* were confirmed by EMSA and DNaseI foot printing results are also pending. Single, double and triple mutants of the three *S. coelicolor* genes in the NsrR regulon have been produced and we predict that the strain will suffer growth defects when grown in the presence of various nitrogen sources. Results from the ChIP-seq analysis of *S. venezuelae* will determine whether the NsrR regulons is conserved between streptomycetes.

Keywords: Nitric oxide, NsrR, *Streptomyces* chip-seq, RNA-seq

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THE NON-CODING REGULATORY RNA LANDSCAPE IN *MYCOBACTERIA*

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Non-coding small RNAs serve as key regulatory molecules in bacteria that, through base-pairing with their mRNA targets or modifying protein activity, control many physiological processes within a cell. Despite playing this pivotal role in regulating gene expression their mechanisms and functions are still poorly understood and in mycobacteria they remain almost completely uncharacterized.

With the focus on mycobacterial regulatory networks we set to identify small RNAs and determine their function in the fast-growing *Mycobacterium abscessus*, which has emerged as a significant health concern in developed countries. In individuals suffering from chronic lung disease, like cystic fibrosis patients, this pathogen leads to respiratory infection resembling that caused by *Mycobacterium tuberculosis*. Its high intrinsic and acquired antibiotic resistance considerably limits therapeutic options and, therefore, necessitates deeper understanding of regulatory pathways, especially those involved in virulence and host-pathogen interactions.

Our previous research pinpointed a subset of highly conserved small ncRNAs in a few mycobacterial species. They included, for example, regulatory transcript with the 6C motif, as well as the structural tmRNA element. Both of these sRNA had been also identified in other Actinomycetes tapping into evolutionary aspects of regulatory networks in this group of microorganisms. This study focused on the transcriptional landscape of *M. abscessus* generated by combining transcription start sites mapping with transcription profiling via RNAseq. We searched for orphaned promoter and terminator sequences that can be used as indicators for detection of putative small ncRNAs. A number of transcripts overlapping non-annotated regions of the genome, many located in the intergenic spaces or antisense to annotated genes was identified. These elements were characterized by their small size, from a few to a few hundred nucleotides in length, and complex secondary structure. Although the majority of these sRNAs seem to be species-specific, we identified a group that presented high sequence similarities to other non-coding RNA elements and that were located within a conserved genetic context. These molecules seem to constitute a mycobacterial core set of ncRNAs.

Our current efforts are directed towards the development of a CRISPR-based knock-down system that would help in the recognition of molecular targets of these conserved ncRNAs shedding light on their specific functions, as well as on the pathways and global regulatory networks in which they are involved. We plan to investigate their role in stress response, as well as in the human macrophage infection model using transcriptomic and proteomic approaches.

Keywords: Mycobacteria, sRNA, Transcription regulation, Gene expression, Pathogen

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LIMR, A MARR-FAMILY TRANSCRIPTIONAL REGULATOR OF CORYNEBACTERIUM GLUTAMICUM: ITS ROLE AND FUNCTION IN THE LIGHT-INDUCIBLE CAROTENOID PRODUCTION

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Carotenoid production in some non-phototropic bacteria occurs in a light-dependent manner. We previously identified LitR, a coenzyme-B12-bound MerR family, to be the regulator responsible for the light-induced transcription of carotenoid biosynthesis gene cluster in *Streptomyces coelicolor*A3(2). The wide distribution of LitR homolog suggests the general occurrence of photoresponse in non-phototropic bacteria.[1,2] Meanwhile, to date, LitR is the only type of transcriptional regulator known for the involvement in the light-dependent carotenoid production in non-phototrophic bacteria. Here, we report identification of a MarR-family regulator to be involved in the light-inducible carotenoid production of *Corynebacteriumglutamicum*, an amino acid producing gram-positive bacterium.

Our screen for carotenoid producers demonstrated that *Corynebacterium* and related bacteria perform photo-dependent carotenoid production. *C. glutamicum* also exhibited photo-dependence, but its genome did not contain any*litR* homolog. Hence, we studied the MarR-family transcriptional regulator, LimR (light-inducible MarR-family regulator), encoded at the flanking region of carotenoid biosynthesis gene cluster (*crt*). Generally, this family serves as a negative regulator for the transcription of the neighboring region. A null mutant for *limR* performed constitutive carotenoid production irrespectively of illumination, and the photo-dependence was restored by genetic complementation. Transcriptional analysis revealed that the transcription of *crt* is light-dependently regulated in the wild type, while it occurred constitutively in the *limR* mutant. A LimR recombinant protein expressed in *E. coli* host specifically bound to the intergenic region between *limR* and *crt*. Overall, the results indicate that LimR regulates the transcription of *crt* in a light-dependent manner in *C.glutamicum*. The conserved gene organization consisting of *crt* and *limR* is distributed in bacteria closely related to *C. glutamicum*. Probabaly, LimR is involved in the light-inducible gene expression in this group of Actinomycetales.

Keywords: LimR, MarR-family protein, Photosensor

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MULTIPLE ROLES OF NUTRIENT-SENSING GLOBAL REGULATORS INVOLVED IN NITROGEN AND CARBON METABOLISM IN *SACCHAROPOLYSPORA ERYTHRAEA*

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GlnR protein, as a central regulator of nitrogen metabolism, is widely explored; however, until now there have been no data for *Saccharopolyspora erythraea*. The biosynthesis of erythromycin is highly regulated by type and concentration of the nitrogen source. The choice of nitrogen source and its concentration have been shown to have a great influence on the erythromycin production. However, not much is known on the actual mechanisms behind the observed effects. We applied bioinformatics and experimental approaches to identify the GlnR-regulon of *S.erythraea*. Among the 30 GlnR-controlled transcriptional units, 20 of them are directly related to nitrogen metabolism. Some target genes are involved in carbon metabolism, including *gltA-2, acsA, csbX, malR,* and *xylR*. GlnR reveals the regulatory effect on tricarboxylic acid cycle by transcriptionally controlling *acsA* and *gltA-2* genes. Meanwhile GlnR exerts direct regulatory effect on utilization of maltose and xylose by activating the transcription of *malR* and *xylR*. It is also found that PhoP could exert the control on the nitrogen metabolism through its binding to the promoter of *glnR* gene and other nitrogen-related genes. We in silico also searched and experimentally identified the DasR-regulon in *S.erythraea*. 33 binding-sites were found corresponding to 45 transcriptional units, including genes involved in the N-acetylglucosamine and chitin metabolism, antibiotics biosynthesis, transporter systems, and regulators. The DNaseI footprinting-assay and kinetic assay results suggested a competition between DasR and GlnR to the promoter region of *glnA-2*, thus revealed a cross-regulation of these two regulators to the citrate cycle. We also found that the genes in *pks3* cluster were also subject to direct regulation by DasR. These findings provided the bases for uncovering the molecular mechanisms of nutrient sensing, transport and metabolism, and understanding the mechanisms underlying cross-talk between regulatory systems involved in the global coordination of primary metabolism and secondary metabolism.

Keywords: Global regulator, Nitrogen metabolism, Carbon metabolism, Transcriptional regulation

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PATHWAY TO VIRULENCE IN THE PLANT PATHOGEN *STREPTOMYCES SCABIES*

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Scab lesions on economically-important root and tuber crops are mainly caused by plant pathogenic streptomycetes which evolved from a purely saprophytic life style by acquiring specific pathogenic traits while retaining their saprophytic capacity. Production of the phytotoxin thaxtomin A is the main virulence determinant and required for scab disease development. It is the predominant form of a family of nitrated dipeptides formed by non-ribosomal peptide synthases and primarily targets the cellulose synthase complex that is active dividing and expanding plant cell tissue. Although genome sequencing and mining efforts have been able to propose an almost complete map of genetic loci associated with plant virulence shared by *S. scabies,S. acidiscabies* and *S. turgidiscabies*, the molecular mechanisms by which these bacteria integrate environmental signals and interpret them into a decision to trigger the pathogenicity machinery are still poorly understood. We present here how the computational prediction of regulatory networks has been able to highlight the first complete signaling pathway from plant material sensing and transport to induction of virulence in *Streptomyces* pathogens. Our work highlights a remarkable example of how the specific distribution of *cis*-acting elements into loci associated with pathogenicity is sufficient to evolve a *trans*-acting factor which in non-pathogenic species is strictly connected to primary metabolism functions, into a master switch which controls the access to virulence in the pathogenic counterparts.

Keywords:*Streptomyces scabies*, Thaxtomin

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THE TWO-COMPONENT REGULATORY SYSTEM OSDKR CONTROLS OXIDATIVE STRESS AND DEVELOPMENT IN *STREPTOMYCES COELICOLOR* AND OVERLAPS THE DORMANCY CONTROL SYSTEM DOSRS OF *MYCOBACTERIUM TUBERCULOSIS*

Mia Urem, Teunke van Rossum, Giselda Bucca, Joost Willemse, Geri Molenaar, Emma Laing, Magda A. Swiatek, Sébastien Rigali, Nora Goosen, Colin P. Smithand Gilles P. van Wezel

Leiden University

The mycelial soil-dwelling bacterium *Streptomyces coelicolor* requires mechanisms for rapid responses to changes in its environment. One prevalent control system is the one controlled by the two-component signal transduction system (TCS) consisting of the sensory kinase (SK) OsdK and the response regulator (RR) OsdR. Microarray analysis showed that deletion of *osdR* results in the deregulation of many genes involved in development and oxidative stress management. Important developmental genes that were deregulated in the *osdR* null mutant included the genes for SsgB, SsgG, DivIVA, SapB, Chp and Rdl spore-coat proteins, and several Bld regulatory proteins. Genes involved in oxidative stress management that were deregulated included *katA*, *trxA*, *trxB*, *sigR/rsrA,* proteasome-related genes, *clpP*, *hspR*, *dnaK*, *groEL1*, *groEL2*, *groES1* and *uspA*, as well as many cold-shock genes.The nameOsdKR was chosen so as to reflect the functional relationship to oxidative stress and development. OsdR is an autoregulatory protein, and analysis of the S. coelicolor genome using the PREDetector algorithm with the regulatory element upstream of *osdR* as input, identified around 40 putative binding sites for OsdR, many of which were confirmed as true targets by EMSA *in vitro*. Direct targets for OsdR include SCO0200 for the universal stress protein UspA, SCO2967 which encodes a protease related to the tmRNA system that rescues stalled ribosomes, the cell division gene *ssgB* and *whiE* for the polyketide synthase that produces the grey spore pigment.

Interestingly, the OsdR binding site is very similar to the one found for the dormancy regulator OsdR in *Mycobacterium tuberculosis*. OsdR is part of the TCS DosSR, which controls dormancy and thus plays a major role in host defence escape of this pathogenic bacterium. Indeed, purified OsdR binds to the regulatory elements found in promoter regions of known DosR target genes *in vitro*. The functional role of OsdKR in the life cycle of *Streptomyces*, and the overlap with the DosRS system of mycobacteria is discussed.

Keywords:*Streptomyces*, Oxidative stress, Dormancy, TCS, Development, OsdKR

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IDENTIFICATION OF PROTEINS BINDING TO PROMOTER REGIONS OF THE NOVOBIOCIN BIOSYNTHETIC GENE CLUSTER IN THE HETEROLOGOUS PRODUCER STRAIN *STREPTOMYCESCOELICOLOR* M512

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Heterologous expression of biosynthetic gene clusters in Streptomycetes has become a common and widely used method. However, often production rates of the secondary metabolites after heterologous expression stay low. To overcome this problem the understanding of pathway specific regulation as well as of global regulatory networks is of great importance.

Pathway specific regulation of the heterologously expressed novobiocin biosynthetic gene cluster in *Streptomyces coelicolor* M512 is well studied and two pathway specific regulatory genes, *novE* and *novG* have been described by our group[1].

In order to identify further transcriptional regulators, which directly influence the heterologous expression of the novobiocin biosynthetic gene cluster in the model strain *Streptomyces coelicolor* M512 we performed DNA affinity capture assays (DACA)[2]. For this we used cell extracts of *Streptomyces coelicolor* M512 containing the novobiocin biosynthetic gene cluster and tested them on each of the three main promoter regions of the novobiocin biosynthetic gene cluster: P*novE* and P*novG*, which control transcription of the two regulatory genes as well as P*novH*, controlling the transcription of all biosynthetic genes in one big operon. As unspecific control we included the promoter region of the main vegetative sigma factor *hrdB*. Captured proteins were analysed and quantified via label free mass spectrometry.

Due to this very sensitive method we could identify and quantify a total of more than 2000 proteins binding to one or more of the tested promoter regions, most of them being detected only in very low quantities, probably because of unspecific interactions between positively charged amino acids and negatively charged DNA. Amongst those which were detected in higher quantities were some typical DNA binding proteins, as e.g. DNA gyrase, helicase, topoisomerase I, nucleases, DNA polymerases, but also a number of known or putative transcriptional regulators. One of them was as expected the pathway specific regulator of novobiocin biosynthesis NovG. Whereas binding of NovG was only detectable to P*novH*, where its binding site is located, other well-known global regulators as AdpA, NdgR, AbsC were found binding consistently high to all four tested promoter regions. For detailed investigations we selected four putative regulatory proteins which like NovG bound specifically to only one of the promoter regions of the novobiocin biosynthetic gene cluster.

Keywords: Regulation, *Steptomyces coelicolor,* Novobiocin, DNA affinity, Capturing heterologous expression

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A "MUTANT-TYPE" RNA POLYMERASE CONTROLS ONSET OF SECONDARY METABOLISM IN *NONOMURAEA* SP. ATCC 39727

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The genome of *Nonomuraea* sp. ATCC 39727, the producer of the teicoplanin-like glycopeptide A40926 with anti-*Neisseria* activity and precursor of dalbavancin, is the largest among known actinomycete genomes up to date. The size reflects both chromosome expansion mainly caused by gene duplication also affecting many essential loci and a plenty of "contingency" loci including those for secondary metabolites. Duplication of *rpoB* locus represents an interesting example to study since *rpoB(R)* allele, which encodes a mutated "stringent" form of RNA polymerase? chain mimicking (p)ppGpp binding, is involved in regulation of biochemical and morphological differentiation. In this study the possible link between *rpoB(R)* expression and environmental conditions was examined. Results demonstrated that alkaline environments (pH values >9.5) stimulated A40926 biosynthesis, pigmentation and sporulation in wild type strain but not in a strain harboring a mutated *rpoB(R)* gene (REV). Molecular analyses indicated that *rpoB(R)* was highly expressed at high pH values along with the regulatory gene *bldA* and the StrR-like regulator-encoding gene *dbv4*. In contrast, *bldA* and *dbv4* were not activated at high pH values in the strain REV suggesting epistatic control of *rpoB(R)* on both genes. *RpoB(R)* promoter region analysis revealed that this gene is finely regulated both at transcriptional and at post-transcriptional level in response to the growth phase and to environmental pH.

Keywords:*rpoB*(R); A40926; *Nonomuraea* sp. ATCC 39727

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GENE EXPRESSION CONTROL OVER GERMINATION

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Bacterial spore germination is a developmental process during which all required metabolic pathways are restored to transfer cells from their dormant state into vegetative growth. Here, we present a systematic approach that analyzes gene expression data obtained from 13 time points taken over 5.5 h of Streptomyces germination. Streptomyces are soil dwelling filamentous bacteria with complex life cycle, studied mostly for they ability to synthesize secondary metabolites including antibiotics. The study examined significant gene expression differences between consecutive time points. Genes whose expression was significantly enhanced/diminished during the time-course were identified, and their classification into appropriate metabolic pathways revealed timing of the activation of specific pathways during the course of germination. The benefits of the presented method were also tested on the expression analysis of the key protein regulators - sigma factors over the course of germination. Among the factors whose expression was enhanced during the initial part of germination, SigE is suggested to manage cell wall reconstruction, SigR controls protein re-aggregation, and others (SigH, SigB, SigI, SigJ) control osmotic and oxidative stress responses. From the results, we conclude that conditions favoring germination evoke stress-like cell responses.

Keywords: Germination, *Streptomyces*, Microarrays, Sigma factors, Metabolic pathways

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STEPWISE IMPROVEMENT OF PRISTINAMYCIN II BIOSYNTHESIS IN *STREPTOMYCES PRISTINAESPIRALIS* BY COMBINATORIAL METABOLIC ENGINEERING

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Pristinamycin is a streptogramin antibiotic produced by *Streptomyces pristinaespiralis*, which contains two chemically unrelated compounds, pristinamycin I (PI) and pristinamycin II (PII). The semi-synthetic derivatives of PI and PII have been approved in human medicine against a broad range of drug-resistant pathogens. Here, we report a combinatorial metabolic engineering strategy to improve PII biosynthesis. Firstly, an extra copy of PII biosynthetic cluster, which was assembled using an improved Gibson assembly method for cloning large DNA fragments with high GC content, was introduced into the high-producing parental strain *S. pristinaespiralis* HCCB10218, resulting in a maximal increase in PII biosynthesis by 45%. To further optimize PII production, all of the regulatory genes (including *papR1-R6* and *spbR*) situated in the pristinamycin biosynthetic cluster were systematically manipulated. Higher PII titer was achieved by deletion of either of two repressor genes *papR3* and *papR5* combined with overexpression of both activator genes *papR4* and *papR6*. Compared with the parental strain, the resulting mutants Δ*papR3+R4R6* and Δ*papR5+R4R6* showed the maximum 99% and 75% increase in PII production respectively. Finally, the combination of these two different metabolic engineering approaches was employed. We found that integration of the assembled PII gene cluster into the Δ*papR5+R4R6* mutant led to the highest titer improvement, which is about 150% higher than the parental strain. Taken together, the strategy used in this study is an efficient method to improve PII titer in *S. pristinaespiralis* and may also be extended to other industrial *Streptomyces* for strain improvement.

Keywords:*Streptomyces pristinaespiralis*, Pristinamycin, Metabolic engineering, Gibson assembly, Cluster-situated regulator

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THE EFFECTS OF MULTIPLE COPIES OF POLYPHOSPHATE KINASE AND EXOPOLYPHOSPHATASE GENES ON ANTIBIOTIC PRODUCTION BY *STREPTOMYCES COELICOLOR*

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Polyphosphate (polyP), which has very important functions in the cell, is synthesized by polyP kinase (PPK) using the terminal phosphate of ATP as the substrate and degraded to inorganic phosphate by endopolyphosphatase (PPN) and exopolyphosphatase (PPX) enzymes. In this study, we determined the effect of overexpression of *ppk* gene alone and together with *ppx* gene on antibiotic production by *Streptomyces coelicolor*. The results showed that the inhibition of antibiotic production by *ppk* gene is reversed by expressing both *ppk* and *ppx* genes together. Overexpression of these genes not only affected antibiotic production but also resistance of cells to different stress factors.

Keywords: Polyphosphate kinase, Exopolyphosphatase, Antibiotic production, *Streptomyces coelicolor*

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INORGANIC PHOSPHATE IS A TRIGGER FACTOR FOR *MICROBISPORA* SP. ATCC-PTA-5024 GROWTH AND NAI-107 PRODUCTION

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NAI-107, produced by the actinomycete *Microbispora sp.* ATCC-PTA-5024, is a promising lantibiotic active against Gram-positive bacteria and currently in late preclinical-phase [1]. Lantibiotics (lanthionine containing antibiotics) are ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by Gram-positive bacteria belonging to the Firmicutes and Actinobacteria phyla. The biosynthesis of biologically active compounds is developmentally controlled and it depends upon a variety of environmental stimuli and conditions. Inorganic phosphate (Pi) usually negatively regulates biologically active molecule production in Actinomycetes [2], while it has been reported to have a positive control on lantibiotic production in Firmicutes strains [3]. So far, no information is available concerning the Pi effect on lantibiotic biosynthesis in Actinomycetes.

The objective of this study was to investigate the effect of Pi on *Microbispora sp.* ATCC-PTA-5024 growth and NAI-107 production at biochemical and molecular genetic levels.

Starting from the mineral composition of Maltose-Glutamate (MG) medium, already used for other actinomycetes, we changed carbon and nitrogen sources and evaluated *Microbispora* growth and NAI-107 biosynthesis. A suitable defined medium, named NG-20, containing 25 mM ammonium Nitrate as nitrogen source and 20 g/l Glucose as carbon source, was developed. *Microbispora* growth and NAI-107 production were monitored in NG20 medium by varying inorganic phosphate concentrations: 0, 0.1, 0.5, 5, 15 and 30 mM (labelled P0, P0.1, P0.5, P5, P15 and P30). Pi-limiting conditions were established and confirmed by quantitative analysis of polyphosphate accumulation and of expression of selected Pho regulon genes, involved in the Pi-limitation stress response.

Our analyses revealed that phosphate positively influences both growth and NAI-107 production up to a concentration of 5 mM. Higher Pi concentrations were not found to further stimulate *Microbispora* growth and NAI-107 production. Our results are in accordance with several reports concerning the positive effect of Pi on the biosynthesis of other ribosomal post-translationally peptides in Firmicutes (i.e. nisin, micrococcin GO5, Pep5, epidermin, gallidermin).

This study, on one hand, expands our knowledge on *Microbispora* physiology, and, on the other one, could be helpful to develop a robust and economically feasible production process of NAI-107 as a drug for human use.

Keywords: Ribosomal post-translationally modified peptides (RiPPs), Phosphate, Polyphosphate

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SYMBIOSIS AND PATHOGENESIS

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CO-INOCULATION OF *STREPTOMYCES* AND *BACILLUS* ENHANCED *IN VITRO* AUXIN PRODUCTION AND GROWTH OF *TRITICUM AESTIVUM* L.

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Introduction: A variety of rhizobacteria including *Streptomyces* and *Bacillus* species are commonly reside in the rhizosphere. Auxin producing plant growth promoting rhizobacteria (PGPR) are known to beneficial and have stimulatory effect on plants. Rapid colonization of the rhizosphere and promotion of plant growth is currently considerable interest in exploiting PGPR.

Objectives: The main aim of the present study was to evaluate the potential of auxin producing *Streptomyces* and *Bacillus* strains to enhance the growth of wheat (*Triticum aestivum* L.) in co-inoculation experiments.

Methods:*Streptomyces* sp. RSF, *S. macrosporeus* SCF, *S. griseoincarnatus* CTF, *Bacillus cereus* McR-3 and *B. subtilis* McR-7 were screened for auxin production in the presence of 0, 100, 300 and 500 µg ml^{-l} L-tryptophan by using colorimetric method. Presence of auxin in bacterial culture supernatants was also confirmed by high performance liquid chromatography (HPLC). In pot trials, single and mixed cultures of *Streptomyces* and *Bacillus* were used to evaluate the phytostimulatory effect of bacterial strains on the growth of *T. aestivum* under axenic conditions.

Results: For auxin production, *B. cereus* McR-3 and *B. subtilis* McR-7 recorded highest levels of 28 and 30 µg ml^{-l}, respectively, in L-tryptophan amended medium. HPLC also confirmed the presence of auxin in bacterial culture supernatants. In pot trials, rhizobacteria showed variable growth responses in single and mixed culture inoculations. In single cultures, significant increase for root length (48%), shoot length (42%) and shoot fresh weight (31%) were observed with *Streptomyces* sp. RSF and *S. macrosporeus* SCF. For mixed cultures, McR-3+SCF and McR-7+SCF were the most effective to enhance shoot length (35%) and shoot fresh weight (20%), respectively, over water treated control.

Conclusion: Overall, single cultures and a few combinations of mixed cultures gave promising results to enhance the growth of *T. aestivum*. Hence, rhizobacteria with auxin production can be used to inoculate agronomically important crops.

Keywords:*Streptomyces*, Auxin, Plant growth promotion

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SOIL MICROBES- TISSUE INTERACTION: A THEORY FROM *STREPTOMYCES* SPECIES

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Background: Members of the genus *Streptomyces* are generally considered non-pathogenic, but few species are causal agents of diseases in man *(S. somaliensis and S. sudanesnsis);* animals (*Streptomyces* species) and plants *(Streptomyces scabies)*. The mechanism of how these saprophytes cause such diseases is not fully understood.

Objectives: To investigate the role of saprophytic soil *Streptomyces* (Actinobacteria) in causing actinomycetoma in man and animals and whether different species instigate consistently the same disease.

Methods: The present study analyzed previously published data that involved isolation of *Streptomyces* from cases of actinomycetoma in human (madura foot) and donkeys (fistulous withers).These data included phenotypic profile, cell wall chemotaxonomic markers, antimicrobial susceptibility tests and 16S rDNA gene sequencing. Data were used to establish a hypothesis that these strains were dissimilar but when get into tissue (subcutaneously) they cause a similar disease (mycetoma).

Results:*Streptomyces* (n = 19) isolated from actinomycetoma cases showed aerobic growth, were gram-positive, non-acid-alcohol fast, non-motile and have typical actinomycete morphology. They form extensively branched substrate mycelia and sometimes aerial mycelia on standard media. Cell wall analysis yielded LL-diaminopimelic acid (LL-DAP) which approved that these isolates are members of the genus *Streptomyces*. The isolates, though they are *Streptomyces*, but exhibit variations in phenotypes and susceptibilities to antibiotics; moreover, preliminary 16S rDNA sequence analyses suggest their placement in new species ranks.

Conclusions: The study was a limited one, but clearly indicated that diverse soil saprophytic *Streptomyces* spp. when inserted in tissue possess mechanisms to cause mycetoma. These mechanisms, inoculum sizes and the success rates of causing the disease upon entrance are not known.

Keywords: Soil, Tissue, Saprophytes, *Streptomyces*, Actinomycetoma, Sudan

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THE USE OF MORPHOLOGICAL AND CELL WALL CHEMICAL MARKERS IN THE IDENTIFICATION OF *STREPTOMYCES* SPECIES ASSOCIATED WITH ACTINOMYCETOMA

Mohamed Hamid

King Khalid University

Background: Microbiologic diagnosis of actinomycetes such as the causal agents of actinomycetoma is often difficult and challenging. Diagnosis and laboratory identification commonly require combined phenotypic and genotypic testing but the search for simple and cost-effective methods are always needed especially in low equiped settings.

Objectives: To study the utility of few phenotypic characteristics along with some cell wall chemical markers in identifying agents of actinomycetoma cases in man and animal.

Methods:*Streptomyces* strains (n = 19), presumptively identified as *S. somaliensis*, were isolated from purulent materials of patients with mycetoma (human) or fistulous withers (donkeys) and studied for phenotypic and chemotaxonomic characteristics following standard methods.

Results: Isolates were tentatively identified as *Streptomyces* species based on morphological and cultural characteristics and were easily differentiated from other actinomycetes and from agents of eumycetoma. Isolates were found variable in their macroscopic and microscopic features and in their sensitivities to antimicrobial agents. Cell wall analysis of isolates yielded LL-diaminopimelic acid (LL-DAP) which authenticates that the isolates are members of genus *Streptomyces*.

Conclusions: The application of few morphological and chemical markers were found useful is assigning unknown mycetoma agent to the genus level namely *Streptomyces*. Although these streptomycetes cause similar lesions but were found distinctive phenotypically. Such variations make is necessary to perform routine *in vitro* antibiotic testing for the sake of patient health and it would be necessary to fully describe such isolates.

Keywords: Actinomycetoma, *Streptomyces* species, Phenotypes, LL-DAP, Madura foot, Sudan

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2D-INFRARED SPECTROSCOPY: A NOVEL METHOD TO STUDY THE ESSENTIAL ENOYL-REDUCTASE (INHA) FROM *MYCOBACTERIUM TUBERCULOSIS*

Paul Hoskisson

University of Strathclyde

InhA is an NADH-dependent enoyl reductase responsible for the biosynthesis of mycolic acids; an essential component of *Mycobacterium tuberculosis* (*Mtb*) cell walls. InhA is also the main target for the frontline anti-tubercular therapeutic Isoniazid. Although the inhibition of the wildtype (WT) InhA active site (hydrophobic pocket) is known to occur through oxidised isoniazid covalently binding to NADH to form an adduct, there is still much ambiguity about which residues within the hydrophobic pocket contribute to the mechanism of drug inhibition and why the clinically important S94A point-mutation confers isoniazid resistance. To this purpose, we employed site-directed mutagenesis (SDM) to generate a series of InhA single-point mutations (S94A, W222A, P193A, M155A, F149A) which, through time-dependent UV-Vis monitoring of NADH depletion, allows us to directly and unambiguously compare the dependence of the InhA activity and inhibition kinetics on to each specific residue.We found that the W222A and wildtype kinetics are almost identical, indicating that the Tryptophan residue plays no role in either enzyme activity or drug inhibition. Conversely, the P193A mutation results in a tenfold decrease in enzymatic activity highlighting its importance in hydrophobic pocket architecture. To fully link structure-function and dynamics, we have employed a novel spectroscopy method (2D-Infrared spectroscopy; 2DIR) that allows us to examine enzymatic function at the femto-second level. These observations may aid in developing new anti-tuberculosis treatments and understanding the dynamics of inhibition.

Keywords:*Mycobacterium*; Drug resistance; Structure-function-dynamics

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MINING THE HUMAN SKIN MICROBIOME FOR SMALL MOLECULE INTERACTORS BETWEEN COMMENSAL ACTINOBACTERIA AND *STAPHYLOCOCCUS AUREUS*

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The healthy microbiome of the human nostrils and most other skin sites is dominated by three genera: *Corynebacterium*, *Propionibacterium* (both Actinobacteria) and *Staphylococcus* (Firmicutes). While most of these bacteria (including *Corynebacterium sp.*, *Propionibacterium sp.* as well as *Staphylococcus epidermidis*) live as commensals that generally do not cause disease, *Staphylococcus aureus* is a medically important bacterium involved in hospital- and community-acquired infections. Nevertheless, *S. aureus* is found as an asymptomatic colonizer in the nasal cavity of at least a quarter of healthy people. Upon hospitalization however, its presence can act as a source of secondary infections. For example, in an estimated 80% of *S. aureus* bacteremia cases, the strain isolated from a patient's bloodstream is a match for one that was already present in the nostril. Eradication of colonizing *S. aureus* can thus be an important preventive measure to reduce the chance of subsequent infection.

A pathogen that tries to invade the human body encounters the skin and mucosal membranes as a first defense barrier, but also has to compete with members of relatively stable communities of commensal bacteria. We therefore explored this undermined niche of commensals that are already naturally involved in interspecies interactions for diffusible small molecules. We analyzed skin and nostril isolates using a combination of activity-guided fractionation, structural characterization and genetic analysis. This led to the identification of several small molecules produced by *Propionibacterium sp.* and *Corynebacterium sp.* that influence *S. aureus* in different ways; including growth inhibition or stimulation, but also aggregation and subsequent biofilm formation.

The identification of small molecule interactors produced by human skin commensals contributes to a better understanding of the complex interplay that is taking place in the communities from our microbiome. This will facilitate the development of therapeutic strategies to influence community structures with the aim to eliminate already established pathogens or inhibit their colonization prior to hospitalization.

Keywords:*Corynebacterium*, *Propionibacterium*, *Staphylococcus*, Skin microbiome, Small molecule natural products

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HYPERVIRULENT ROUGH MORPHOTYPE OF *MYCOBACTERIUM ABSCESSUS* MODULATES CYTOKINE AND CHEMOKINE PRODUCTION BY HUMAN MACROPHAGES

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Mycobacterium abscessus (Mab) is an emerging pathogen frequently encountered in a number of human infections, particularly it represents a serious threat in cystic fibrosis patients. Moreover the treatment is complicated by its high natural and acquired antibiotic resistance.

Mab is able to switch from a smooth morphotype (Mab-S) which is considered the wild-type, to a rough one (Mab-R), characterized by a reduction of glycopeptidolipids in the cell wall, which is associated with more severe and persistent human infections.

Most pathogenic mycobacteria are intracellular pathogens that reside and multiply within macrophages of their host. To create an environment that is favorable to their survival and proliferation, these pathogens have evolved sophisticated mechanisms to modify macrophage functional properties in particular they are able to manipulate the production of pro-inflammatory cytokines.

We challenged THP-1-derived-macrophages with Mab-R or Mab-S and we analysed the profile of secreted cytokines and chemokines, which are known to play a key role in the polarization and initiation of the specific T-cell response, in order to study the possible relationship between disease severity and the cytokine-inducing capacities of these strains.

Although IFN-g did not play a major role in the early *in vitro* response to both Mab-R and Mab-S, we observed that, compared to Mab-S, Mab-R induced significantly more TNF-a, a cytokine involved in macrophage activation and granuloma formation, suggesting that it may elicit a prominent immediate Th1 response. On the other side, the secretion of IL-1b, an important costimulator for maturation of Th17 cells, was reduced in Mab-R infected macrophages, suggesting that it may impair the balance between Th1 and Th17 cells.

Moreover, we reported that the production of a selected panel of chemokines (MIP-1a, MIP-1b, RANTES and IL-8) was significantly lower in Mab-R infected cells. Since these chemokines are known to recruit or activate T cells and monocytes at inflammatory *foci*, we speculate that the reduced ability of Mab-R to induce them might lead to defects in the acquired immune response.

In conclusion, the Mab-S to Mab-R switch seems to lead to modulation of the host innate immune response by altering the cytokine and chemokine production. These studies provide new insights to our knowledge of Mab virulence and suggest potential targets of therapy.

Keywords:*Mycobacterium abscessus*, Infection, Cytokines, Chemokines

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FRANKIA NODULATION STUDY OF *HIPPOPHAE SALICIFOLIA* IN RIVERINE AND NON-RIVERINE AREAS OF LACHEN VALLEY IN NORTH SIKKIM

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Introduction: Sea buckthorn belongs to family Eleagnaceae (2n=24) is an actinorizal angiosperm which colonizes in a new soil (Basistha *et.al*, 2009). Sea buckthorn (*Hippophae* L) has been shown to be superior to any other tree in afforestation, soil conservation, waste land reclamation (Zhou *et al*. 1990) especially on fragile slopes, due to its extensive root system. Akkermans, 1975 reported that the highest nodulation was found in 1 to 2 year old plants. Older plants are poorly nodulated, though they were frequently connected by stolons with nodulated young shoots. The nitrogen fixation effect of sea buckthorn root nodules varies with seasons, precipitation, age of plant and environmental factors. Changes occur even in one day, between morning and evening (Zhou *et al*., 1985).

Objective: Since nodulation of *H.salicifolia* appeared to be highly variable, the present study was undertaken to determine the nodulation frequency of Reverine and Non-Reverine areas of Lachen North Sikkim.

Methodology: Sea buckthorn study area is approximately 300 ha, located in Lachen Valley (North District of Sikkim), which lies between 27°47'32.1", and 27°43'57.2" North latitude and 88°33'07" and 88°33'13" East logititude. The study of nodulation was carried out in the trees aged between 3-6 years in riverine and non-riverine areas, thirty trees each from both the areas were selected for study. The area of 1.5 m around the tree and 6-8 inch below the soil surface was dug carefully and the root system was extracted and position of nodules was mapped. The root nodules numbers, weights, soil moisture, plant age, plant height, plant canopy and sizes of the root nodules were determined with an altitude ranging from 2540 to 3099m.The tree was then tagged by numbering it using aluminum plant tag and wire for further studies of the same trees in different seasons.

Result and Discussion: In *Hippophae salicifolia* maximum number of nodulation was observed in the taproot with some in lateral roots too. Healthy and maximum numbers of nodules were found at a depth range of 4-6 inches. The nodule on the top was matured ones and dried as compared with inner nodules. It is observed that nodules found at the sandy soil are larger in size (clumped) and less in number as compared with nodules found in humus soil, which nodules are scattered, having more in number but small in size. Total number of nodules varied from 4 to 107 in different trees. ANOVA of riverine and non-riverine areas of number of root nodules and weight of root nodules found highly significant *P*-0.00096, *P*-0.0066 respectively. Positive correlation was analyzed between number of nodules and the soil moisture content in both the areas. No relationship was found between the weight and the age of the nodules. Soil analysis (org C, K_2O, P_2O_5, S and Cl) found to be less in riverine areas when compared to non-riverine areas. Maximum number of nodulation was found in riverine area (580 nos.) as compared to non-riverine area (224nos.). This may be due to deficiency of nutrient in rhizosporic soil, thus larger nodules are formed to compensate their requirement of nutrients.

Acknowledgements: Authors are grateful to the Department Of Biotechnology, Government of India for finical support and also to Secretary, Department of Science and technology, Government of Sikkim for providing necessary facilities**.**

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Keywords: Lachen, Hippophae salicifolia, Frankia, North Sikkim, Riverine and Non Riverine.

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ISOLATION OF ACTINOMYCETES FROM ARABIDOPSIS THALIANA AND NICOTIANA BENTHAMIANA ROOTS

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RIKEN Center for Sustainable Resource Science

Actinomycetes are known to promote plant growth and productivity, as well as protection against plant pathogens. To define the actinomycetes community and thus understand the role in plant protection against pathogens, we aim to isolate the bacteria from the roots of *A. thaliana* (Brassicaceae) and *N. benthamiana* (Solanaceae) grown on natural soil under controlled environment. Based on partial 16S rRNA sequence, many of the isolates belong to the family Micromonosporaceae (8 genera, 38 species) while the others were the family Streptosporangiaceae (6 genera, 17 species), Streptosporangiaceae (1 genus, 11 species) and Thermomonosporaceae (2 genera, 3 species). Thirty of all 65 isolates showed less than 99% sequence identity between each isolate and type strain. Of these 30 isolates, 5 isolates showed less than 98% identity, suggesting these are likely to be novel actinomycetes. To clarify the role these root-associated actinomycetes in plants, 22 isolates were tested for antibacterial and antifungal activities. Two isolates showed an antibacterial activity against *Kocuria rhizophila*. One isolate showed an antibacterial activity against both *K. rhizophila* and *Escherichia coli*, as well as against a plant pathogen *Ralstonia solanacearum*. Two isolates strongly inhibited the growth of a strawberry pathogen, *Colletotrichum fructicola*, pointing toward their potential as a biocontrol agent against fungal plant pathogens. These preliminary findings pave a way for further analysis of other isolates and advance our understanding on the importance of actinomycetes-plant association.

Keywords: Isolation, Symbiosis, Plant, Pathogen, 16S rRNA

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LACTATE METABOLISM OF THE PATHOGENIC ACTINOMYCETE *RHODOCOCCUS EQUI*

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The pathogenic actinomycete *Rhodococcus equi* grows rapidly using the short-chain carboxylic acids L-lactate, propionate and acetate as growth substrate. L-lactate is present throughout the vertebrate body, and may therefore be an important source of carbon and energy. In silico analysis of the *R. equi* genome identified a number of potential pathways for the uptake and oxidation of L-lactate. Using a functional genomics approach we show that uptake of L-lactate depends on both lactate permease (LutP, REQ16290) and a protein belonging to the sodium/solute symporter family (MctC, REQ10610). Disruption of the former reduced the growth rate on L-lactate by 38%, whereas disruption of the latter had no effect. Disruption of both REQ16290 and REQ10610 completely prevented growth on L-lactate, however growth on propionate and acetate remained unaffected. In silico analysis identified a number of genes encoding potential L-lactate oxidising enzymes, including two 2-hydroxy-acid dehydrogenases (REQ36260, REQ43160), 2-hydroxy-acid oxidase (REQ15040) and three genes (REQ16300, REQ16310, REQ16320) encoding proteins homologous to a recently identified L-lactate oxidation system (LldEFG/LutABC). A triple mutant in which the former three genes were deleted was not affected in its ability to utilise L-lactate. In contrast, deletion of the latter three genes completely abolished growth on L-lactate and also affected intracellular proliferation of *R. equi* within macrophages.

Keywords:*Rhodococcus equi*, Virulence, Lactate Metabolism, Macrophage

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SYSTEMATICS

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TAXONOMIC DIVERSITY OF ACIDOPHILIC ACTINOBACTERIA AS A ROADMAP TO DRUG DISCOVERY

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Filamentous actinobacteria are the source of almost half of all known natural products and typically have whole-genome sequences which contain > 20 biosynthetic gene clusters that code for known or predicted secondary metabolites[1]. Although clearly an underdeveloped resource, it is difficult to discovery new chemical entities from known actinobacteria as screening them leads to the costly rediscovery of known compounds. Consequently, new strategies are needed to isolate, dereplicate and recognise new taxa for screening purposes. This taxonomic approach to drug discovery was used to selectively isolate and highlight novel acidophilic actinobacteria from litter and mineral horizon of a spruce forest soil. Four novel / putatively novel acidophilic species of *Actinospica*, *Nocardia* and *Streptacidiphilus* were discovered some of which produced new natural products thereby providing further evidence that filamentous actinobacteria from extreme habitats are a potentially rich source of new bioactive compounds for healthcare[2].

Keywords: Actinomycetes, Acidophiles, Polyphasic taxonomy, Natural products

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ONGOING THREE RESEARCH PROJECTS OF ACTINOMYCETE DIVERSITY FROM DIFFERENT HABITATS

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Introduction: Actinomycetes are widely distributed in terrestrial and aquatic ecosystems, especially in soil, where they play a crucial role in the recycling of refractory biomaterials by decomposition and humus formation (Ventura et al., 2007). They are also of industrial importance for the formation of a wide variety of secondary metabolites notably potent antibiotics. Actinomycete taxonomy was formerly thought to be associated with morphology, which is inadequate in distinguishing different species of many genera. The use of phylogenetic and molecular evolutionary approaches has been of great importance to the classification methods (Babalola et al., 2009; Hozzein and Goodfellow, 2011).

Objectives: This study was aimed to identify the possible novel actinomycetes that are potentially secondary metabolite producers, and to gain novel species for the literature. For this purpose, different soil samples were used for isolation of different actinomycete strains, and totally 897 isolates were obtained with selective and conventional isolation methods.

Methods: Various methods such as cesium chloride density gradient ultracentrifugation (Karwowski, 1986), modified paraffin bait method (Kurup and Schmitt, 1971) and sucrose-gradient centrifugation method have been applied to isolate actinomycetes from different habitats.

Results andConclusions: Analyses of its morphological, physiological and bichemical characteristics, together with DNA–DNA relatednes data, confirmed that strain FMN03^T represents a novel *Nonomuraea* taxon that is distinguished from closely related reference strains. Strain FMN03^T (= DSM 45913^T= KCTC 29233^T) is proposed as the type strain of the new species, for which the name *Nonomuraea muscovyensis* sp. nov. is proposed (Kocak-Ozdemir et al, May 6, 2014 as doi:10.1099/ijs.0.061291-0, IJSEM) and also isolate Z1R7^T (=KCTC 29434^T = DSM 42126^T) should be classified in the genus *Streptomyces* as *Streptomyces burgazensis* sp. nov. (Saricaoglu et al .2014, under revision IJSEM).

In conclusion, we are hopefull that our isolates have a great potential to merit species status in their related genera in ongoing three research projects on actinomycetes diversity.

Keywords: Selective isolation, Actinomycetes, Polyphasic taxonomy

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PHYLOGENETIC ANALYSIS OF THE FAMILY *STREPTOSPORANGIACEAE* BASED ON THE *RecA* GENE

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The family *Streptosporangiaceae* (suborder *Streptosporangineae*) comprises 13 genera and 96 species with validly-published names. Most of the genera contain fewer than ten species and three genera contain only one species. The genera with the highest numbers of species are *Nonomuraea* and *Streptosporangium*. In a recent study, *gyrB* gene sequences were obtained for members of the family *Streptosporangiaceae* and the GyrB amino acid sequences were analysed for molecular signatures. The *gyrB* gene phylogenetic trees provided taxonomic support for the composition of the currently recognised genera in the family. Inspection of the GyrB protein sequence alignments revealed genus-defining amino acid insertions for the genera *Herbidospora* and *Microbispora*. Genus specific amino acid sequences were identified for most of the multi-species genera. Furthermore, GyrB signature amino acids were identified, which distinguish the family *Streptosporangiaceae* from the related family *Nocardiopsaceae*. The RecA protein has a key cellular function, as it is involved in genetic recombination to repair damaged DNA. In this study, *recA* gene sequences (897 nt) were determined for the type strains of the species in all the genera in the family *Streptosporangiaceae*. The sequences used represent 81% of the full-length *recA* gene of *Streptosporangium roseum* DSM 43021^T. The *recA* gene sequences were used for phylogenetic analyses and the trees were compared to the corresponding trees for the 16S rRNA and *gyrB* gene trees. RecA amino acid alignments (299 amino acids) were generated and inspected for unique amino acid signatures to distinguish the genera in the family from each other. As was observed for the *gyrB* gene trees, the *recA* gene trees generally supported the division of the members of the family *Streptosporangiaceae* into 13 genera. The genus *Nonomuraea* was not monophyletic in any of the *recA* gene trees, while the genus *Planomonospora* was not monophyletic in the maximum likelihood and maximum parsimony trees. No insertions or deletions were observed in any of the *recA* gene sequences. Examination of the RecA sequence alignments for genus-specific amino acid sequences showed that the genera *Herbidospora* and *Planomonospora* contain unique amino acids that distinguish these genera from all other genera within the family *Streptosporangiaceae*. The shorter length of the *recA* gene sequences compared to the previously used *gyrB* gene sequences and the generally high *recA* gene sequence similarities between genera limit the usefulness of RecA protein sequences in distinguishing between genera in the family. Nevertheless, the results of this study extend the results of the GyrB study and will be useful in future taxonomic studies in the family *Streptosporangiaceae* by providing additional genus-specific molecular signatures.

Keywords: Family *Streptosporangiaceae;* RecA; Molecular signatures

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MULTILOCUS SEQUENCE PHYLOGENETIC STUDY OF THE GENUS *STREPTOMYCES* WITH DESCRIPTION OF *STREPTOMYCESTUNISIENSIS* SP. NOV.

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Filamentous soil bacteria belonging to the genus *Streptomyces* are industrially important because of their ability to produce many kinds of novel secondary metabolites including antibiotics. Taxonomic characterization of Streptomyces strains has been a challenge due to the large number of described species. The identification of new *Streptomyces* species is achieved via a polyphasic taxonomic approach, including molecular, biochemical and phenotypic characteristics. Phylogenetic analysis has provided a number of genotypic approaches to investigate the taxonomy of *Streptomyces*, including rRNA sequence comparison, DNA–DNA hybridization and DNA fingerprinting. However, due to the drawbacks of each method all have their limitations in routine use. Multilocus Sequence phylogenetic study has been successfully applied to the unambiguous characterization of new *Streptomyces* isolate. Our previous work was developed to study the taxonomic position of the isolate CN-207^T. On the basis of its phenotypic and molecular properties, strain CN-207^T is considered as a novel species of the *Streptomyces* genus, for which the name *Streptomyces tunisiensis* sp. nov. is proposed. The type strain CN-207^T (=JCM17589^T = DSM 42037^T) belongs to clade 100 as described by Labeda et al (2012). Here we report the results of a multilocus sequence analysis scheme developed to address the phylogeny of this clade. Sequence fragments of six housekeeping genes, atpD, gyrB, recA, rpoB, trpB and 16S rRNA, were determined for type strain CN-207^T and 35 reference type strains including its closest phylogenetically neighbours. Analysis of each individual locus confirmed the suitability of loci and the congruence of single-gene trees for concatenation. Concatenated trees of three, four, five and all six genes were constructed, and the stability of the topology and discriminatory power of each tree were analysed. It can be deduced from the results that phylogenetic analysis based on multilocus sequences is more accurate and robust for species delineation within *Streptomyces* species. A multilocus phylogeny of six genes proved to be optimal for elucidating the taxonomical position of the type strain CN-207^T and the relationships between the reference type strains 16S rRNA gene clade. Phylogenetic analysis of single gene alignments and a concatenated 6-gene alignment confirmed that strain CN-207^T was delineated from its phylogenetically related *Streptomyces* species and so should be considered to represent a new species of the genus *Streptomyces*, for which the name *Streptomyces tunisiensis* sp. nov is proposed.

Keywords: Multilocus sequence phylogeny, *Streptomyces tunisiensis* sp., nov

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16S RRNA-BASED TAXONOMY UNDERESTIMATES THE DIVERSITY OF BIOACTIVE STREPTOMYCETES

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One of the reasons attributed for the slowdown in discovery of novel bioactive compounds is the repeated recovery of previously isolated taxa. This has led to common practice of grouping together isolates with similar 16S rRNA gene sequences. Usually, whenever the SSU sequence data are identical to known species the isolates are discounted from further screening. The study presented here aimed at examining the validity of 16S rRNA gene sequence both in terms of bioprospecting and species-biodiversity.

In this work 10 isolates that were 100% identical to each other and to type strain of *Streptomyces cyaneofuscatus* were analysed. The isolates originated from three coastal lichens *Lichina confinis, Lichina pygmeae* and *Roccella fuciformis*. A detailed polyphasic approach was used to study the diversity between these strains. This included phenotypic assays, microscopic morphological examination, genetic analyses and genomic comparisons. Despite the fact that these 16S rRNA gene sequences were 100% identical, these strains had markedly different properties. Based on the analyses the 10 strains could be distinguished into 5 different species with 3 of them novel. The secondary metabolic profiles of these strains were also found to be significantly different.

It is clear that SSU-phylospecies even with the most stringent cut-off can include multiple bacterial species within them. Thus this study demonstrates that valuable bioresources could be easily lost when 16S rRNA gene sequence data are used for dereplication. Furthermore, it is evident that the genus *Streptomyces* is under-speciated. Most significantly, the results raise serious questions about the contemporary use of 16S rRNA gene sequence based OTUs to examine prokaryotic biodiversity and to extrapolate microbial ecological functions.

Keywords: 16S rRNA, Species concept, Diversity, Streptomycetes

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TAXONOMIC CHARACTERIZATION OF A NOVEL *SACCHAROPOLYSPORA SP*. CR3506, ISOLATED FROM SOIL

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Objectives: The genus *Saccharopolyspora* was established by Lacey & Goodfellow (1975) and was assigned to the family Pseudonocardiaceae (Embley et al., 1988; Warwick et al., 1994). Most species of the genus were isolated from soil samples, but *S. gregorii* and *S. hordi* were isolated from fodder (Goodfellow *et al*., 1989), *S. cebuensis* from a marine sponge (Pimentel-Elardo *et al*., 2008) and *S. halophila* from a saline lake (Tang *et al*., 2009). The aim of the present polyphasic study was to clarify the taxonomic position of the novel strain CR3506 of the genus *Saccharopolyspora* isolated from a soil sample, Corum.

Materials and Methods: *Saccharopolyspora* sp. CR3506 was picked after 4 weeks of incubation at 28°C on SM3 agar supplemented with nalidixic acid (10 µg/ml) and cycloheximide (50 µg/ml). Genomic DNA isolation was performed by Pitcher *et al*. (1989). The 16S rRNA gene was amplified by PCR using universal primers 27f and 1525r. Phylogenetic analyses were performed by using Neighbour Joining algorithm. DNA-DNA hybridisation was carried out as described by De Ley *et al.* (1970). Isomers of diaminopimelic acid in whole-cell hydrolysates and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed by thin layer chromatography (Staneck & Roberts, 1974). Respiratory quinones were extracted from freeze dried cells based on the two stage method described by Tindall (1990a; 1990b). Cellular fatty acids were extracted, methylated and analysed by gas chromatography with the standard Sherlock Microbial Identification (MIDI) system (Sasser 1990; Kämpfer & Kroppenstedt, 1996).

Results: CR3506 shared highest 16S rRNA gene sequence similarity with *Saccharopolyspora spinosa* NRRL 18395^T (99.11 %). Strain CR3506 showed DNA relatedness values 50.4 % to *Saccharopolyspora spinosa* NRRL 18395^T. Chemotaxonomic analyses showed that the cell wall contained *meso*-DAP. MK-9(H_4) (66 %) was the predominant menaquinone. The polar lipids detected were diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, phosphatidylglycerol and six unknown phospholipids.

On the basis of the genotypic and phenotypic data presented that isolate CR3506 represents a novel species within the genus *Saccharopolyspora*.

Acknowledgements: This study was supported by Ondokuz Mayis University (PYO. FEN.1901.12.014).

Keywords: *Saccharopolyspora*, 16S rRNA gene, Sequence, Taxonomic characterization

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AN ESTABLISHED MULTILOCUS SEQUENCE ANALYSIS FOR THE GENOME-BASED SYSTEMATICS OF STREPTOMYCETES

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Streptomycetes have adapted to various natural environments such as soil, oceans, plants and the extreme environment in Antarctica, and exhibit high levels of genetic and functional diversities. With the increasingly accepted theoretical species concept, microbial systematics is required to perform comprehensive analysis on genetic information for species diversity and evolutionary relationship. In this study, the core gene sets from 101 *Streptomyces* genome strains were compared to the five-gene multilocus sequence analysis (MLSA) to determine whether the MLSA scheme (*atpD*, *gyrB*, *recA*, *rpoB* and *trpB*) is sufficient to construct a refined framework for streptomycetes, and the genetic features of MLSA that may correlate to different environments were also assessed.

The five-gene MLSA reconstructions presented a clear framework of the *Streptomyces* genome strains, having a high resolution even for the closely related strains. Sixty-five core genes were retrieved from the *Streptomyces* genome strains, covering series of vital cellular functions in streptomycetes and providing a relatively larger span of nucleotide divergence. The 65-core-gene trees showed a better-supported branching pattern than MLSA, nevertheless, as MLSA, still contained a few uncertain branches including the typical single-membered clusters, implying that these strains may be sensitive to both concatenation operations. Moreover, MLSA showed a high correlation with whole-genome ANI values (R^2=0.93). For individual genes, except for that *rpoB* had a relatively low correlation with the ANI (R^2=0.56), the other four markers of *atpD* (R^2=0.70), *gyrB* (R^2=0.88), *recA* (R^2=0.77) and *trpB* (R^2=0.76) were satisfied with the whole-genome relationship, confirming that MLSA is a powerful and pragmatic alternative to unravel *Streptomyces* diversity at the genome level.

Moreover, a relative rate test was conducted on two fully sequenced strains of *Streptomyces pratensis*, ATCC 33331 and PAMC 26508. The results showed that at both MLSA and core-gene sequence levels, *S. pratensis* failed to reject the restriction models that assuming different evolutionary rates in lineages. When assessing evolutionary constraints on genes, a weak negative relationship was found between the evolutionary rate of d*N*/d*S* and codon usage bias at the 65-core-gene scale. And strain ATCC 33331, adapted to grassy field, showed a low general codon adaptation index (CAI) values in *atpD* and *recA* compared with strain PAMC26508 that was isolated from Antarctic lichen. It is probable that the recombination-related genes such as *atpD* and *recA* may be involved in the initiations in response to environmental changes.

In light of the genome-based analyses, we conclude that the MLSA scheme has great superiority in defining the phylogenetic structure and evolutionary history of *Streptomyces*, and is useful to explore the population parameters shaping the species.

Keywords: Streptomycetes, Multilocus sequence analysis, Genome-based systematics, Core genes, Evolution

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A REVISED VIEW OF THE PHYLOGENY OF VERRUCOSISPORA AND ITS RELATIVES BASED ON MULTIPLE GENE SEQUENCES

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The actinobacterial family *Micromonosporaceae* represents a rich resource of bioactive compounds second only to *Streptomycetaceae*. As a member of this family, *Verrucosispora* is one of the most promising genus, producing many compounds with unique structures and interesting activity, such as abyssomicins and proximicins. Recently, a number of new members of or related to this genus were isolated and described, but few of them showed the expected discriminative phenotypes. Current methods based on 16S rRNA gene phylogeny in prokaryotic systematics failed to divide *Verrucosispora* and its relatives into meaningful units of biology and ecology. So, a clear phylogenetic framework is needed for the systematics of this group of microorganisms.

In this study, we investigated 26 *Verrucosispora* strains, including all the nine type strains, and another 26 type strains of related genera in the family *Micromonosporaceae*. We got the almost full-length sequences of six housekeeping genes, i.e. 16S rRNA, *rpoB*, *gyrB*, *recA*, *atpD* and *trpB*, and employed an MLSA scheme to analyse the phylogenetic structure. The single-gene and the concatenated phylogenies indicated that all the abyssomycin-producing strains stably formed a single cluster within the the genus *Verrucosispora*, suggesting that the antibiotic-producing cluster might be formed by periodic selection. *Verrucosispora* was phylogenetically closely related to other single-spore-forming genera of the family *Micromonosporaceae* (e.g. *Jishengella*, *Xiangella*, *Micromonospora* and *Salinispora*), forming a well-supported cluster clearly seperated from other genera of this family, and this result was supported by the previous study of SALPs (Geneviève Girard et al. 2013).

Widespread gene exchange within the genus *Verrucosispora* was found, and the interspecies recombination rate was as high as that in *Streptomyces*. We also detected a large amount of HGT events between *Verrucosispora* and other genera, and therefore infer that *Verrucosispora* might gain new phenotypic traits through HGT. Remarkably, we found that the 16S rRNA genes of *Verrucosispora* and most of other *Micromonosporaceae* strains are recombinant molecules, and this may explain why people always fail to build a well-resolved 16S rRNA gene-based hierarchical system in this family.

Keywords: *Verrucosispora*, *Micromonosporaceae*, Multiple gene sequences, Phylogeny, Recombination

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A STUDY OF *STREPTOMYCES* SPECIES ISOLATED FROM ROOT SOIL OF *PHASEOLUS VULGARIS* GEVAŞ, VAN

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Root of common bean (*Phaseolus vulgaris L.*) is a soil-borne disease that is incited by several pathogens including fungi and bacteria spp. It occurs in all bean-growing areas of the world leading to enormous crop losses. The pathogen is known to be very persistent in soil and capable of surviving in infested fields for very long period and is difficult to control.

The aim of this study is to isolate, identifiy and characterise Streptomyces bacteria isolated from root soil samples of *Phaseolus vulgaris* cultivated abudantly at Gevaş town, Van. Physicochemical characters those were pH, organic matter content and humidity of 10 soil samples collected from 10 different villiages of Gevaş were measured and then dilution of soils were inoculated on plates of starch casein agar and raffinose histidine agar to isolate *Streptomyces* bacteria. Total 52 *Streptomyces* strains were purified and colour grouped on after growing on oatmeal and peptone yeast extract iron agar and identified using computer identification program. 9 *Streptomyces* strains represent colour groups were characterized using whole cell protein profiles.

Identified test strains were 8 *S. anulatus*, 6 *S. xanthochromogenes*, 3 *S. prasinosporus,* 2 *S. alboflavus,* 2 *S. aurantiacus*, 2 *S. griseoflaus*, 2 *S. langiporoflavus* and 2 *S. californicus* species. *S. roseus*, *S. tubercidius*, *S. glaucescens, S. phaeochromogenes, S. flaveolus* and *S. luridus*. 9 test strains were divided into 7 groups on dendogram generated from analysis whole cell protein profiles of test organisms by SDS-PAGE electrophoresis method. Our results suggested that abundance of Streptomyces have biologic control effect on pathogenic and nonpathogenic microoorganisms growing in the root soils plant species.

Keywords:*Streptomyces*, Isolation, Identification

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REVERSE ENGINEERING OF A NON-PELLETING LINEAGE OF *STREPTOMYCES LIVIDANS* EVOLVED IN CONTINUOUS CULTURE CREATES NEW DERIVATIVES WITH ENHANCED PRODUCTIVITY

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Classical strain improvement has generated many phenotypes connected to growth properties that are relevant for industrial production. In the pre-genomics era, the random nature of these improvements prevented the ready transfer of desirable characteristics to other strains. Here we report the reverse engineering of a evolved lineage derived from the enzyme producer *Streptomyces lividans* 66, which grows as dispersed mycelium with a growth rate that is up to three times higher than that of the wild-type strain. Surprisingly, this strain failed to secrete tyrosinase, suggesting that besides a nonpelleting phenotype, the strain also failed to secrete enzymes secreted via the Twin Arginine Transport (Tat) system. Genome comparison via SNP analysis revealed mutations in 40 ORFs. A subset of 11 ORFs with functions related to morphogenesis was selected and deletion mutants prepared in the wild-type strain *S. lividans* 66. Deletion of a gene for a novel cell-wall enzyme, gave the same morphological changes as seen for the spontaneous mutant, but the secretion defect was not found. The latter is therefore most likely due to a yet undiscovered second-site mutation. The new deletion mutant in fact showed an increased capacity in the production of the enzyme tyrosinase, while fermentation times were reduced. This implies a major improvement of productivity. Our work shows not only the successful reverse engineering of a complex mutant, but also illustrates the genetic tug-of-war the strains in the lineage undergo toward their evolved phenotype.

Keywords: Inverse engineering, Morphology, Whole genome sequencing, Enzyme production

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CHARACTERIZATION OF *NOCARDIA FUSCA* SP NOV., OBTAINED FROM HUMAN CLINICAL ISOLATES FROM NORTH AMERICA

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Nocardia fusca was originally isolated from a soil sample in 1983, but the species has never been validly named. Following the retrospective analysis of 16S rRNA gene sequence data obtained from clinical isolates submitted to the Special Bacteriology Reference Laboratory for identification, we identified seven clinical isolates that were closely related to *N. fusca* NBRC 14340 (=JCM 14340). The seven clinical isolates were obtained from the sputum of seven patients from different states and from Puerto Rico; four isolates were obtained from males and the age of patients range from 24 to 66 years old. All the clinical isolates exhibited aerial and substrate hyphae. Five of the seven isolates (X0018, W9231, X0035, W7803 and W9728) had 16S rRNA gene sequences that were 100% identical to each other and showed 99.93% sequence similarity to isolates W7209 and X0699. BLASTn searches of the GenBank database using near full length 16S rRNA gene sequences (1439-bp) showed the highest sequence similarity of the clinical isolates to *N. fusca* JCM 14340^T (100 – 99.93% similarity), *Nocardia rhamnosiphilia* (99.66%), *Nocardia carnea* (99.58%), *Nocardia testacea* (99.52%) and *Nocardia flavorosea* (99.52%). Genome-to-genome distance was further delineated by calculating the average nucleotide identity (ANI) values obtained from whole genome sequences. The ANI values calculated between *N. fusca* NBRC 14340 and the type strains of *N. rhamnosiphila, N. grenadensis* and *N. sienata* were 93.28, 92.22%, and 91.87%, respectively. These values were below the ANI value of 95-96% and suggest the clinical isolates represent a new species. MICs to 10 antimicrobial agents were determined following the guidelines and interpretative breakpoints recommended for the genus *Nocardia* by the CLSI. All isolates were resistant to clarithromycin but susceptible to amikacin, ceftriaxone, trimethoprim/sulfamethoxazole, ciprofloxacin and linezolid. To our knowledge this is the first report of clinical isolates in North America resembling *N. fusca*. Based on their morphologic, phenotypic and phylogenetic characteristics, our analyses suggest the clinical isolates represent a novel species of the genus *Nocardia*. Further polyphasic testing including phenotypic and genotypic analysis, DNA-DNA hybridization analysis and additional whole genome sequence analysis will be required to confirm the identity of the isolates as *N. fusca*.

Keywords:*Nocardia fusca*, Average nucleotide identity, Clinical isolates, Antimicrobial resistance

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ISOLATION, CHARACTERISATION AND IDENTIFICATION OF *AMYCOLATOPSIS* AND *STREPTOMYCES* SPP. FROM SOME SOIL

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Introduction: Currently different bacteria species are used against particular chemical components and pathogen bacteria as biological agents. Amycolatopsis and Streptomyces species are known to have significant potential due to their ability to produce antibiotics and different bioactive substances. The species of Actinobacteria are extremely diverse and contain a variety of subdivisions as well as yet-unclassified isolates. The conventional methods that are used in identification and classification of Actinomycetes are supported by chemotaxonomic and molecular techniques to get more precise results. Studies of isolation, identification and characterization on Actinomycetales particularly on the Streptomyces species, have increased due to their products that are important in terms of biotechnological studies. Although the studies carried out on the Amycolatopsis species have been increasing, their numbers are insufficient in the worldwide and too few in Turkey.

Objective: The objective of this study is to isolate, diagnose and characterize the bacteria that belong to Amycolatopsi**s** and Streptomyces types, from 30 soil samples taken from different locations around Erçek Lake in the Van Lake Basin.

Methods: Physico-chemical analyses of the 30 soil samples have been performed. Numeric taxonomic analyses have been performed after color grouping of insulated, purified 60 Amycolatopsis and 60 Streptomyces isolates by using a selective nutrition environment. The diagnoses of all of the purified Streptomyces test isolates have been made by using Bacterial diagnosis programme including phonetic data (IDENTAX). The numeric analyses of 10 Amycolatopsis and 10 Streptomyces test isolates, which have been chosen for representing color groups for molecular characterization studies have been performed, by determining all the cell protein profiles with SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis), and the results have been presented as dendrograms.

Results: While the pH of the soil samples have been around neutral values, the organic material content has changed between %0,494 and 14,944, and the humidity has been measured under %10. As a result of the insulation study, Amycolatopsis colony numbers changed $0{,}3x10^4$ and $12{,}4x10^4$ cfu/gr between dry soil and Streptomyces colony numbers changed $2{,}4x10^4$ and $33{,}4x10^4$ cfu/gr between dry soils. Purified Amycolatopsis test isolates have been sorted into 11 color groups and Streptomyces test isolates have been sorted into 12 color groups. 42 of 60 Streptomyces test isolates have been diagnosed. Test organisms have been put in the same groups in the characterization studies that have been made by using SDS-PAGE and RAPD techniques.

Conclusion: Classification and characterization of Amycolatopsis and Streptomyces species that are purified were done according to our study isolation. From the test isolates 70% of Streptomyces have been diagnosed. The representatives isolates have taken for molecular characterization studies show the great similarity and fall in the same groups when using SDS-PAGE and RAPD techniques. Future studies will aim to find precise results by comparing 16S-rDNA sequence analysis.

Keywords: Amycolatopsis, *Streptomyces*, Isolation, Characterization, RAPD

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DIVERSITY OF CULTURABLE ACTINOBACTERIA

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In order to evaluate the diversity of culturable actinobacteria in desert soil crusts, we collected 50 samples from Shapotou region of Tengger Desert, China, and four different media were chosen to isolate actinobacteria. Biolog GEN III (MicroPlate), API 50CH and API ZYM test kits (bioMerieux) were employed to investigate the metabolic characters of the isolates. As a result, 376 actinobacterial strains were purified from these samples. According to the 16S rRNA gene information, these isolates belong to 29 genera of 18 families, among which the members of Geodermatophilaceae were predominant. We got 48 strains affiliated to the genus Blastococcus, 28 Geodermatophilus satrains and 39 Modestobacter strains. These isolates were screened using 6 models for screening new antibiotics, and the fermentation broths from 17.4% of theses strains exhibited activity on at least one screening model. Combined the 16S rRNA gene information and the physiological data, 9 isolates representing hitherto unknown species were discovered. The results showed that there was abundant actinobacterial species and antibacterial activity diversity in the desert soil crusts.

Keywords: Actinobacteria, 16S rRNA, Isolation, Diversity

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PROMISING ACTINOMYCETES FROM MEKE LAKE, A SALTERN IN TURKEY

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Introduction: Actinomycetes are a group of microorganisms which are important economically and biotechnologically. They are known to be researched for discovery of new drugs and bioactive metabolites (Ramesh and Mathivanan, 2009). Many of pesticide, herbicide and antiparasitic compounds as well as antibiotics are obtained from actinomycetes. These compounds are primarily used in pharmaceutical industry and agriculture. Recently, great advances have been gained in use of biocatalyzers and secondary metabolites isolated from terrestrial actinomycetes. However, research and development programs have focused on isolation of novel actinomycete strains from underexplored habitats (Ghosh et al., 2013). Extreme habitats have extraordinary conditions in terms of temperature, acidity, pressure or salinity. Microorganisms inhabiting such environments attract high interest because they have special mechanisms to adapt harsh conditions and are able to produce novel compounds. However, there are few reports on actinomycetes isolated from extreme environments. Due to re-isolation of known compounds, searching for new actinomycete groups such as halophiles from underexplored habitats as sources of novel chemical compounds is essential. Halophilic actinomycetes are accepted as valuable resources for many industrial products having antimicrobial, cytotoxic, neurotoxic, antimitotic, antiviral and antineoplastic activities (Subramani and Aalbergsberg, 2013).

Objects: This study was aimed to isolate novel actinomycete strains from coastal soil and sediment samples of Meke Lake an extreme saline crater lake in Turkey. Being an unstudied habitat in terms of actinomycete diversity, Meke Lake has a great potential for novel actinomycetes producing bioactive secondary metabolites.

Materials and Methods: Coastal sediment and soil samples from Meke Lake were kept in sterile plastic bags and stored at $+4^0$C. Actinomycetes were isolated by spreading dilutions of samples on Czapek-Dox (Weyland, 1969), Raffinose-Histidine (Vicker et al., 1984), Starch-Casein (Küster and Williams, 1964) and SM3 (Tan et al., 2006) agar plates supplemented with nystatin (50 µg/ml) and rifampicin (5 µg/ml). A total of 54 isolates were obtained from sediment and soil samples. Eighteen isolates out of 54 were selected for 16S rDNA PCR amplification by using 27f and 1525r primers. PCR amplicons were purified and sequenced by Macrogen Inc. using MG3f, MG5f and 800r primers. Obtained 16S rRNA gene sequences were aligned in MEGA6 programme. The isolates were identified using EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012) on the basis of 16S rDNA sequence data. Phylogenetic trees were inferred by using neighbour-joining (Saitou and Nei, 1987) and maximum likelihood (Felsenstein, 1981) methods.

Results and Conclusions: According to almost complete 16S rRNA gene sequence analysis, 18 isolates were identified to belong to four different genera: *Nonomuraea* (5 isolates), *Streptomyces* (6 isolates), *Micromonospora* (6 isolates) and *Nocardia* (1 isolate). The isolates MTCD26 and MKTC17 having 98.23% similarity with *Nonomuraea monospora* PT708^T and 98.09% similarity with *Streptomyces marokkonensis* Ap1^T, respectively, are regarded having potential to represent novel species. A polyphasic approach will be adopted to determine exact positions of both isolates. In conclusion, we are of the opinion that Meke Lake, a crater lake having extreme salinity, is a promising source inhabited by potentially novel actinomycetes.

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Keywords: Actinomycetes, Extreme habitats, Novel species

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RAMAN SPECTROSCOPIC FINGERPRINTING OF ACTINOMYCETES

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The molecular information obtained from microorganisms using spectroscopic, chromatographic, or any other means can be used for identification, discrimination, or classification. However simplicity, speed and reliability are preferable characteristics of these techniques. Recently, there is a high interest in the use of Raman spectroscopy to investigate biological structures. Although it is advantages in terms of its low sensitivity to water, easy sample preparation and short spectral acquisition time, it suffers from inherently weak scattering and background. Surface-enhanced Raman Scattering (SERS) offers a solution to this problem.

Although there are many studies of use of Raman spectroscopy for bacterial and eukaryotic identification and characterisation, no study was observed on actinomycetes.

In this study, we aim to characterize the different actinomycetes genera using colloidal silver nanoparticles (AgNPs) as the SERS substrate and investigate the use of SERS spectra obtained from actinomycetes fort he identification and discrimination of five genera namely, *Actinoplanes abujensis*, *Actinomadura geliboluensis*, *Nocardia sungurluensis*, *Nonomuraea muscovyensis* and *Pseudonocardia kujensis*.

Keywords: Fingerprinting, Raman, SERS

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RELAXED SELECTION DRIVES A NOISY NON-CODING TRANSCRIPTOME IN MEMBERS OF THE *MYCOBACTERIUMTUBERCULOSIS* COMPLEX (MTBC)

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Related species are often used to understand the molecular underpinning of virulence through a shared set of biological features attributable to a core genome of orthologous genes. An important but understudied question, however, is the extent to which the regulatory architecture is similarly conserved. A small number of studies have compared primary transcriptomes between bacterial species but few have compared closely related species with clearly divergent evolutionary histories.

Using high-resolution transcription start site (TSS) mapping, we addressed the impact of differing modes of evolution within the genus *Mycobacterium* through comparison of the primary transcriptome of *M. marinum* with that of a closely related lineage, *M. bovis*. Both are thought to have evolved from an ancestral generalist species, with *M. bovis* and other members of the *M. tuberculosis* Complex (MTBC) having subsequently undergone downsizing of their genomes during the transition towards obligate pathogenicity. *M. marinum*, in contrast, has retained a large genome, appropriate for an environmental organism, and is a broad host range pathogen.

We identify evolutionarily conserved transcriptional attenuation and highlight its potential contribution to multiple drug resistance mediated through the transcriptional regulator *whiB7*. We show that a species' population history is reflected in its transcriptome and posit relaxed selection as the main driver of an abundance of canonical -10 promoter sites in *M. bovis* relative to *M. marinum*. It appears that transcriptome composition in mycobacteria is driven primarily by the availability of such sites and that their frequency diverges significantly across the mycobacterial clade. Finally, through comparison of *M. bovis* and *M. tuberculosis*, we illustrate that SNP-driven promoter differences likely underpin many of the transcriptional differences between MTBC lineages.

Keywords: Selection, evolution, Transcription, Non-coding RNA, TSS

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PHYLOGENETIC RELATIONSHIPS OF 65 *NOCARDIA* SPECIES WITH SODA GENE

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Introduction:*Nocardia* are soil saprophytic, microorganisms related to opportunistic diseases as nocardiosis and actinomycetoma. The *rrs* gene (16S RNAr) has been used for identification and taxonomic classification of *Nocardia* species, however, it has been reported that it does not have enough polymorphism to distinguish between certain species within the genus. Other genes have been used (*hsp65*, *secA1*, *gyrB*, *rpoB*) and the 16S -23S intergenic region (ITS) as genetic targets. Another interesting gene is *sodA*, that it has been used to identify *Mycobacterium* species, it seems that the method is fast and accurate, representing an interesting alternative for differentiation and identification within the genus *Nocardia*.

Objective: To analyze the specific variability of *sodA* gene for *Nocardia* species.

Materials and Methods: We used 65 type strains of genus *Nocardia* to get corresponding sequences of *sodA* gene (440 bp), and 55 clinical and 34 environmental strains of *Nocardia*. The most frequently encountered species on clinic and soil (11 *Nocardia* species), were obtained from Mexican Hospitals and the French Observatory of Nocardiosis (OFN) in order to validate the technique.

Results: Among the analysis of *sodA* gene sequences we were able to obtain values of similarity within 79.8% to 100%, 24 phylogenetic nodes, with bootstraps ≥ 90, and 38.71% of resolution compared with the 17.74% of the *rrs* gene, which allowed us to construct a robust phylogenetic tree. It was analyzed heterogeneity and polymorphism of 89 *Nocardia* strains of eleven *Nocardia* species; *N. paucivorans*, *N. farcinica* and *N. ignorata,* showed a 100% similarity to the corresponding type strain with both genes. In contrast, strains of *N. abscessus*, *N. beijingensis*, *N. cummidelens*/*N. soli*, *N. brasiliensis*, *N. transvalensis*, *N. cyriacigeorgica* and *N. vinacea* showed divergent similarity to 100% with *sodA* gene. Were identified isolates belonging to other species (*N. wallacei*, *N. araoensis*, *N. aobensis*) and it was observed heterogeneity of sequences within de same species.

Conclusions: The *sodA* gene used as a molecular marker of *Nocardia* showed a significant genetic polymorphism, high resolution, specificity and reliability to identify to species level. Gene *sodA* showed a clear specificity to differentiate between clinic and soil strains**.**

Keywords: Phylogeny, *Nocardia*, SodA, Gene

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POLYPHASIC IDENTIFICATION OF *LECHEVALIERA FRADIA* SUBSP. IRANICA, A RARE ACTINOMYCETE ISOLATED FROM LOSHAN REGION OF IRAN

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Introduction: Actinomycetes are widely distributed in natural and man-made environments, and have capability for degradation of organic matter. They are also well known as a rich source of antibiotics and bioactive molecules, and are of considerable importance in industry.

Material and methods: In this study, a rare actinomycete was isolated and subjected to polyphasic identification. Identification of this rare actinomycetes was carried on according to polyphasic taxonomic approach which have been outlined by International Committee on Systematics of Prokaryotes (ICSP).

Results: The cell wall of strain LO5 contained *meso*-diaminopimelic acid as diaminoacid and galactose, mannose and rhamnose as diagnostic sugars. The phospholipids consisted of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Phylogenetic analysis based on nearly complete 16S rRNA gene sequence comparison revealed affiliation to the family*Pseudonocardiaceae* and its similarity to the most closely related neighbor the *Lechevalieria fradiae* CGMCC 4.3506^T was 98.6%. The level of DNA–DNA relatedness between the novel strain and *Lechevalieria fradiae* CGMCC 4.3506^T was only 75%. So according to the recommendations of a threshold value of 70 % DNA-DNA similarity, LO5 belongs to the species *Lechevalieria fradiae* CGMCC 4.3506^T. On the basis of genomic and phenotypic properties, the subspecies *Lechevalieria fradiae* subsp. *Iranica* IBRC-M 10378 is proposed.

Discussion and conclusion: A polyphasic approach based on phenotypic, chemotaxonomic and phylogenetic investigations has been proposed for categorizing of a rare actinomycete in subspecies level. The techniques used to obtain the data required for determination of the taxonomic status of this isolate are based on minimal standards that have been established by some of the taxonomic subcommittees of the International Committee on Systematics of Prokaryotes (ICSP) for specific groups of organisms.

Keywords: Polyphasic taxonomy, Rare actinomycetes, Chemotaxonomy, Phylogenetic analysis

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ALLOACTINOSYNNEMA IRANICUM SP. NOV., A NOVEL RARE ACTINOMYCETE ISOLATED FROM HYPERSALINE WETLAND AND EMENDED DESCRIPTION OF THE GENUS *ALLOACTINOSYNNEMA*

Mahdi Moshtaghi Nikou[1]

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A Gram-staining-positive actinobacterial strain Chem10^T was isolated form soil around Inche-Broun hypersaline wetland in the north of Iran. Strain Chem10^T was strictly aerobic, catalase and oxidase positive. The isolate grew between 0-3% NaCl, at 20-40 °C and at pH 6.0-8.0. The optimum temperature and pH for growth were 30 °C and pH 7.0, respectively. The cell wall of strain Chem10^T contained ***meso***-diaminopimelic acid as diaminoacid and galactose, ribose and arabinose as whole-cell sugars. The polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. It synthesized cellular fatty acids of the straight-chain saturated, mono-unsaturated, iso and anteiso-branched types $C_{14:0}$, $C_{16:0}$, iso-$C_{16:1}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$ and iso-$C_{15:0}$, and the major respiratory quinone was MK-9(H_4). The G+C content of the genomic DNA was 70.7 mol %. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Chem10^T belongs to the family *Pseudonocardiaceae* and showed the closest phylogenetic similarity with *Alloactinosynnemaalbum*KCTC 19294^T(98.3%) and *Actinokineospora cibodasensis*DSM 45658^T (97.9%). DNA–DNA relatedness between the novel strain and strains *A. album*KCTC 19294^T and *A. cibodasensis*DSM 45658^{T}were only 52.5 % and 23.5% respectively. On the basis of phylogenetic analysis, phenotypic characteristics and DNA-DNA hybridization data, a novel species of the genus *Alloactinosynnema*is proposed, *Alloactinosynnema iranicum*sp. nov. The type strain is Chem10^T (= IBRC-M 10403^T = CECT 8209^T).

Keywords: *Alloactinosynnemairanicum*, *Pseudonocardiaceace*

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APPLICATIONS AND BIOTECHNOLOGY OF ACTINOMYCETES

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FRANKIA AND OTHER FREE - LIVING SYMBIONTS

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NECESSITY OF VARIOUS ROOTING HORMONES IN SEED GERMINATION OF ALNUS – A POSSIBLE CAUSE OF ACTINORHIZAL ASSOCIATION

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The seed germination of *Alnus* and its survival, in both *in vitro* and *in vivo*, is a serious challenge to scientists as the germination process is very slow. Mature seeds of *Alnus* were collected from the healthy plants of Darjeeling hills. Some of the surface sterilized seeds were overnight soaked in aerated water, three different media WPM, MS and Hoagland in half and full strength was used. For the study of effects of hormones in seed germination, the media were separately supplemented with 1-5mg/l NAA and IBA singly or in combinations. Maximum germination was observed in Hoagland media for both treated and untreated seeds, whereas minimum in ½ MS for treated and ½ WPM for untreated seeds. IBA and NAA+IBA were found to be more effective than NAA in terms of germination percentage and rooting. Good rooting and its growth were maximum in the media supplemented with IBA(@ 3, 4 & 5mg/L) These experiments showed that germination of *A. nepalensis* seeds have specific hormonal requirements, which is fulfilled by actinorhizal association with *Frankia*.

Keywords: Frankia, Alnus, Seed germination, Root hormones

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DIVERSITY OF *FRANKIA* POPULATIONS IN THE ROOT NODULES OF ACTINORHIZAL MORELLA OF THE CAPE FLORA OF SOUTH AFRICA

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Actinorhizal plants are major contributors to global terrestrial biological nitrogen fixation. Nitrogen-fixing actinorhizal plants constitute more than 220 species of woody plants belonging to eight families which are able to enter into symbiosis with saprophytic soil-dwelling *Frankia* species. *Frankia* can be classified into four host-infection groups: host-infection group (HIG) 1 *Alnus*-infective; HIG 2 *Dryas*-infective; HIG 3 Eleaegnaceae-infective; and HIG 4 non-infective/non nitrogen fixing. Actinorhizal plants typically associate with *Frankia* from a single host-infection group. *Morella* (family Myricaceae) species are defined as being promiscuous as under laboratory conditions they readily nodulate with *Frankia* from different host-infection groups. However, results from field studies, conducted under natural conditions, have revealed a narrower spectrum of association which may be influenced by a variety of factors including soil chemistry and strain availability. *Morella* is the dominant actinorhizal genus on the African continent and is the only genus with species endemic to the continent. Of the 31 accepted *Morella* species, at least sixteen occur in Africa. The Cape Floristic Region of South Africa houses seven *Morella* species (all endemic to Africa), of which three are endemic to the Cape flora. These species occupy distinct and sometimes overlapping habitats including coastal dunes, riverbanks, coastal flats and montane islands. In terms of their natural symbiotic associations, to date only six *Morella* species have been studied in their natural habitats. While the seven Cape Flora Morella species are known to be nodulated in the wild and are capable of nitrogen fixation, the diversity of their endosymbionts had never been investigated prior to this study.

Nodules were collected from natural populations of six of the seven Cape species in parks and reserves within the western reaches of the Cape flora and a partial *nifH* gene sequence was amplified from field-collected nodules. Of the 26 unique 606 bp *nifH* sequences recovered from 202 nodules, 7 could be assigned to *Alnus*-infective frankiae and 19 to the Eleaegnaceae-infective frankiae. The sequences derived from endophytic *Frankia* could be assigned to seven clusters (defined at 97% sequence identity), two grouping with the *Alnus*-infective strains and five with Elaeagnaceae infective strains. Identical *nifH* gene sequences were recovered from different *Morella* species and from sites distributed across the entire sampling region. Of the Cape Flora *Morella* species, three (*M. diversifolia, M. quercifolia* and *M. integra*) were found to be promiscuous in the field. *M. cordifolia* was nodulated by Elaeagnaceae-infective strains only, while *M. kraussiana* and *M. serrata* were exclusively nodulated with *Alnus*-infective strains. Host infection group appears to be correlated with soil pH, with *Alnus*-infective strains detected in nodules from acidic soils only, with Elaeagnaceae-infective strains being ubiquitous. Rhizosphere soil libraries from selected sites indicated the presence of *Alnus*-infective *FrankianihH* genes in neutral soils, which were absent from nodules collected from the same sites. Comparatively high diversity was detected in the Elaeagnaceae-infective strains, which were assigned to four of the five previously defined clusters. *Alnus*-infective strains from nodules have much lower diversity with only two clusters found, including a previously undescribed cluster. In addition, six *Frankia* strains were isolated from four *Morella* species (*M. quercifolia*, *M. integra, M. kraussiana, M. cordifolia*), of which two strains belong to the *Alnus*-infective cluster and four

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to the Eleaegnaceae-infective cluster. The *nifH* genes amplified from these isolates corresponded to *nifH* gene sequences recovered from nodules. This study is the first to investigate the diversity of *Frankia* strains naturally associated with *Morella* of the Cape Flora in their natural habitats and the first to isolate *Frankia* strains from endemic African *Morella*.

Keywords: Frankia, Morella, Cape Floral region

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MARINE BIODISCOVERY AND BIOTECHNOLOGY

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ANTICOLORECTAL CANCER BIOACTIVE COMPOUNDS FROM *ACTINOBACTERIA* ASSOCIATED WITH INTERTIDAL MARINE SPONGES

Demes C. Martantiningtyas, Noer Kasanah, Umar A .Jenie

Actinobacteria is a very important group of bacteria that has considerable value as prolific producers of antibiotics, anticancer agents and other pharmaceutically useful compounds.As a part of our investigation to discover anticancer agent, we explore marine actinobacteria associated with sponges collected in intertitidal zone of Yogyakarta coast line, Indonesia. Due to the extreme condition of intertidal zone we expected to isolate unique marine Actinobacteria as source of anticancer agent. Isolate *Actinobacteria* were screen for their possesing enes encoding polyketide synthases (PKS) and nonribosomal peptide synthetase (NRPS). Bioactivity screening was carried out using MTT assay and WiDr cell lines on 96 well format assay. Potential isolates that contain gene of interest and exhibit promising bioactivity were selected for further bioassay guided isolation to obtain bioactive compound. The phylogenetic diversity of the actinobacteria was assessed by analyzing the sequence of 16S rDNA. Results showed that actinobacteria harboring NRPS gene and profiling secondary metabolites indicated as source of anticancer agent.

Keywords:*Actinobacteria*, Intertidal sponges, Anticolarectal cancer, NRPS, PKS

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FLUOROACETATE BIOSYNTHESIS FROM THE MARINE-DERIVED BACTERIUM *STREPTOMYCES XINGHAIENSIS*

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Organofluorine compounds have been widely exploited by the pharmaceutical industry. Well over 20% of current drugs in clinical trials contain a fluorine atom. Fluorinated entities have also found extensive use in agrochemicals and in tuning the properties of performance high-value organic materials. In contrast, nature has hardly evolved a biochemistry of fluorine, and fluorinated natural products are extremely rare. Fluoroacetate is the most ubiquitous fluorometabolite found as a toxic component of many tropical and sub-tropical plants.[1] In 1986, a soil bacterium *Streptomyces cattleya* was shown to have the capacity to produce fluoroacetate and the antibiotic, 4-fluorothreonine when grown in the presence of fluoride ion.2 Subsequently the origin of the fluorometabolites of *S. cattleya* has been studied. Fluorinase genes remain sparse since and *S.cattleya*has remained the only genetic host characterized that encodes a C-F bond forming enzyme until 2014. We have recently reported the characterization of a new fluorinasefrom one terrestrial actinomycete *Streptomyces sp* MA37 since more than a decade after the first identification.[1] Morerecently we characterized one new fluorometabolite in the MA37 strain. It is worth to note that it is the first time for almost 30 years that a new fluorometabolite has been identified.

Herein we reported the identification of the first fluoroacetate producing microorganism from the marine environment, *Streptomyces xinghaiensis*, through microbial fermentation and genetic interrogation[2].

Keywords: Fluorometabolite, Fluorinase, Marine organism, Soil bacterium

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SCALE-UP OF KERATINASE PRODUCTION BY *STREPTOMYCES* SP. 2M21: FROM 250 ML TO 5 L

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In this study, keratinase production from *Streptomyces* sp.by utilizing chicken feather as a sole carbon and nitrogen source was carried out from 250 ml shaking flasks to 2 and 5 L stirred tank reactors, respectively. Screening and optimization of production conditions were implemented in 250 ml shake flask; mixing and aeration rates were optimized in 2 L bioreactor under the optimized production conditions. Subsequently, constant impeller tip speed, constant oxygen mass transfer coefficient and constant geometric similarity were used as scale up parameters in order to maximize enzyme production in 5 L bioreactor. Mixing time was experimentally determined using the pH-response technique. Impeller tip speed (u_{tip}) was calculated as a function of tip speed and reactor diameterwhereas; oxygen mass transfer coefficient (k_La) was measured using the unsteady state method. Optimum keratinase activity was 330, 351 and 339 U/ml at 250 ml shaking flask, 2 L and 5 L stirred tank reactor, respectively.When compared with 5 L stirred tank reactor, mixing time was12.5 % lower in 250 ml shaking flask consequently, cells were not uniformly distributed within the flasks. Most of the cells were captured in the lower part of the flask surrounded by feathers and this resulted in decreased enzyme production. Moreover, the results showed that when only constant impeller speed was used as a scale up parameter, lower enzyme production was yielded in 5 L reactor due to the lower k_La value. By changing the place of the upper impeller to a constant diameter of impeller/liquid height value of 2 L reactor, the same k_La value was achieved and 13% higher keratinase production was obtained in 5 L stirred tank reactor. This study showed that, impeller tip speed and k_La value were the key parameters for a successful keratinase production at lab scale using chicken feathers by *Streptomyces* sp.

The authors wish to thank The Scientific and Technical Research Council of Turkey (TUBITAK, 112M250) and Ege University Science and Technology Center (EBILTEM, 13BIL020) for the financial support.

Keywords: Keratinase production; Scale-up; *Streptomyces* sp.;Kla; Impeller tip speed; Chicken feather

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EXPLORATION OF MARINE ACTINOMYCETES FROM THE BAY OF BENGAL, INDIA FOR ANTIMICROBIALS AND ENZYMES

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Introduction: As the marine actinomycetes are surviving in the challenging marine environment, it is hypothesized that they might have developed unique metabolic and physiological capabilities different from terrestrial actinomycetes. This aspect could make them excellent candidates to produce novel secondary metabolites and various useful enzymes. The Bay of Bengal, India is one of the arms of Indian Ocean, stretching on 1,300 miles long and 1,000 miles wide. But, the vast area has not been intensively explored for marine microbial wealth.

Aim: To isolate marine actinomycetes from the Bay of Bengal, India and explore their potential as producers of antimicrobials and various enzymes.

Methods: Marine samples were collected from the Bay of Bengal, India during the cruise programmes with the help of National Institute of Ocean Technology, Chennai, India. Serial dilution technique was followed to isolate the marine actinomycetes using starch casein agar. Morphological and physiochemical characterization was carried out as per the standard protocols and documented. Antimicrobial activity was done against human and plant pathogens by dual culture technique. Secondary antimicrobial screening was carried out using their crude extracts. Standard qualitative and quantitative methods were used to determine the activity of different enzymes. Fermentation parameters were standardized to optimize the antimicrobial metabolites and an alkaline thermostable protease production. Colum chromatography was followed to purify the metabolites and protease.

Results: Totally 345 different marine actinomycetes were isolated from the Bay of Bengal. Majority of them exhibited pronounced antimicrobial activity against human and plant pathogens. Polyphasic taxonomy of the selected promising marine actinomycetes revealed that eight isolates are belonging to the genus *Streptomyces*. Of which, an isolate, MML1614 was identified as *Streptomyces fungicidicus*. Their 16S rRNA sequences have been deposited in the GenBank database of NCBI. An antimicrobial protein purified from *Streptomyces* sp. MML1703 showed strong antibacterial activity against methicilin resistant *Staphylococcus aureus* (MRSA). In addition, eight compounds were purified from *S. fungicidicus* MML1614. Of which, only four compounds were characterized owing to their antimicrobial activity. They were identified as N*β*-acetyltryptamine, anthranilic acid, 3-acetyl indole and N-acetyl pyrrol. Interestingly, all the four compounds have not been reported in *S. fungicidicus* before. Furthermore, 3-acetyl indole and N-acetyl pyrrol have not been found in microorganisms before and it is the first report. Among 208 marine actinomycetes screened for different enzymes, 183, 157, 116, 72 and 68 isolates produced lipase, caseinase, gelatinase, cellulase and amylase, respectively. Interestingly, *S. fungicidicus* MML1614 was found to produce high amount of protease. The partially purified an alkaline thermostable protease was able to remove blood stain effectively from cloths, when it was used along with the commercial detergents. Further, this alkaline thermostable serine protease was purified by ion-exchange and gel filtration chromatography up to 6.6-fold with 26.5% recovery and characterized.

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Conclusion: Results of the diversity of marine actinomycetes, their antimicrobial activity and enzymes production have increased the scope of finding industrially important marine actinomycetes from the Bay of Bengal, India as these organisms could be the vital sources for the discovery of various bioactive molecules and enzymes.

Keywords: Marine actinomycetes, Secondary metabolites

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EXPLORING THE DIVERSITY OF THE SECONDARY METABOLOME OF A MARINE STREPTOMYCETE USC633 USING THE NMR-GUIDED METABOLIC FINGERPRINT APPROACH

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Recently we have reported on the creation of proton nuclear magnetic resonance (^{1}H NMR) metabolic fingerprints of a natural product fraction library and its effectiveness in the identification and isolation of new and novel natural products.[1] By identifying unique spectral patterns, the NMR fingerprint approach enables a non-targeted interrogation of the drug-like natural product universe and has the potential to simplify and accelerate the current efforts in the isolation and identification of new small molecules. This methodology is exemplified on the exploration of biosynthetic capacity of a novel marine streptomycete species USC633, collected from the near-shore marine environment of the Sunshine Coast region of Australia. Through ^{1}H NMR metabolic fingerprints, we show the expression of natural products by the microbe is media dependant and give examples of the various secondary metabolites produced.

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ISOLATION, SELECTIVE SCREENING, IDENTIFICATION AND METABOLIC PROFILE STUDY OF THE ENDOPHYTIC MARINE ACTINOMYCETE STRAIN *STREPTOMYCES SUNDARBANSENSIS* WR1L1S8

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Although strongly studied, actinomycetes continue to prove themselves as reliable sources of new bioactive compounds. During the last decade, research was oriented towards the study of marine origin actinomycetes strains in order to identify new bioactive molecules for the treatment of infections caused by MRSA.

The main objective through this study was to evaluate the metabolic potential of *Actinomycetales* strains isolated from two marine algae species *Fucus* sp. and *Ulva lactuca* as well as marine sediments in the region of Bejaia (North East of Algeria). The use of selective five different culture media resulted in the isolation of 22 isolates. Preliminary screening based on the antibacterial activity of the strains against some pathogenic bacteria, led to the selection of an efficient strain denoted WR1L1S8. The combination of morphological, chemotaxonomic, physiological and molecular parameters via the sequencing of the 16S rDNA, revealed its relationship to the species *Streptomyces sundarbansensis*. In addition, the selected strain was subject to the production of these bioactive secondary metabolites on SCA medium previously optimized and an analysis of the metabolic profile of the crude extract by HPLC-DAD-ELSD coupled with mass spectrometry (ESI-MS). This analysis helped to highlight, first, the presence of polyketides of phaeochromycines group, isolated for the first time from the species *Streptomyces sundarbansensis*, but also the influence of culture conditions (culture medium, concentration seawater, pH) on their production. It was thus possible to isolate a novel compound having an anti MRSA activity confirmed by the use of pure compounds previously isolated and structurally characterized.

Keywords: Endophytic actinomycetes, Marine actinomycetes, *Streptomyces sundarbansensis*, Polyketides, Phaeochromycines, HPLC-DAD-ELSD/ESI-MS.

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EXTRACTION OF *JONESIA DENITRIFICANS* XYLANASES PRODUCED ON AGRICULTURAL COPRODUCTS BY PEG 4000/K_2HPO_4/KI

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Interest in xylanases from actinomycetes has increased markedly in the past decade, in part because of the application of these enzymes in the pulp and paper industry. The extensive use of enzymes in industrial applications necessitates the development of efficient and economical methods for their large scale separation, concentration and purification, since the most expensive part of biomolecule production is recovery and purification.An attractive and a simple method for enzyme separation is the utilization of an aqueous two phase system (ATPS) providing a mild environment for sensitive biopolymers like proteins, easy scale-up, short process time, high enzyme recovery, low capital and operating costs. The aim of our study is to choose the best ATPS for the extraction of *Jonesia denitrificans* xylanases. *Jonesia denitrificans*, an actinomycete witch grow well on agricultural co-products: orange peel, alfa and Retama, xylanase activities obtained using the three co-products were 0.662, 0.711 and 0.835 U/ml. in the system containing alfa and Retama, 57.5% and 72% of xylanase was located in the bottom phases and the purification factor was respectively 1.78 and 1.63. When we use orange peel as substrate in the fermentation medium, the ATPS composed of 3.5% PEG 4000 / 14% potassium phosphate/9% KI was favorable for partition of xylanases to the top phase, 82% xylanase recovery was obtained; the zymogram results verified partitioning of xylanases to the top phase. In conclusion, the best ATPS was made of 3.5% PEG 4000/14% K_2HPO_4/9% KI and 10% orange peel medium.

Keywords: Aqueous two phase system, Xylanases, *Jonesia denitrificans*actinomycetes

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MYCOBACTERIUM

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MYCOBACTERIAL STEROIDS FOR INDUSTRY (MYSTERI)

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Steroid hormones play a vital role in cell proliferation, influencing gene expression and tissue differentiation. Steroid based pharmaceuticals are therefore extremely important for health, with uses in disease prevention and therapies ranging from cancer and obesity treatments to human fertility. Conversion of cheap agricultural or food waste, containing phytosterols into these highly valuable steroids is therefore an attractive prospect. Previous studies have indicated that in order to achieve this goal several areas need improvement; including culture broth solubility, the microbial strains used for bioconversion as well as product recovery. By utilizing the bioconversion bacterium *Mycobacterium* sp. NRRL B-3805 we aim to replace the current microbial and chemical multistep processes with a single-step production of several steroids including androst-4-ene-3,17-dione (AD), 11-alpha-hydroxyandrost-4-ene-3,17-dione (11-alpha-OH-AD), 3-beta-hydroxyandrost-5-ene-17-one (DHEA) and testosterone from a cheap phytosterol feedstock. We have developed genetic engineering tools and procedures to manipulate *Mycobacterium* sp. NRRL B-3805 to improve its bioconversion of phytosterols. Mycobacterium sp. NRRL B-3805 metabolises steroids through the action of cholesterol oxidases, which modify the A-ring of the steroid core before cleaving the side chain through multiple enzymatic steps to produce AD, a precursor for C19 steroids. Optimisation of this innate process through manipulation of the existing catabolic enzymes is our initial goal, requiring a greater understanding of the phytosterol catabolic pathway. Genomic technologies have been implemented in order to increase our knowledge of these processes to assist rational strain development for an improved phytosterol bioconversion. Overall the use of single-step bioconversion will reduce production costs, loss of yields and be environmentally cleaner.

Keywords: Microbial bioconversion,Steroid production

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NOCARDIOSIS IN HIV-POSITIVE AND HIV-NEGATIVE INDIVIDUALS CLINICALLY SUSPECTED OF HAVING TUBERCULOSIS IN SUDAN

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Background and objective: In many developing countries chronic lung diseases, particularly tuberculosis is quite prevalent, but nocardial infections are either missed or misidentified in laboratory specimens. Correct identification is important in deciding the clinical relevance of a species and in management of patients. This study aimed to determine the frequency of nocardiosis in HIV-positive and HIV-negative individuals clinically suspected of having tuberculosis (TB).

Methods: The study population (n = 171) were those who attended chest hospitals in Khartoum State, Sudan, between January and March 2010. The patients suffered from pulmonary infections with positive acid-fast bacilli. Blood (n = 171) and sputum (n = 171) samples were collected simultaneously. Blood samples were tested serologically for the presence of antibodies using HIV/Intensified Combination Prevention (ICP) test and sputum were cultured onto Lowenstein Jensen slants according to standard methods. Isolates showing rapid growth characteristic of nocardiae were subcultured and subsequently identified using glucose yeast extract agar medium.

Results: All candidates in the study population (n = 171) suffered from pulmonary infections, nocardiosis was diagnosed in 4% (n = 7), HIV-positive cases were 17 (9.9%). Five *Nocardia* species were isolated from HIV-negative patients whereas two were from HIV-positive patients. *Nocardia* spp. cause pulmonary infections (4.09%) in both immunocompetent (2.92%) as well as immunocompromised (1.17%) patients who attend chest clinics in Sudan.

Conclusion: Ability of *Nocardia* species to cause diseases in human seems to be not necessarily affected by the immune status of individual, as it is evident from the results of the present study.

Keywords: Nocardia, HIV, Sudan

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PROTEOMICS

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DIFFERENTIAL PROTEOMICS OF WILD TYPE AND ENGINEERED STRAINS OF *S. CLAVULIGERUS*

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Introduction: *Streptomyces* genus of *Actinomycetales* are Gram positive bacteria renowned for producing highly diverse secondary metabolites including antibacterial, antifungal, antiviral, and antitumor compounds[1]. *Streptomycesclavuligerus* can produce several β-lactam antibiotics such as cephamycin C, penicillin N and clavulanic acid[2].

Proteome is "the analysis of the entire protein complement expressed by a genome, or by a cell or tissue type" and proteomics is the study of quantitative changes of protein expression levels[3].

S. clavuligerus NRRL3585 was genetically manipulated in our lab to construct one transformant strain (TB3585) and one recombinant strain (AK39) to increase cephamycin C production. TB3585 contains multiple *ask* genes and AK39 is the *hom*-deleted mutant strain of *S. clavuligerus* [4].

Objectives: Aim of the study is to investigate the effects of multiple copies of the *ask* gene and *hom* deletion on cell physiology of *S. clavuligerus* at proteomic level.

Methods: *S. clavuligerus* strains are grown at 28°C at 220 rpm in TSB medium. For TB3585, TSB medium is supplemented with 50 µg/mL thiostrepton and, for AK39 TSB medium is supplied with 200 µg/mL kanamycin. Protein extraction method is modified from Yin et al. (2013)[5]. Concentration of proteins is determined according to Ramagli and Rodriguez (1985)[6]. IEF was performed with "Ettan IPGphor3" System (GEHealthcare). The voltage applied to the gel was at least 70,000 V.h. Spot pattern analyses are accomplished by using the 2D image analysis software Delta2D version 3.4 (Decodon, Germany).

Results: In comparison to WT protein spots, AK39 strain displayed 187 differentially expressed protein spots with 115 spots down-regulated and 72 spots up-regulated. Furthermore, 268 protein spots were shown to be differentially expressed with 74 up-regulated spots and 194 down-regulated spots for TB3585.

Conclusion: 187 protein spots were differentially regulated for AK39 and 268 protein spots were differentially regulated for TB3585.

Distinct pattern of expression levels among the strains may be attributed to the changes in global expression patterns as a result of genetic manipulations on the strains.

Identification of the proteins by mass spectrometric analyses will aid in elucidation of the exact mechanisms behind the differential protein expression levels.

Keywords:*Streptomyces clavuligerus*, Cephamycin C, Proteomics

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A NOVEL BIOMATERIAL DESIGN TO INCORPORATE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY, USING A NOVEL *STREPTOMYCETE TYROSINASE*

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Tyrosinases (EC 1.14.18.1) are Type III copper proteins that utilize molecular oxygen to catalyse two types of reactions: monophenolase and catechol oxidase type reactions [1; 2]. Their relevance to biotechnology spans a number of applications, from bioremediation (e.g. phenol-contaminated wastewater) to biosensor technology and applications in biocatalysis reactions (e.g. production of L-3,4-dihydroxyphenylalanine or L-DOPA). Traditionally, tyrosinases from *Agaricus bisporus* (common button mushroom) are used for these applications, however, due to their limitations under certain reaction conditions, researchers have turned to bacterial tyrosinases due to the ease of their genetic manipulation and enzyme production [3;4]. Actinomycetes are predicted to produce tyrosinases for protection against the potential toxic effect of phenolic compounds and for the production of melanin.

It is the application of an actinomycete tyrosinase in cross-linking reactions that is the focus of this study.

In this study, a tyrosinase from *Streptomyces pharetrae* CZA14^T was used in the formation of a novel biomaterial. This biomaterial combines the biodegradable biopolymer chitosan with gelatin and L-DOPA to create a cross-linked product that provides a scaffold for the incorporation of rooibos (*Aspalathus linearis)* tea antioxidants and silver nanoparticles, in order to produce a biomaterial with both antioxidant and antibacterial activity. The majority of cross-linking reactions are carried out through chemical applications (e.g. using harsh chemicals such as glutaraldehyde). By using an actinomycete tyrosinase, we aimed to create a more bio-friendly product that has applications in both the medical and industrial fields.

The biomaterials generated contained specific combinations of the following as final reaction concentrations: 0.32% (w/v) chitosan solution, 5% (w/v) gelatin solution, 1mM L-DOPA, 1U/ml tyrosinase enzyme and 1% (v/v) unfermented rooibos tea extract. The final reaction volume was 1ml and all reactions took place in 1.5ml microcentrifuge tubes placed on a rotary carousel, incubated at 37^{o}C overnight. The antioxidant capacity of the biomaterials was tested using three different antioxidant assays: FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl) and TEAC (Trolox Equivalent Antioxidant Capacity).

Preliminary results suggest that after overnight cross-linking, the biomaterials retain their antioxidant capacity (based on the results of the antioxidant assays). Those biomaterials that were cross-linked with L-DOPA showed the highest antioxidant capacity, which may be due to the presence of melanin (produced as a by-product by the tyrosinase enzyme). Antioxidant capacities as high as 2724 µmol AAE/L (ascorbic acid equivalent per litre) and 1799 µmol AAE/L were recorded for the FRAP and DPPH assays, respectively, while the TEAC assay showed capacities as high as 6330 µmol TE/L (trolox equivalent per litre).

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As this project is still in its early stages, the silver nanoparticles have yet to be incorporated into the final product. Once these have been included, the biomaterials will be tested for their antibacterial activity against a number of test bacterial strains, using disc diffusion assays as well as testing in microtiter plate format using the MTT assay. Future work will involve the use of Fourier transform infrared spectroscopy to analyse the various biomaterials generated.

Keywords:*Streptomyces*, Tyrosinase, Biomaterial, Rooibos, Silver nanoparticles

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SEARCH AND BIODISCOVERY

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BIO-PROSPECTING FOR NOVEL METABOLITES FROM RARE THERMOPHILIC ACTINOMYCETES FROM UNDEREXPLORED THAR DESERT OF RAJASTHAN, INDIA

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The likelihood of isolating undiscovered strains from the terrestrial habitats has almost diminished. Therefore the search for novel products from actinomycetes has switched from normal habitats to the discovery of rare genera found in unusual habitats like deserts. Rare actinomycetes have usually been regarded as strains of actinomycetes whose isolation frequency by conventional methods is much lower than that of streptomycete strains. Most of the land area in Rajasthan state in India is covered with the Thar Desert. The full potential of the Thar domain with regard to the diversity of rare actinomycetes as the basis for biotechnology remains largely underexplored. The present study describes conventional and a completely novel *in situ* method for selective cultivation of actinomycetes directly from soil belonging to the Thar Desert. Such isolated actinomycetes strains have been characterized based on their phenotypic, molecular and broad spectrum antimicrobial specificities. A few promising strains have been screened to identify their phylogenetic relationship with actinomycetes and identify their fermentation products. The comprehensive results from the above study will be discussed.

Keywords:*In situ* cultivation, Selective isolation, Specific growth media, 16S rRNA sequencing

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GENOME OF THE NYSTATIN PRODUCER *STREPTOMYCESNOURSEI* ATCC 11455: SECONDARY METABOLITE BIOSYNTHESIS POTENTIAL AND ACTIVATION OF A SILENT GENE CLUSTER FOR NOVEL POLYENE MACROLIDE

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Streptomyces noursei ATCC 11455 is known as a producer of the medically important polyene macrolide nystatin used in treatment of superficial fungal infections. Earlier studies identified several gene clusters for antibiotic biosynthesis in this species, including nystatin[1], an antibacterial glycosylated macrolide[2] and albonoursin[3]. A high-quality draft of the *S. noursei* genome sequence was obtained using a combination of Illumina and PacBio technologies, yielding 17 contigs spanning over 9.66 Mb. antiSMASH 2.0 analysis of the genome revealed the presence of at least 38 gene clusters (ca 14.5 % of the whole genome) potentially governing biosynthesis of secondary metabolites. Among these, seven gene clusters harbouring genes for polyketide synthase type I were identified, including those for nystatin, the antibacterial glycosylated macrolide, and a putative polyene macrolide. Analysis of the latter gene cluster suggested that it is involved in the biosynthesis of a tetramycin-like molecule with an expanded macrolactone ring. Since no tetraenes other than nystatin and shunt products of its biosynthetic pathway have previously been identified in *S. noursei*, this cluster was probably not expressed under the conditions tested. A putative transcriptional regulator gene from the tetramycin-like cluster was cloned and introduced into the nystatin non-producing mutant under control of a strong constitutive promoter. The recombinant strain was shown to produce a tetraenic polyene macrolide, termed notrondomycin. Structure elucidation confirmed this compound as related but structurally distinct from tetramycin. The presented study provides an example of genome-based discovery of bioactive compounds that can revitalize antibiotic discovery programs.

Keywords: Secondary metabolites, Silent gene cluster, Polyene macrolide

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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF TETRANGOMYCIN ISOLATED FROM TERRESTRIAL *STREPTOMYCES* SP. CAH29

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A rhizosphere isolate *Streptomyces* sp. CAH29 showed strong antibacterial and antifungal activity against various test organisms. Based on 16S ribosomal RNA sequence homology studies, it was found to be similar to with *Streptomyces stramineus* (gene sequence similarity 99 %).

The major active metabolite was extracted, purified and identified as tetrangomycin. This angucycline metabolite exhibited inhibitory activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* with the inhibition zones 14, 10, 12 and 8 mm, respectively.

The antioxidant activities of crude ethyl acetate extract and tetrangomycin were evaluated by DPPH radical scavenging activity assays. The percentage DPPH scavenging activity of crude ethyl acetate extract ranged from 15.0% to 856.4% and tetrangomycin ranged from 10.0% to 30.4%.

The present study suggests that moderately halophilic strain *Streptomyces* sp. CAH29 may be a novel biological source for the discovery of antimicrobial and antioxidant agents.

Keywords:*Streptomyces*, Natural products, Tetrangomycin, Antimicrobial activity, Antioxidant activity

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MARINE ACTINOBACTERIA ASSOCIATED WITH INDONESIAN SPONGES: BIOSYNTHETIC CAPACITY, BIOACTIVITY AND BIODIVERSITY

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Indonesia is one of the biodiversity hotspots in the world and has become a leading target for biodiscovery and bioprospecting. Marine Actinobacteria receive our attention due to their biological diversity and their ability to produce novel metabolites and enzymes for wide purposes in biotechnology. The objectives of the research are to access and biosynthetic capacity, bioactivity and biodiversity of marine Actinobacteria associated with sponge as sources of new chemical entities for human health, aquaculture and agriculture.

Marine Actinobacteria were isolated from diverse sponges collected from the area of coral triangle: Ternate, Tulamben and Manado. The isolates were subjected for profiling their biosynthetic capacity, metabolites and bioactivities. Screening of particular genes for secondary metabolites was carried out using PCR and degenerate primers for NRPS, PKS, halogenase and HMGCoA reductase. High throughput bioassay screening using 96 well formats was employed to evaluate the activity for antibacterial, fish pathogen and anti cancer. Potential marine actinobacteria were selected based on gene of interest, diversity of metabolites and bioactivity. Identification of marine Actinobacteria was completed by amplifying of 16S rDNA gene using specific primers for Actinobacteria.

The results expected from this project are profiles of biosynthetic capacity, bioactivity and diversity of marine actinobacteria associated with sponges from Indonesia. The strategies presented here are combination of genetic screening, profiling metabolites and bioactivities to discover the genetic potential of sponge associated Actinobacteria to produce novel target compounds.

Keywords: Indonesia, Marine Actinobacteria, Diversity, Biosynthetic capacity and Bioactivity

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HEAVY METAL TOLERANCE OF *AMYCOLATOPSIS CIHANBEYLIENSIS*

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Introduction*:* The genus *Streptomyces* comprises a group of bacteria species with high economic importance. Several of these species are employed at industrial scale for the production of useful compounds. In addition to natural environments, strongly contaminated areas often are a result of industrial activity or mining. Due to the active secondary metabolism, streptomycetes also may be a good source for the identification of heavy metal binding components with possible future biotechnological application. Some *Streptomyces* species exhibit multiple tolerances against different metals and metalloids whereas others are more sensitive to them.

Objectives: The aim of the present study was to determine the level of heavy metal resistance of *Amycolatopsis cihanbeyliensis* isolated from saline environment in Turkey.

Material and Methods*:* *A. cihanbeyliensis* was tested on Bennet Agar (NaCl 5gr, Yeast extract 1gr, Beef extract 1gr, Casamino acid 2gr, Glucose 10gr, Agar 15gr, dH_2O 1000ml) plates supplemented with various concentration of each metal [$FeSO_4.7H_2O$, $CuSO_4$, $Hg(NO_3)_2$, $Ni(NO_3)_2$.6H2O, $Pb(NO_3)_2$]. Plates were dried at 37 ºC for 30 min and inoculated with 0.1 ml from exponentially grown cultures. Plates were incubated at 28°C and pH 7.2 for 7 days. Plates containing media with no added metal were inoculated in the same manner to serve as controls. MICs were determined as lowest concentrations of metal ion preventing growth.

Results and Conclusion*:* *A. cihanbeyliensis* demonstrated resistance to Pb, Fe and Ni indicating potential application for removal of these heavy metals.

Keywords:*Amycolatopsis cihanbeyliensis*, Heavy metals, MICs

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DEGRADATION OF AZODYE AZORUBINE E122 CI AND PONCEAU 4R CI16255 E124 BY A HALOTOLERANT ACTINOMYCETE *AMYCOLATOPSIS CIHANBEYLIENSIS*

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Introduction: Azo dyes are widely used for the coloration of products and generally believed to be toxic and carcinogenic or prepared from other known carcinogens. The discharge of these dye stuffs from industries into rivers and lakes results in a reduced dissolved oxygen concentration causing anoxic conditions, which subsequently affect aerobic organisms. Azorubine E122 CI and Ponceau 4R CI16255 E124 are synthetic food dye from the azo dye group. Because they are azo dyes, it may elicit intolerance in people allergic to salicylates. Additionally, it is a histamine liberator, and may intensify symptoms of asthma. Azo dyes can still be removed from wastewater by the microbial biomass via the process of biosorption. Biosorption by actinomycetes strains is also becoming a promising azo dyes removal process from wastewaters. Actinomycetes strains have been identified which decolorize effluents containing different types of dyes. But there has been no report on *Amycolatopsis cihanbeyliensis.*

Objectives: The objective of this study was to investigate the capacity of decolorization of the two food dyes Azorubine E122 CI 1472014720 [2-(4-Sulfo-1-naphthylazo)-1-naphthol-4-sulfonic acid disodium; 4-Hydroxy-3-((4-sulpho-1-naphthalenyl)azo)-1-naphthalenesulphonic acid disodium; Disodium 4-hydroxy-3-((4-sulphonatonaphthyl)azo)naphthalenesulphonateis] and Ponceau 4R CI16255 E124 [1-(4-sulpho-1-napthylazo)- 2-napthol- 6,8-disulphonic acid, trisodium salt] by *Amycolatopsis cihanbeyliensis* (type strain, BNT52T).

Material and Methods: The decolorization efficiency of *Amycolatopsis cihanbeyliensis* isolate was screened in Bennet broth containing 50g/L NaCl and 50mg/L dye. The broth medium was inoculated with 1 ml of exponantially grown culture and incubated at 27°C on a rotary shaker at 200 rpm for six days. The broth culture was then centrifuged and the optical density of the supernatant was measured. Biosorption percentage was calculated.

Results and Conclusion: The preliminary results indicate that *Amycolatopsis cihanbeyliensis* decolorized both of the dyes offering potential studies of optimization.

Keywords:*Amycolatopsis cihanbeyliensis*, Azo dyes, Degradation

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METAGENOMIC SEARCH FOR THE GENUS *KRIBBELLA* IN INDIGENOUS SOUTH AFRICAN PLANTS

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Kribbella is a small actinobacterial genus (family *Nocardioidaceae*) consisting of 20 species, three of which were isolated in South Africa. The majority of type strains were isolated from soil samples, while only *Kribbella lupini* and *Kribbella endophytica* were isolated from plant material. The goal of this study was to perform a targeted search for endophytic *Kribbella* strains within the Cape Floral kingdom of South Africa. We amplified *Kribbella* 16S rRNA gene sequences from five indigenous South African *fynbos* plants using a genus specific PCR primer.

A *Kribbella* specific 16S rRNA gene PCR primer (Krb977F) was designed from 16 *Kribbella* type strains, as well as 16S rRNA gene sequences from members of the families *Nocardioidaceae* and *Propionibacteriaceae*. The primer, when used with the universal 16S rRNA gene primer R5, amplifies a 535-bp fragment of the 16S rRNA gene. Total DNA was extracted directly from a soil sample using the Zymo Research Soil Microbe DNA Mini Prep kit, as well as from phosphate-buffered extracts of root and leaf samples of the five plants. An initial PCR experiment utilized universal 16S rRNA gene primers F1 and R5 to amplify all 16S rRNA gene sequences in the sample followed by a second, touchdown PCR experiment using the *Kribbella* specific 16S rRNA gene primer Krb977F with universal primer R5 to amplify *Kribbella* 16S rRNA gene sequences. The amplified products were then ligated into the pGEM-T Easy vector and transformed into *Escherichia coli* DH5α cells. White colonies were selected and screened by colony PCR (using primers Krb977F and R5), for inserts of the correct size. The cloned metagenomic DNA samples were purified and sequenced. Results were analysed using the Ez-Taxon-e database to determine the closest matches.

Kribbella 16S rRNA gene sequences were successfully amplified from the soil sample and each of the five leaf and five root samples. The Krb977F primer proved to be highly specific for *Kribbella* sequences as only eight sequences belonging to cultured type strains of other genera were amplified. Of a total of 320 metagenomic 16S-rRNA gene sequences, 178 (55.6%) were identified as belonging to *Kribbella*, eight (2.5%) were identified as non-*Kribbella* cultured strainsand 134 (41.9%) were most closely related to uncultured strains. Plate isolation techniques were also employed to isolate *Kribbella* strains from the plant samples. Although the low percent sequence similarity of some of the amplified environmental sequences indicated that the plants could contain new *Kribbella* species, no *Kribbella* colonies were obtained.

Kribbella strains appear to be ubiquitous in soil and in the leaves and roots of plants and this PCR-screening method can be utilized to screen environmental samples for the presence of *Kribbella* strains before culture-dependent methods are used. The fact that this study established the presence of *Kribbella* endophytes in all the plants screened provides motivation to search for and isolate more endophytic *Kribbella* species.

Keywords:*Kribbella*, Endophyte, Metagenomic, 16S rRNA

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DEREPLICATION AND IDENTIFICATION OF ANTIBIOTICS FROM ACTINOMYCETES

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Resistance to all antibiotic classes together with the recent development of multi-drug resistance in pathogens demonstrate that new antibiotics with novel scaffolds are needed and should be a priority. Unsuccessful strategies including high throughput screens against individual targets resulted in decline of the rate of antibiotic discovery in recent years. Good historical evidence that rare actinomycetes can produce novel antimicrobial compounds and estimates that only 1-3% of antibiotics produced by Streptomyces have been discovered so far suggest that returning to a validated method of screening natural products from actinomycetes is a promising approach in novel antibiotic discovery.

The objective of this experiment was to dereplicate and/or identify novel antibiotics produced by prioritized strains of Actinomycetes.

Extracts of Actinomycetes strains were screened against MDR gram-positive and gram-negative bacteria (resistant to over 15 different antibiotics) in order to select strains producing antibiotics with potential novel chemistry. Compounds with high inhibition cut-off values were prioritized for dereplication and identification. Two *Streptomyces* strains, DEM20663 and DEM30049, were identified as producers of broad-spectrum antibiotics. Selected strains were grown on a laboratory scale in both liquid and solid cultures. Disk diffusion bioassay coupled to a three-step chromatography purification protocol resulted in purification of four active compounds. These were subsequently analysed using high resolution mass spectrometry. DEM30049 produced in liquid culture two compounds which were identified as niphimycin – a potent antifungal, and ionomycin – potential anti-cancer drug. DEM20663 produced in liquid culture a novel antibiotic DEM20663/B which was identified using tandem mass spectroscopy as a novel derivative of streptothricin D. Compound DEM20663/A was demonstrated to possess a broad spectrum antibiotic activity and remains to be identified.

Here, we will report on the results of isolation, dereplication and identification of broad-spectrum antibiotics from prioritized Actinomycetes strains.

Keywords: Actinomycetes, Antibiotics, Natural products

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SIMULTANEOUS CR(VI) AND LINDANE BIOREMEDIATION BY *STREPTOMYCES SP*. M7: ENVIRONMENTAL FACTORS INFLUENCE EVALUATION

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The co-contaminated environments with metals and organic compounds are difficult to treat because of the mixed nature of the contaminants. Lindane is an organochlorine pesticide with high persistence and toxicity. The Cr (VI) is used by several industries, and it is toxic and mutagenic. Bioremediation is a low cost technology, which could be an alternative to remove these compounds. *Streptomyces* sp. M7, previously isolated and characterized, showed ability to remediate soil samples co-contaminated with Cr (VI) and lindane.

In this study, the effects of various environmental factors (physical, chemical and biological) on bioremediation of soils contaminated with Cr (VI) and lindane were studied using *Streptomyces* sp. M7, to optimize the process. Besides, the usefulness of *Lactuca sativa* as biomarker was evaluated.

Determination of optimum inoculum concentration of *Streptomyces* sp. M7: Soils were contaminated with 25 mg kg^{-1} of lindane and 50 mg kg^{-1} of Cr (VI), and inoculated with *Streptomyces* sp. M7: 0.5; 1.0; 2.0 and 4.0 g kg^{-1} soil. Controls non-contaminated or uninoculated with *Streptomyces* sp were performed. Flasks, with 200 g of soil were incubated for 14 days at 30°C. Residual contaminants concentrations were determined. The lowest concentration of *Streptomyces* sp. M7 that simultaneously allowing greater removal of Cr (VI) and lindane, in co-contaminated samples was 1 g kg^{-1}.

The influence of physical and chemical parameters on the soil bioremediation by *Streptomyces* sp. M7 was assessed using a full factorial design. The tested factors and levels were: Temperature: 25, 30, 35ºC; Humidity: 10, 20, 30%; Initial concentration of Cr (VI): 20, 50, 80 mg L^{-1}; Initial lindane concentration: 10, 25, 40 mg L^{-1}. The model, with 4 factors, demonstrated to be suitable for studying s soil bioremediation by *Streptomyces* sp. M7. It was possible to predict the performance of *Streptomyces* sp. M7 during bioremediation, knowing the initial conditions of the system. The higher Cr (VI) removal was produced under elevated temperature and low humidity. The higher lindane removal under high Cr (VI) initial concentration and humidity.

Effectiveness of both toxic removal by *Streptomyces* sp. M7 was evaluated using, ecotoxicity bioassays on *Lactuca sativa*. Germination and radicle and hypocotyl elongation were evaluated. The toxic effects were partially reversed after bioremediation process. Tested biological parameters on lettuce seedlings improved, which would indicate an effective decrease in the contaminant concentrations.

These results represent a significant advance in the study of co-contaminated environments bioremediation, and the next step would be scaling the process to achieve bioremediation of large environments that suffer mixed contamination.

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Keywords: Actinobacteria, Bioremediation, Cr, Lindane

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MULTIPLE ANTIBIOTICS PRODUCED FROM *AMYCOLATOPSIS* ISOLATE DEM30355

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New antibiotics are urgently needed to combat the rise of resistant bacteria. About two thirds of the clinically used antibiotics are produced by Actinomycetes or are semisynthetic versions of such. The genus *Amycolatopsis* is known to produce important antibiotics such as Rifamycin and Vancomycin. The Demuris screening programme identified promising activity from the *Amycolatopsis* isolate DEM30355. Dereplication using mass spectrometry identified putative novel compounds. To gain enough material for structure elucidation a scale up to 500L wv at the CPI was performed. The multiple step downstream method yielded in 235g of crude extract. Bioassay guided fractionation of this extract revealed three structurally unrelated antibiotics (DEM30355/A, DEM30355/B1 & DEM30355/C). DEM30355/C was identified as Vancoresmycin via tandem mass spectroscopy. DEM30355/B1 is a novel derivative of the Ansamycin type antibiotic Kanglemycin A, which was dereplicated using X-ray crystallography and mass spectrometry. DEM30355/A is a novel tricyclic, partial aromatic polyketide which was elucidated via X-ray crystallography and NMR experiments. The MIC against MRSA is 4-8µg/mL. Even though toxicity was observed towards a mammalian cell line, an epimer of DEM30355/A named DEM30355/A* showed little toxicity. The mode of action of this compound is currently under investigation.

Keywords: Natural product, Antibiotic, Scale up

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BIOACTIVE POTENTIAL OF ACTINOMYCETES ISOLATED FROM A DARK CAVE

Mathivanan Naryanasamy, Aroumougame Souprayane, Balaji Ravichandran, Ramkumar Dharmaraj

University of Madras

The dark cave habitat differs from other environments due to low level nutrients, temperature, and light intensity but has very high humidity. Many microbiologists believes that microbes collected from pristine sites that unexplored or rarely visited by humans were likely to be novel taxa or strains, which could produce unique beneficial chemical compounds and various enzymes. With this background, the present study was aimed to investigate the cave actinomycetes and their bioactive potential. Soil samples were collected from a dark cave in Tamil Nadu. The soil samples were treated at 50°C for 60 min and 14 different cave actinomycetes were isolated using actinomycetes specific agar media and these actinomycetes isolates were designated as MML2601-MML2614. The 16S rRNA sequencing was carried out to identify the cave actinomycetes. Among the 14 actinomycetes, 9 belong to the genus *Streptomyces*, 3 belong to *Nocardiopsis,* 1 belongs to *Micromonospora* and 1 belongs to *Prauseria.* All the isolates were able to grow in 50% $CaCO_3$. Among the 14 terrestrial actinomycetes, 10 actinomycetes showed antimicrobial activity against human pathogens, 11 showed antifungal activity against two plant pathogens. All the isolates were screened for industrial and therapeutic enzymes. Interestingly, all the isolates were capable of producing L- asparaginase enzymes, which is used for the treatment of acute lymphoblastic leukemia (ALL), since it catalyses the irreversible conversion of asparagine to L-aspartic acid thereby depriving malignant lymphoblastic cells. Further study is in progress to isolate, purify and characterize the L-asparaginase produced by the cave actinomycetes.

Keywords: Actinomycetes, Dark cave, Asparaginase

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ISOLATION OF HERBICIDE PRODUCING *STREPTOMYCES* SP. KR-004 AND INVESTIGATION OF ITS HERBICIDAL PROPERTIES

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With environmental problems arose from long-term use of synthetic chemical herbicides, microbe-originated herbicides were considered fascinating alternatives in current agriculture. In this study, we isolated herbicidal metabolites producing *Streptomyces* sp. KR-004 from soil near a lake in South Korea. Crude culture broth of the *Streptomyces* sp. KR-004 caused complete leaf burn-down and necrosis on a grass weed *Digitaria ciliaris* (southern crab-grass) in 7 days in concentration dependent manner. Above this, under greenhouse condition, the culture broth of *Streptomyces* sp. KR-004 showed over 90% of herbicidal effect on the other weed species such as *Echinochloa crus-galli*, *Sorghum bicolor*, *Aeschynomene indica*, *Abutilon avicennae*, *Xanthium strumarium*, *Alopecurus myosurodies*, *Viola mandshurica* and *Sicyos angulatus* respectively. It is meaningful that the *Streptomyces* sp. KR-004 culture broth killed noxious weeds *V. mandshurica* and *S. angulatus* living near water supply where is not allowed using synthetic chemical herbicides. The herbicidal activity of the culture broth of *Streptomyces* sp. KR-004 also lead to deccication of diverse weeds including *Erigeron annuus*, *A. indica*, *Persicaria dissitiflora*, *Rorippa indica*, *Artemisia annua* and many more in semi-field condition. These results suggest that the herbicidal metabolites produced from *Streptomyces* sp. KR-004 can be a bio-herbicide candidate with further research and development.

Keywords: Herbicidal metabolite, Bioherbicide

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ACTINOBACTERIAL DIVERSITY IN UNIQUE BIOTOPES: LIMESTONE HABITATS AND ETHNOMEDICINAL PLANTS IN MANIPUR, INDIA

Debananda Ningthoujam, Salam Nimaichand, Keishing Tamreihao, Rakhi Khunjamayum, Sarita Athokpam

Manipur University

137 actinomycetes were isolated from limestone habitats in Hundung, Manipur, India. ARDRA analysis placed these in 31 phylotypes of *Streptomyces*, *Micromonospora*, *Arthrobacter*, *Amycolatopsis*, *Janibacter*, *Rhodococcus*, *Nocardia*, and *Pseudonocardia*. *Streptomyces* was the dominant taxon and this paper is the first instance of *Janibacter* and *Kitasatospora* from limestone biotopes. Based on 16S sequencing, 10 putative novel strains were found, of which 3 were characterized as *Micromonospora kangleipakensis*, *Streptomyces manipurensis* (**MBRL 201**), and *Streptomyces hundungensis*. Some isolates esp. MBRL 201 showed promising antimicrobial, biocntrol and plant growth promoting (PGP) potential. In addition, MBRL 201 and some endophytic strains esp. TgR6 (teak endophyte) and SxF1 and SxF2 (*Solanum xanthocarpum* isolates) exhibited significant antimycobacterial capacities.

Keywords: Actinomycete diversity, Unique biotopes, ARDRA, 16S, Antimicrobial, Biocontrol, PGP.

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BIOSYNTHETIC PATHWAY OF NONPROTEINOGENIC HERBICIDE FROM SOIL-DERIVED *STREPTOMYCES* SP. KR-004

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The abuse of Synthetic pesticides cause severe environmental and health problems such as residual toxicity and increased resistance in pests. Therefore needs for development biopesticides have been increased to substitute synthetic pesticides. Biopesticides called as biocontrol agents or green pesticides protect crops from harmful pests and pathogens. The biopestidice which comes from natural materials or organisms have advantages in term of minimizing dexterous effects of existing pesticides in terms of good resistance management, restricted entry interval, safe residues on plants. Previous study proved that *Actinomycetes* strain *Streptomyces* sp. KR-004 showed herbicidal activity against weed species such as *Echinochloa crus-galli*, *Sorghum bicolor*, *Aeschynomene indica*, *Abutilon avicennae*, *Xanthium strumarium*, *Alopecurus myosurodies*, *Viola mandshurica* and *Sicyos angulatus.* Furthermore we could isolate and characterize the herbicidal metabolites from *Streptomyces* sp. KR-004 cultures. Previous study revealed that *Enterobacteria*, *Photohabdus luminescens* produced same metabolites through nonproteogenic synthetic pathway. We found conserved regions which share similar amino acid sequences between *Streptomyces sp*. and *Photorhabdus sp*. in the nonproteogenic synthetic pathway. The reduced amino acid sequences which matched with functional genes in the nonproteogenic synthetic pathway of *Photohabdus luminescens* suggested that the similarity of a genetic structure among different species contribute to produce the same herbicidal molecule. This is the first study to reveal the synthetic pathway of an herbicide in *Streptomyces* sp.

Keywords: *Streptomyces* sp. KR-004, Biopesticides

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MICROBIAL LIFE AT HIGH ALTITUDE: ACTINOBACTERIA DIVERSITY OF SURFACE AND SUBSURFACE ENVIRONMENTAL SAMPLES FROM THE ATACAMA DESERT

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Culturable studies based on the use of selective isolation media showed that low, but taxonomically diverse actinobacteria are present in hyper-arid and extreme hyper arid Atacama Desert soils despite prevailing harsh environmental conditions. In this study, we used a culture-independent approach to determine the actinobacterial diversity in surface (2cm depth) and subsurface (30cm depth) soils collected from three different high altitude sites (3000m, 4000m and 5000m) in the vicinity of the Atacama Large Millimeter Array (ALMA) Observatory (23^{O}04'39''S 67^{O}57'43''W). Community DNA was extracted from soil samples and 16S rRNA gene fragments amplified by primers specific for actinobacteria, Com2xf/Ac1186r, each labelled with individual barcodes. The products were purified, quantified and sent for pyrosequencing prior to analysis. PCR amplified actinobacterial 16S rRNA gene sequences yielded 52300 reads (average length ~270bp) which were grouped into 42 genera belonging to 30 families. The dominant actinobacterial genera detected in all soil samples were *Amycolatopsis,Geodermatophilus, Kribbella, Mycobacterium, Modestobacter, Pseudonocardia, Rathayibacter* and *Streptomyces; Pseudonocardia* was the predominant genus. Differences were detected in relative sequence abundance of actinobacteria in subsurface compared to surface samples. Genera *Modestobacter* and *Geodermatophilus* showed higher abundance in surface samples compared to subsurface samples. In contrast, some genera, like *Kribbella* and *Pseudonocardia* showed higher abundance in subsurface samples while others such as members of the genera *Amycolatopsis* and *Rhodococcus* were discontinuously distributed. The study demonstrates that high altitude Atacama Desert soils contain taxonomically diverse actinobacterial communities that provide a novel resource exploited for biotechnological purposes.

Keywords: Pyrosequencing. Atacama Desert. Culture-independent

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L-ASPARAGINASE PRODUCTION BY ACTINOMYCETES ISOLATED FROM CALCIUM CARBONATE RICH DARK CAVE SOIL OF A LIMESTONE HILL

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Introduction: Dark caves are peculiar habitats with low level nutrients, temperature, and light intensity but have very high humidity. Presently, research on different cave environments is focused to explore rare or novel microorganisms especially actinomycetes. Some of them include isolation of actinomycetes from gold mine in Korea, Reed Flute cave in China, Grotta Dei Cervi Cave in Italy, Thai caves and caves occupied by bats in Spain. Actinomycetes are in the limelight for more than 60 year in academic and industrial arena as they are prolific producers of various bioactive compounds and industrial enzymes. Importantly, actinomycetes were already reported to produce the therapeutic enzyme, L-asparaginase in the presence of calcium carbonate.

Objective: The present study was aimed to isolate the cave actinomycetes from a dark cave soil of a limestone hill and investigate their potential to produce L-asparaginase.

Methods: Soil samples were collected from a dark cave of a limestone hill at Batu Cave, Malaysia, which is around 400 million years old with 400 meters long and 100 meters high. Soil samples were treated at 50°C for 60 min, serially diluted, plated on actinomycetes specific agar media and incubated at 28±2°C. Development of actinomycetes colonies was observed periodically and subcultured on starch casein agar. Morphological and physiochemical characteristics of all the isolated cave actinomycetes were documented. Genomic DNAs were extracted from all the isolates, the 16S rRNA gene was amplified, sequenced and submitted in the GenBank data base of NCBI. Polyphasic taxonomy was carried out to identify the cave actinomyetes. All the isolated cave actinomycetes were screened for the production of different enzymes with special emphasis on L-asparaginase. Culture conditions were standardized for the promising isolate, MML2604 to optimize the enzyme production.

Results: A total of 14 different actinomycetes were isolated from a dark cave soil samples and designated them from MML2601 to MML2614. All the 14 isolates were capable of growing in 10% $CaCO_3$ amended agar medium without any carbon and nitrogen sources. Interestingly, all the isolates were capable of growing in 50% $CaCO_3$ amended medium with the supplement of carbon and nitrogen sources and seven of them were able to grow even up to 75% $CaCO_3$. All the 14 isolates were capable of producing L-asparaginase, which is used for the treatment of acute lymphoblastic leukemia (ALL). Carboxymethyl cellulose and casein were enhanced the L-asparagine production by the cave actinomycetes isolate, MML2604, when used as carbon and nitrogen, respectively sources. Further, high L-asparaginase activity was measured when 48 h old seed culture was used at 5% as inoculum to culture the isolate, MML2604 at pH 8 and at 35°C for 5 days.

Conclusion: There is an ample possibility to identify rare or novel actinomycetes from the dark caves with diverse bioactive potential. The capacity to grow at high concentration of $CaCO_3$ is a unique feature of these cave actinomycetes. As all the 14 cave actinomycetes were able to produce the therapeutic enzyme, L-asparaginase, there is a tremendous scope for their industrial exploitation.

Keywords: L-asparaginase, Dark caves, Actinomycetes

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MOLECULAR AND CULTURAL CHARACTERIZATION OF ANTIBIOTIC PRODUCING SOIL ACTINOMYCETES RECOVERED FROM DIFFERENT HABITATS IN UAE

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In this study six different soil samples in the mid-region of the UAE were screened for the presence of inhibitory compounds-producing streptomycetes. Soil samples were enriched to recover streptomycetes on starch casein nitrate (SCN) agar medium. The recovered colonies were macroscopically and microscopically examined, and then their potential to inhibit the growth of different clinical multi-drug resistant Gram positive and negative pathogens was tested by the agar diffusion method. A total of 19 different actinomycetal isolates were recovered then characterized and assessed for their antagonistic activity. Results indicated that only one strain (strain 73) was active against all tested pathogens with an inhibition zone ranging between 10 and 15 mm in diameter. The most potent antibiotic-producing isolates were subjected to genomic DNA extraction in order to perform PCR amplification using specific primers targeting actinomycetes-specific sequences as well *Streptomyces*-specific sequence. Overall, three isolates (43, 73, and 74) were confirmed to be actinomycetes which showed a single band of an approximate size of 350 bp, indicating that all 3 isolates have the actinomycetes 16S ribosomal DNA-specific sequence. Also, PCR results confirmed that two isolates (73 and 74) to be *Streptomyces* strains with an approximate size of 1000 bp. Based on the results of this study it can be concluded that the molecular characterization using PCR amplification and PCR-specific primers correlates with what was obtained from the microbial and cultural characterization, and they can be applied for differentiation in screening projects, taxonomic characterization, and phylogenetic analysis. The inhibitory effect of the recovered streptomycetes isolates from unique habitats in the UAE may suggest the novelty of the inhibitory compound(s) produced by the *Streptomyces* sp. 73 and the other promising strains.

Keywords: Actinomycetes, Activity, Antibiotics, *Streptomyces*, 16S rDNA

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STRUCTURE ELUCIDATION OF PERSIPEPTIDES, NEW CYCLIC PENTAPEPTIDES FROM *STREPTOMYCES* SP. UTMC 1154

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Introduction: Many cyclopeptides exhibiting unique structures and interesting pharmacological activities have been isolated from microorganisms namely actinomycetes. Actinomycetes and in particular *Streptomyces* genus have been the source of many antibiotics including those which are presently the 'last line of defense' against multi-resistant bacteria. Despite intensive investigation of *Streptomyces*, still new antibiotic structures are discovering form this genus.

Objective: During our ongoing search for new antimicrobial natural products, *Streptomyces* sp. UTMC1154 was subjected to a chemical and biological screening leading to the isolation of new secondary metabolites.

Methods: After 7 days fermentation, the culture was filtered and extracted using Amberlite XAD16. Obtained crude extracts were subjected to column chromatography on silica gel using a CH2Cl2/MeOH gradient.

Their structures were established using 1D and 2D NMR experiments. 2D TOCSY experiments were applied to identify the amino acid residues, while HMBC correlations were used to determine their sequence. The chemical shifts were expressed in d values using CDCl3 and DMSO as solvent and TMS as internal standard.

Configuration of the amino acid units were determined by comparing the chromatograms with those of derivatives of commercially available amino acids.

Result: Peptides A and B were isolated from the fractions with 5% and 10% MeOH, respectively. They were purified on Sephadex LH20 (MeOH) and further by repeated preparative TLC (CH2Cl2/MeOH, 9:1) to yield 12 mg of A and B mg of 2, respectively. Persipeptides were isolated as a colorless oil of the molecular formula C35H49N5O5, determined by HRESIMS. Analysis of its 1H and 13C NMR and HSQC data revealed three amide protons, eight methyl groups including two N-methyls, two methylene groups, five methines, two mono-substituted phenyl rings and five carbonyl carbons. Detailed interpretation of 1H–1H COSY, TOCSY, HSQC and HMBC data established the amino acids as two valines, one N-methylvaline, one phenylalanine and one N-methylphenylalanine residue. Their sequence was determined using HMBC correlations between the NH or N-CH3 protons, respectively, with the two amide carbons in their vicinity. According to Marfey's method, all amino acids had the L-configuration. Molecular identification experiments introduced the producing strain most closely related to *Streptomyces coerulescens* DSM 40146^T (99.2 %).

Conclusion: The two cyclic peptides had the same ring size and amino acid composition, but differed in their sequence. The main difference between persipeptides and related cyclopeptides lies in the amino acid composition, whereby the persipeptides have basically only two types of amino acids while others are composed of at least three or more amino acids.

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The N-methylation of peptides results in modified pharmacokinetic properties. Hydrogen bonding and aggregation are reduced, and the increased lipophilicity allows a facile penetration of plasma membranes besides stability of the amide bonds against endopeptidases.

In the disk diffusion test, a mixture of persipeptide A and B showed activity against *Staphylococcus aureus* in concentration of 40 µg/disk. Although still not experimentally validated but antiviral and acetyl esterase inhibitors are amongst their predicted biological activity based on structural prediction analysis.

Keywords:*Streptomyces*, Cyclic peptides, Persipeptides, Antibacterial activity

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ANTIOXIDANT ACTIVITY OF SECONDARY METABOLITES PRODUCED BY *KRIBBELLA SHIRAZENSIS* UTMC 693

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Introduction: Regarding to oxidative stress role in numerous diseases, finding new compounds with antioxidant activity is still necessary. Actinobacteria especially rare genera and among them new species are promising candidate to screen their secondary metabolite for finding antioxidant compounds. In order to seek compound with antioxidant activity, *Kribbella shirazensis* UTMC 693 was investigated as a recognized new species which is capable of producing a number of secondary metabolites.

Objective: In this study we investigated antioxidant activity of *K. shirazensis* extract by in vitro bioassay of DPPH, DNA damage tests and xanthine oxidase inhibition.

Methods: Strain UTMC 693 was isolated on AV agar from treated sample by with UV at 254 nm for 10 min in 20 cm distance. The strain was characterized by a polyphasic approach including morphological, physiological, molecular and chemotaxonomical approache. In order to assess the antioxidant activity, crude extract of its secondary metabolites prepared by ethyl acetate extraction and concentrated to dryness.

The antioxidant activity of the extract and ascorbic acid (200µg/ml) as standard was assessed by DPPH method. DNA damage protection activity of extract was evaluated by DNA damage which was generated by hydroxyl radical in Fenton reaction and glutathione used as positive control (200µg/ml). Negative control wells contained no extract. The extract also was screened for possible antioxidant activity by xanthine oxidase inhibition assay. Allopurinol was used as positive control (1mg/ml) and negative control contained no extract.

Result: Strain UTMC 693 was isolated from a non-rhizospherical soil sample taken at a depth of ~10 cm in Shiraz, Fars Province, Iran. Strain UTMC 693 showed the typical morphology of *Kribbella* and could be differentiated from closely related species by genotypic characteristics, chemotaxonomical results and other phenotypic markers. Based on these results, strain UTMC 693 was considered the type strain of a novel species in the genus *Kribbella*, for which the name *K. shirazensis* UTMC 693^T (JN038072) was proposed. The *K. shirazensis* ethyl acetate extract showed 59% antioxidant activity in DPPH test in 2.5mg/ml concentration, this extract display 19%, 20, 23% antioxidant activity in 200, 400 and 800µg/ml respectively. Ascorbic acid has 80% antioxidant activity in 200µg/ml concentration. Also its metabolites demonstrated protection ability against DNA damage which was induced by hydroxyl radical in 3mg/ml concentration. The extract showed 30% xanthine oxidase inhibitory activity in concentration of 1mg/ml compared to 66% for allopurinol.

Conclusion: It is a more promising approach to search for new bioactive compounds among metabolites of new species in *Actionobactria*, especially rare genera and new species, like the target species of this work. This study showed that extract of *K. shirazensis* contains compounds that have at least three kinds of antioxidant activities. Although in comparison, the antioxidant activity of *K. shirazensis* was slightly lower than that some other antioxidant from plant or animal source, for example 50 µg of quercetin, rhamnetin, isorhamnetin which has the DPPH radical scavenging rate of 79.2%, 62.5% and 60.1%, respectively. Further work on purification and structure elucidation of antioxidant compounds of *K. shirazensis*is are under progress.

Keywords: New species, *Kribbella*, Secondary metabolite, Antioxidant

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PHENOL TOLERANCE, ENZYME PRODUCTION AND DYE DECOLORIZATION CAPACITIES OF ACTINOMYCETES FROM DUZKIR CAVE (ALADAGLAR, TURKEY)

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In this study, actinomycetes which isolated from rock wall and speleothem surfaces of a newly discovered Düzkır cave with 3344 m elevation from Aladağlar, Turkey were used. Totally 95 isolates were tested for their phenol tolerance, laccase and amylase production, and dye decolorization (cibacron blue, remazol black, remazol brilliant blue R) capacities on solid media. Guaiacol, gallic acid and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were used as indicator for laccase production. Out of 95 isolates, 65 isolates exhibited amylase production. On the other hand cibacron blue, remazol black, and remazol brilliant blue R were decolorized by 21, 20, and 18 isolates, respectively. Phenol tolerance and laccase production were determined in five and two isolates, respectively. The results indicated that the isolates obtained from this extreme habitat have great potential for enzyme production and biodegradation studies.

Keywords: Düzkır cave, Dye decolorization, Enzyme, Phenol tolerance

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IN VITRO ANTIOXIDANT PROFILE OF ACTINOMYCETES FROM AYVACIK SINKHOLE (ODEMIS, IZMIR, TURKEY)

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As a part of our efforts to determine antioxidant and antitumor activities of actinomycetes isolates from caves, 42 isolates from Ayvacık sinkhole were studied for their antioxidant activity. As a first step, to select best growth medium and culture type, four growth media and two culture type were compared for their effects on the antioxidant activity of fermentation medium of the actinomycetes isolates. For that purpose, representatively selected four isolate were grown on Starch Casein Broth (SCB), glycerol peptone broth (GPB), International Streptomyces Project Medium 1 (ISP 1), and soy flour broth (SFB) at static and submerged culture types at 125 rpm, 27°C, 7 days. The antioxidant activities of fermentation fluids of actinomycetes isolates were investigated using the total phenolic content and DPPH radical-scavenging activity assays. As a result SFB medium and submerged culture conditions supported to the best antioxidant activity.

As a second step, to determine total phenolic content and DPPH radical-scavenging activity of their fermentation fluids, all of the actinomycetes isolates from Ödemiş sinkhole were studied with the selected culture conditions. The results showed that 9 and 19 actinomycetes isolates gave equally effective or higher DPPH Radical-Scavenging Activity than butylated hydroxy toluene (BHT) and ascorbic acid which were used as positive controls, respectively. Consequently, the results of antioxidant activity produced by fermentation fluids clearly indicated that caves can be a good source to determine new and/or strong antioxidant compounds from actinomycetes.

Keywords: Antioxidant activity, Ayvacık sinkhole, Total phenolic content

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STUDY OF THE ADHESIVE POWER OF ACTINOMYCETES ON DIFFERENT SUBSTRATES

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Actinomycetes are regarded as the major producers of industrial, agricultural and environmental interest bioactive molecules, especially in the soil where it can contribute to its fertility by nitrogen fixation, decomposition of organic and recalcitrant compounds. The adhesion of bacterial cells to substrate (first stage of biofilm formation) is governed by physicochemical properties of both surfaces (cells and substrata). The aim of this work was to examine the adhesion of actinomycetes cells isolated from different soils to different surface models.

The objects of the study strains are isolated from soils of the the region Beni Amir Morocco, Streptomyces strain youssoufiensis from phosphate rock of khouribga Morocco. Middle Olson, Bennet and minimal synthetic medium were used for the isolation and growth of actinomycetes. Three substrates are selected for this study: the glass, widely used substrate in this type of study and clay, constituting major soils and the Moroccan phosphate rock as a special case. Characterization of the surfaces of bacteria was obtained by the method of MASTS (Microbial adhesion to solvents) and the adhesive behaviour estimation is based on sedimentation of the bacteria adsorbed to substrata power (clay and rock) and expressed as the percentage of cells adhered to the substrata. The observation of bacteria adhered to the glass is made by optical microscope after Gram staining.

Physicochemical characterization of actinomycetes isolated from several soils revealed a hydrophilic character and basic (electron donor) very marked. Nevertheless, the hydrophilic character is variable depending on environmental conditions and the physiologic stage of the bacterium Stadium. A very marked adhesive character opposite clay says the colonization of this group of microorganisms in very diverse environments and even very hostile. The hydrophobicity of *Streptomyces youssoufiensis* changes during growth. The bacterium begins very hydrophilic and presents a peak on the third day to decline thereafter. This increase exceeds war the threshold of the hydrophilicity (affinity with hexane < 50%).

Colonization of rock phosphate by *Streptomyces youssoufiensis* strain seems to be linked to the metabolic activities of bacteria that would development a biofertilizer in promoting the formation of biofilms.

Keywords: Actinomycetes ısolation, Physical chemistry, Mats, Adhésion, Biofilm, Soils, Clay, Phosphate rock, Biofertilizer

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L-ASPARAGINASE AND FIBRINOLYTIC ACTIVITIES OF SOIL ACTINOMYCETES

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Microbial enzymes play important role in nature degrading the organic residues. They are also promising agents for treatment of cardiovascular diseases and cancer that occupy the leading position among diseases causing human death. Fibrinolytic proteases that can directly degrade the fibrin in the blood clots have been discovered from bacteria, actinomycetes and fungi. Microbial L-asparaginase used as the effective agent in chemotherapy of such cancer as acute lymphoblastic leukemia and lymphosarcoma were found to be produced by actinobacteria from the genera *Amycolatopsis, Corynebacterium, Mycobacterium, Nocardia, Pseudonocardia* and *Streptomyces*. Actinobacteria of Mongolian soils are known for their high potential for biosynthesis of antibiotics and enzymes. Therefore, they can be a promising source for screening of producers of anticancer enzyme L-asparaginase and fibrinolytic proteases. However, no study has been done to explore these activities among Mongolian actinomycetes. Here we present the results of evaluation of 19 strains isolated from Mongolian soils for production of these enzymes. Activity for L-asparaginase production was identified by a rapid plate assay method with use of asparagine-dextrose salts agar (ADS) supplemented with phenol red (0.009% final concentration). Strains forming a pink zone were considered as positive for production of L-asparaginase. Fibrinolytic activity was estimated by measuring the diameter of a clear zone formed on plasminogen-free fibrin plates after incubation at 37^0C for 6, 12 and 24 hours. Results of PCR amplification with the primer set AMY-2 and ATOP specific for the genus *Amycolatopsis* and the 16S rDNA sequencing showed that 8 strains belonged to that genus. Three strains were assigned to the genus *Streptomyces* based on results of 16S rDNA sequencing. Other strains were filamentous actinomycetes. L-asparaginase activity was found in 9 strains after 24 hours and 14 strains after 48 hours. All 8 strains assigned to the genus *Amycolatopsis* were able to produce L-asparaginase after 48 hours. As for fibrinolytic activity, after 6 hours of incubation 1 strain, after 12 hours – 4 strains and after 24 hours 9 strains showed fibrinolytic activity. From 8 strains of the genus *Amycolatopsis* 3 strains had fibrinolytic activity, but activity was low (7-12.5 mm). The most active strain with unidentified taxonomic position formed the clear zone with diameter 17 mm after 24 hours. It had no L-asparaginase activity. Thus, the results received revealed a high potential for production of L-asparaginase and fibrinolytic proteases among actinobacterial strains studied: 73.7% of strains studied produced L-asparaginase and 47.4% had fibrinolytic activity.

Keywords: Anticancer enzymes, Proteases, Thrombosis, Amycolatopsis, *Streptomyces*

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SELECTIVE ISOLATION

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SCREENING OF AUTOCHTHONOUS *STREPTOMYCES* SPP. ISOLATED FROM WESTERN ALGERIA AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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Isolation and characterization of new strains of *Streptomyces* is a topic of great interest in the search for new antibiotics or new bioactive molecules of interest. This study is devoted to the development of collection of the genus *Streptomyces* strains from samples of different habitats that have undergone different treatments ($CaCO_3$, heat). The isolation and purification of the strains were prepared on ISP2 medium gave better results compared to other media used, their conservation has been done on liquid ISP2 medium 15% glycerol. The best treatment samples with satisfactory results by *Streptomyces* diversified presence and absence of fungal contamination compared to other treatments was that of $CaCO_3$. In total 85 strains suspected be related to the genus *Streptomyces* have been isolated, selected and stored. Antibacterial activity of different isolates were tested against the following pathogenic bacterial strains: *Escherichia coli, Staphylococcus aureus, Candida albicans, Klebsiella pneumonea* ATCC 4352, *Pseudomonas. aeruginosa* ATCC 27853, *Listeria monocytogenes* NCTC. Three strains (TB1, SM2.2 and SM1B1) presented an interesting antibacterial activity on almost all pathogenic strains used, their inhibitory potential was demonstrated by the double-layer technique incubation of 28 C for 12 days. Furthermore, the extraction of these three strains synthesized molecules by fermentation was performed for 7 days. The extraction of secondary metabolites from the float is provided by chloroform and concentration from the organic phase required the use of rotary evaporator. In another step, a thin layer chromatography (TLC) was performed on a silica gel plate to separate the different metabolites synthesized. The samples were placed 1 cm above the boundary of the eluting system chloroform, after migration, visualization was done by a plate Ultra Violet, and the revelation showed the presence of UV-absorbing molecules and the presence alkaloids. Over the word, the research of novel *Streptomyces* strains is important in order to satisfy the increasing request of the market and to obtain new functional products.

Keywords:*Streptomyces*, ISP2 medium, *Staphylococcus aureus*, TLC, Antibacterial activity

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CLONING AND CHARACTERIZATION OF BENZOATE DIOXYGENASE GENE FROM *RHODOCOCCUS RUBER* UKMP-5M

Ainon Hamzah

UKM

Some Gram-positive bacteria, including actinomycetes can also mineralize a wide array of hydrocarbons, including n-alkanes and aromatic compounds. This capability is due to actinomycetes possess enzymes that can degrade these compounds and used it for carbon and energy source. *Rhodococcus ruber* UKMP-5M has been isolated from oil contaminated soil in Malaysia. Preliminary study showed this bacterium able to use crude oil as well as toluene as sole source of carbon and energy. Hence, the objective of this study is to determine benzoate dioxygenase gene that involve in the benzoate degradation.

Total DNA from *R. ruber* UKMP-5M was extracted by Wizard genomic DNA purification kit (Promega). By using specific primers, the benzoate dioxygenase gene (*ben*A) gene was amplified, and the PCR product cloned into pGEM-T vector to construct plasmid pGEMT-benA. The recombinant plasmid pGEMT-benA was digested by double restriction enzymes *BamH*I and *Hind*III and ligated into plasmid pET 28b resulting new plasmid pET28b-benA which was then transformed into *Escherichia coli* BL21 (DE3) as a protein expression host.

The optimum concentration of sodium benzoate for recombinant *E. coli* BL21 growth was also determined. The recombinant *E. coli* BL21 was induced with 0.5 mM isopropyl *β-D*-thiogalactoside (IPTG) at 22°C at different incubation time. The recombinant benzoate dioxygenase was purified by ion exchange chromatography and the product was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. The end product from the benzoate dioxygenase degradation was identified by GC-MS.

The *ben*A gene from *R. ruber* UKMP-5M amplified at 64°C yielding a product of 0.6 kb band. The recombinant plasmid pGEMT-*benA* constructed which produced 3.6 kb. Sequences analysis of inserted DNA showed 70% similarity with aromatic ring dioxygenase *Rhodococcus equi* 103S. The pGEMT-benA plasmid digested by *BamH*I and *Hind*III resulting in two fragments; the linear pGEMT fragment (~3000 bp) and benA fragment (~ 600 bp). The recombinant pET 28b-benA was ~ 6 kb which included pET 28b (5.4 kb) and benA fragment (0.6 kb). The recombinant *E. coli* BL21 (DE3) produced highest growth in MSM with 0.5 mM sodium benzoate at 24 hours incubation. The recombinant cells *E. coli* BL21 produced benzoate dioxygenase after the growth was induced with 0.5 mM IPTG at 6 hours incubation. The molecular weight of benzoate dioxygenase was estimated 25 kDa with isoelectric point (pI) of 10.38. The highest benzoate dioxygenase activity was 6.54 U/mL at 25°C. The optimal pH and temperature for benzoate dioxygenase activity was 8.5 and 25°C, respectively. The V_{max} and K_m calculated from Lineweaver-Burk plots were 7.36 U/mL and 5.58 µM, respectively. The recombinant benzoate dioxygenase from *R. ruber* UKMP-5M can utilized benzene, ethyl benzene and benzoic acid, as well as sodium benzoate. The end metabolite of benzoate dioxygenase reaction was cyclohexamine dione. Benzoate dioxygenase family showed high similarity in sequences with other aromatic dehydrogenases such as biphenyl and toluene dioxygenase. The recombinant benzoate dioxygenase from *R. ruber* UKMP-5M showed capacity for benzoate degradation.

Keywords: Benzoate deoxygenase, *Rhodococcus ruber*, *benA* gene

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AEROBIC BIODEGRADATION OF HEXACHLOROCYCLOHEXANE ISOMERS BY *STREPTOMYCES* SP. M7

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The Hexachlorocyclohexane (HCH) isomers are highly toxic and persistent organochlorine pesticides, which have caused serious environmental problems since they began to be produced at the beginning of the1940s. During the synthesis of lindane (g-HCH), other isomers were also synthesized. There are seven isomers (a, b, d, e, g, q, h), but only g-HCH possesses insecticidal activity. However, the other isomers are still toxic for humans and in the environment they cause an accumulation of the more persistent form, the β-HCH. For this reason we have focused on the ability of biotransformation of HCH isomers by Gram-positive microorganisms and particularly by actinobacteria species.

Therefore the objective of this work was to study the optimum conditions for the degradation of a- and b- isomers and the determination of the a-HCH isomer intermediaries during the first steps of the degradation by *Streptomyces* sp. M7.

Streptomyces sp. M7, previously isolated from pesticide contaminated soils, was grown in Minimal Medium (MM), supplemented with each HCH isomer (a, b and g). They were aseptically added to the autoclaved medium at different final concentrations (1.66, 8.3, and 16.6 mg L^{-1}). The strain was also grown in presence of glucose as sole carbon source. All cultures were incubated on a rotatory shaker (100 rpm) at 30 ºC, for 96 h. The biomass produced was measured by dry weight, washing the pellets with 25 mMTris-EDTA buffer (pH 8.0) and drying to constant weight at 105ºC.

Streptomyces sp. M7 has the ability to use a- and β -HCH isomers as carbon source, and to remove them from the culture medium, at 30 ºC and an initial pH of 7, and 30ºC and initial pH of 9, respectively, until the maximum concentration studied. The removal capacity of the a-HCH was not affected to the highest concentration tested, while the β -HCH removal capacity was affected at a concentration of 16.6 mg L^{-1}.

When tested with isomers mixtures (a-, β -, g-HCH) the removal capacity decreased to 40% less than in studies with individual isomers.

The metabolic intermediates detected by Gas Chromatograph-Mass Spectrometry in cell free extracts from cultures of *Streptomyces* sp. M7 with a-HCH as carbon source were: 1,2-dichlorobenzene; 1,3 - or 1,4-dichlorobenzene and 1,2,3-, 1,2,4- or 1,3,5-trichlorobenzene compounds that were also detected in *Sphingobium*.

These results represent a significant advance in the study of the growth of actinobacteria in presence of HCH-isomers. For this reason *Streptomyces* sp. M7 could be considered as promising strains for HCH isomers degradation from contaminated areas.

Keywords: Biodegradation, *Streptomyces*, Isomers HCH, Lindane

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IDENTIFICATION OF TRICHOSTATIN DERIVATIVES FROM *STREPTOMYCES* SP. CPCC 203909

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CLA-1/SR-BI plays important roles in hepatic uptake of HDL-C and HDL-mediated cholesterol efflux. Therefore, CLA-1/SR-BI has been suggested as a novel preventative and/or therapeutic target for atherosclerosis.

To search for active compounds that can increase CLA-1 transcription, we screened for new up-regulators of CLA-1 from microbial products; Streptomyces sp. CPCC 203909 was found to present potent activity in elevating CLA-1 transcriptional levels in 6,000 microbial secondary metabolite crude extracts. In our previous study, three known trichostatin analogues, including trichostatin A, FL657C and trichostatin D were isolated from the liquid fermentation of the strain CPCC 203909, and showed up-regulating CLA-1 transcriptional activity in the preliminary in vitro experiments. In our ongoing search for novel CLA-1 up-regulators, chromatographic separation of the chemical components of the rice fermentation extract of the strain CPCC 203909 led to the isolation of four novel trichostatin analogues **1**-**4** and six known analogues, namely trichostatin A (**5**), FL657C (**6**), trichostatin RK (**7**), t richostatin acid (**8**), trichostatin D (**9**), and trichostatin C (**10**). Compounds **1**-**3** and **4** are the first natural products of trichostatin derivatives substituted in the 6 position with a hydroxyl group, and possessing a cis-double bond as 4Z, respectively. The structures and absolute configurations of these compounds were determined by extensive spectroscopic analysis including 2D NMR and electronic circular dichroism (ECD) calculations based on the quantum-mechanical time-dependent density functional theory (TDDFT). Compounds **2**, **5**-**7**, **9**, and **10** up-regulated the transcriptional activity of human high density lipoprotein receptor (CLA-1) with EC_{50} values of 0.38-78.8 u*M.*

Keywords:*Streptomyces* sp., CLA-1 expression up-regulator, Trichostatin analogues, Anti-atherosclerotic agent

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ISOLATION OF SALT-TOLERANT ACTINOMYCETES FROM DIFFERENT SALINE-ALKALI SOIL OF BENI AMIR (MOROCCO)

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Actinomycetes are an important class of microbial resources, they are important producers of antibiotics and other important bioactive substances. These saprophytic bacteria are able to colonize various ecological niches including the most extreme. Halophilic or salt-tolerant actinomycetes are a kind of extreme environment actinomycetes, which are increasingly concerned around the world as research materials of microbial physiology from adverse circumstance.

The purpose was to explore the actinomycete ecological distribution characteristics in extreme environment from different sites of saline-alkali soil of Beni Amir region (Beni Mellal, Morocco). Mesophilic actinomycetes were isolated from 11 different soil samples through the perimeter of Beni Amir by spreading samples on two kinds of agar media. 51 actinomycete isolates were isolated and were characterized according to routine methods, and detected their salt-tolerance. The results indicated that there were salt-tolerant, mesophilic actinomycetes more abundant in the site 6 (56.5 % of total) followed by the site 4 (20 % of total) and the site 5 (17.4 % of total) than the other sites studied. All of the salt-tolerant actinomycetes identified were *Streptomyces* (92 % of total), and they were all albsporus. And 45 % and 10 % of the tested actinomycetes could tolerant NaCl concentration at 50 g/L and 70g/L respectively.

This preliminary study could contribute to the isolation of the best group of actinomycete strains in saline conditions and may reflect the importance of the isolation conditions to uncover less-explored microorganisms that could produce interesting biological activities.

Keywords: Actinomycetes distribution, Isolation, Salt-tolerant, Beni Amir.

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ISOLATION OF INDIGENOUS *STREPTOMYCES* SSP. FROM ARID AREA AND EVALUATION OF THEIR ANTIFUNGAL ACTIVITY

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Actinomycetes are the main sources of secondary metabolites reflecting an anti-cellular activity, species belonging to the genus ***Streptomyces*** constitute 50% of the total population of soil actinomycetes and 75% of molecules with antibiotic activity are produced by this genus. In addition, rare Actinomycetales bacteria are a potentially important source of unexplored new secondary metabolites with antibacterial and antifungal activity. This study was conducted from thirteen samples in total of which the majority were collected in arid regions. Some have undergone treatments to allow enrichment of bacteria in the initial sample, such as a thermal shock for a week. Methods for decimal dilution in physiological water to 0.9% NaCl with an incubation of 28 °C for three days led to the isolation. Most selective media used in this work are as follows: Starch modified casein (pH 7.0), Bennette (pH 7.3) and ISP2 (PH 7.3). The modified starch-casein medium proved to be the most suitable for the selective isolation of actinomycetes. The isolated strains were screened for their macroscopic appearance on solid medium and their filamentous appearance under an optical microscope. A total of 54 strains were selected and stored in LB broth medium supplemented with 20% glycerol and stored at -20 °C. Stirred broth cultures were performed on GLM medium at room temperature, the strain 1P made a highly viscous medium, the strain 15 G determined the formation of an ovoid gel structure, these two strains are certainly responsible for the formation of exopolysaccharides. In addition, three other strains (5L,15R and 9R) disseminate yellow pigments, red and purple. Interactions were performed with respect to three genus of fungi (***Mucor ssp.***, ***Penicillium*** ssp. and ***Aspergillus niger***). The results showed antifungal activity as follows: ten strains of ***Streptomyces*** neutralized one fungus, eight had an inhibitory effect on both fungi and finally two neutralization exerted on the growth of three fungi. Finally, 15 ***Streptomyces*** strains showed no antifungal inhibition.

Keywords: Arid area, *Streptomyces*, Starch modified casein, *Mucor*, antifungal activity

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ISOLATION AND ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES FROM TEA (*CAMELLIA SINENSIS*) AND VERBENA (*ALOYSIA TRIPHYLLA*)

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Actinomycetes were one of the major groups of the soil population having high G+C content gram positive bacteria. They are capable to grow both on rich substrates and on those containing a minimum or even an apparent lack of macro/micro nutrients. Actinomycetes can produce an array of secondary metabolites having antibacterial or antifungal properties were applied for the human pharmaceutical and the industrial use.

The intention of the present study was to isolate and characterize Actinomycetes from Tea (*Camellia sinensis*, China) and Verbena (*Aloysia triphylla,* Morocco) plants for the production of bioactive secondary metabolites. The antimicrobial activity of the purified strains were studied by performing test using selected human, Tea and Verbena plant pathogenic microorganisms. The results indicated the presence of mesophilic Actinomycetes on Tea and Verbena plants and their amount in Verbena plant was seven told larger than in Tea plant. A total of 45 representative isolates, including 40 Streptomyces species and 5 Actinomycetes from other taxa, were screened for the production of antimicrobial activities against a panel of bacteria, filamentous fungi and yeasts, including some of clinical relevance. Production of antimicrobial activities was detected in 43 strains. In the case of the genus Streptomyces, 36 antimicrobial activities (83.7% of the tested isolates) were recorded. The activities observed among the other Actinomycetes taxa were lower (12% of the tested isolates).

The results demonstrate that these Actinomycete strains isolated from Tea and Verbena plants present a significant capacity to produce compounds with antibacterial and antifungal activity that has the potential to control the pathogens.

Keywords: Actinomycetes, Antibacterial and antifungal compounds, Antimicrobial screening, Tea, Verbena.

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ACTINOBACTERIA BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE: ECOLOGICAL STRATEGY FOR SOLUBILIZING PHOSPHATE AND PROMOTE PLANT GROWTH

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Phosphorus is the second largest agricultural chemical after nitrogen, required by the plants for their growth and development. Majority of the inorganic P applied to soil gets rapidly fixed as insoluble forms (Iron-phosphate, aluminium-phosphate and calcium phosphates) depending on soil pH and becomes unavailable to the plants. The soil microbes have the ability to convert fixed form of P (in soil) to soluble forms that can be easily taken up by the plant roots These soil microorganisms convert insoluble P to soluble P by releasing various organic acids (oxalic acids, succinic acids and gluconic acids etc.), chelation and ion exchange methods.

Phosphate solubilizing bacteria (PSB) are known to convert the inorganique insoluble forms of phosphate to soluble one and make them available for plant uptake. The present study aimed to isolate PSB actinobacteria from Moroccan phosphate mine.

In the present study, the occurrence of tricalcium phosphate(TCP) solubilizing bacteria has been confirmed. Actinomycetes isolates were preliminary characterized by morphological and biochemical tests.

Screening for phosphate solubilization actinobacteria (PSA) was done by inoculating the isolates of actinobacteria on NOTTIAL and MMS media using (TCP) as a sole P source. The growth of colonies was considered a PSA. Sixty isolates were retained for further studies. Twenty two actinobacteria isolates were grown in NOTTIAL and MMS liquid medium at selected pH and temperature. The released phosphate content in the culture filtrate and the acid phosphatase activities were evaluated. The PSA isolates were also examined for production of indole acetic acid (IAA), salinity tolerance and chitinase activity.

The present study indicates the actinomycetes of phosphate mine potential as plants growth promoting.

Keywords: Biofertilizer, Actinobacteria, Phosphate solubilizing Bacteria, Plant growth promoting

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INSECTICIDAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM SALT RANGE, PAKISTAN AGAINST MOSQUITOES AND RED FLOUR BEETLE

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A total of fifty-one actinomycetes strains were selectively isolated on glycerol casein KNO3 agar andactinomycetes isolation agar (AIA). The isolates were screened for the production of insecticidal and larvicidal compounds against 3rd instar larvae of mosquitoes *Culex quinquefasciatus* and *Tribolium castaneum* (Red flour bettle). The biological screening revealed the metabolites of three isolates exhibit 100% mortality of the tested larvae. In chemical screening by thin layer chromatography (TLC) and HPLC-UV, the crude extracts obtained from the culture broths of these isolates, exhibited an impressive diversity of the chemical constituents. The selected isolates SA-10BC, SA-9K and SA-9L were characterized for their morphological, biochemical, physiological and cultural characteristics and by 16S rRNA gene sequencing. The selected isolates show maximum genetic similarity with *Streptomyces rochei* (99%), *Streptomyces minutiscleroticus* (98%) and *Streptomyces phaeoluteigrisseus* (92%) respectively.

Keywords: Insecticidal activity, Larvicidal activity, Saline actinomycetes, 16S rRNA gene sequencing

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STUDY OF INVITRO AND INVIVO OF ACTIVE SECONDARY METABOLITES OF *STREPTOMYCES LEVIS* ISOLATED NORTH WEST SOIL AND THEIR ANTIBACTERIAL EFFECTS

Ali Reza Dehnad

Agriculture research education organization

Introduction: *Streptomyces levis*is a member of Actinomycetes family. Chemical compounds extracted from this bacterium have been used as antibiotics, anti-tumoral, anti-bacterial, anti-fungal, antiviral, anticancer factors. The purpose of this study, was to investigate different metabolites of Streptomyces strains isolated from North West soils, Iran and antimicrobial effects of them.

Methods & Materials: The soil samples were taken from North West in spring of 2010. The isolated bacteria were purified and identifiedby common methods. The metabolites of this bacterium were extracted by 7 different solvents (diethyl ether, dichloromethane, hexane, ethylacetate, chloroform, methanoland water). The antimicrobial effects of metabolites were tested by disk diffusion agar method on Gram-positive and Gram-negative microorganisms. The metabolites with antibacterial effects were analyzed by GC/MS.

Results:Metabolites produced from diethyl ether solvent affected on gram- positiveand gram- negativestrains and15different compounds identified in the active metabolite of the bacterium. The main ingredients were: Docosanoic acid, methyl ester (11.578%), 9-12-octadecadienoic acid (2.961%), 5-Tetracosenois acid, methyl ester (8.389%), Bis (2-ethylhexyl) phthalate (2.153%), and D-alpha-Tocopherol (0.959%).

Conclusion: The results of this study were shown that docosanoic acid, methyl ester (11.578%) and 5-Tetracosenois acid methyl ester (8.389%), are the main metabolites of *Streptomyces levis* isolated from North West soils. Antimicrobial effects of extracts could be due to the presence of 1, 2-Benzenedicarboxylic acid diisooctyl ester compounds and Bis (2-ethylhexyl) phthalate in the metabolite.

Keywords:*Streptomyceslevis*, GC/ MS, Antibacterial effects, Histopathology

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INVESTIGATION OF CAPABILITY OF BIOLOGICAL DISSOCIATION OF NAPHTHALENE USING SOIL BACTERIA IN TABRIZ REFINERY

Alireza Dehnad and Akbar Mohammadzadeh

Agriculture Research Education Organization

Polycyclic aromatic hydrocarbons (PAHs) are important contaminants and carcinogenic. In most cases these contaminants such as anthracene, naphthalene and benzene are released in the wastewater of petroleum and petrochemical industries, which could cause a great damage to environment. In order to remove these substances from soil, some biological methods along with potential of soil's microorganisms are utilized.

These microorganisms metabolize the contaminants by using carbon and yield water, carbon dioxide and biomass.

In the present study the capability of isolated microorganisms from soil in Tarbiz refinery has been investigated.

The above microorganisms were isolated and purified in YGM culture medium. Naphthalene (for model of PAHs) with concentration 1000mgL^{-1} was prepared in Mueller Hinton Broth and constant amount of bacteria was added and kept for one week at 28 and 120r.p.m. Then the destruction level was evaluated with double beam spectrophotometer. 14 types of bacteria were isolated and in the above concentration 13 effective bacteria were identified and destruction level was achieved from (9.2-57.1) percent. There fore this method is capable of removing naphthalene from contaminated oily solid.

Keywords: Soil microorganism, Biodegradation, Aromatics poisons

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STUDYING THE ANTIFUNGAL POTENTIAL OF *STREPTOMYCES* SPECIES FOR PRODUCTION OF BIOLOGICAL FUNGICIDES

Alireza Dehnad and Akbar Mohammadzadeh

Agriculture Research Education Organization

Phytopathogenic fungi are among the most important damages of agricultural products causing a notable loss of crops every year. In sustainable farming, biological control is considered as an alternative for chemical methods of controlling different plant pathogens, specially terricolous fungi. Bacteria belonging to genus *Streptomyces* are able to hydrolyze chitin cell wall of fungi because of secretion of several secondary metabolites such as chitinase family 19 enzyme. 60 samples were collected from different points in Eastern Azarbaijan Province, Iran to identify an efficient *Streptomyces* isolate. The primary identification of *Streptomyces* isolates was done based on morphological properties. After DNA extraction, 16S rDNA gene was amplified using (AF/AR) primers and 31 isolates were isolated. The occurrence of chitinase 19 was proved in four isolates using (CH19F/CH19R) specific primers. Chitinase-producing were antagonistically cultured with pathogenic fungus of genus *Alternaria*. Three antagonistic bacterial isolates and two pathogens were cultured antagonistically on Potato Dextrose Agar medium. Isolate AE09 gave the biggest growth inhibition area against both pathogens and was selected as the efficient isolate. Enzymatic extract was prepared from isolate AE09and its effect on colloidal chitin was measured under different conditions on pH and temperature. The optimum enzymatic activity was observed at pH 6 and 37°C. The chitinase gene of isolate AE09 was cloned on pGEM-T expression vector and in *E.coli* DH5α host cells. The sequencing was done after cloning and bioinformatic examinations showed that the purified chitinase gene had 96% similarity to chitinase C hene from *Streptomyces griseus*. At the end, the PCR product of 16S rDNA gene from isolate AE19 was cloned on pTZ57R/T expression vector and in *E.coli* DH5α host cells and sequencing was done for molecular confirmation of genus *Streptomyces* identification. It was determined that the genus *Streptomyces* had 99% similarity to 43 different strains.

Keywords: Antifungal, *Streptomyces*species

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FIRST REPORT OF ACTINOMYCETOMA DUE TO *NOCARDIA* SPP. IN SUDAN

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Background and objectives: *Madurella mycetomatis* is the main cause of eumycetoma, being responsible for approximately 70% of all mycetoma cases in Sudan. But the situation is ultimately different in countries like Mexico where the predominant etiologic agents are actinomycetes (97.8%), from which *Nocardia brasiliensis* comprises more than 86.6%. In this study we reported for the first time documented cases of actinomycetoma caused by *Nocardia* Spp. in Sudan.

Setting: The present study is a prospective cross-sectional laboratory based study in which clinical samples (n =100) from patients with mycetoma lesions were collected.

Methodology: The samples were cultured on Lowenstein Jensen and Glucose Yeast Extract Agar media. Grown colonies were initially identified using Gram's stain, Ziehl Neelsen stain, and selected biochemical reactions. Confirmation was done by the analysis of PCR amplified strb1 gene.

Results: Actinomycetoma was represented by a high ratio (12%) among the study population. Nine out of the 12 isolates (75%) were found to belong to the genus Streptomyces; whereas three isolates (25%) were identified as Nocardia spp. on the basis of phenotypic and mycolic acid contents.

Conclusion: It could be concluded that nocardia exists with considerable prevalence (25%) among patients with actinomycetoma investigated in the present study.

Keywords: Actinomycetoma, Nocardia, Sudan

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ANTIMICROBIAL POTENTIAL OF HALOPHILIC ACTINOMYCETES ISOLATED FROM A SALINE LAKE AGAINST VARIOUS MULTI DRUG RESISTANT BACTERIAL PATHOGENS CAUSING VENTILATOR ASSOCIATED PNEUMONIA

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Background: The rapid emergence of drug resistance among pathogenic bacteria, especially multidrug resistant bacteria, underlines the need to look for new antibiotics. In the past actinomycetes have remained the prolific sources of the largest number of secondary metabolites. Nowadays, the traditional approaches based entirely on the screening of microbes from soil have been tired and proven to be totally ineffective for the release of novel antibiotics. Due to this reason, many scientists and pharmaceutical industries are actively involved in the extensive utilization of extremophilic actinomycetes from various unexplored habitats with unusual environment including saline lakes, forests, deserts and marine sediments.

Aims and objectives: The present study was designed to screen the halophilic actinomycetes for their antimicrobial potential against multi drug resistant bacteria causing ventilator associated pneumonia.

Material and Methods: In the present study, forty different actinomycetes were isolated from the water and mud samples collected from a saline Kallar Kahar lake. The morphological, biochemical and physiological characterization of these strains was performed to identify them. In prescreening studies, the selected strains were cultivated on small scale, and the crude extracts were screened biologically and chemically. In biological screening the activity of the extracts was determined against a set of pathogenic bacteria including MRSA, Pseudomonas aeruginosa, *Proteus vulgaris*, *K. pneumoniae*, *E. coli*, *Enterobacter spp*., and *Acinetobacter spp*. In chemical screening the crude extracts were analyzed by TLC and HPLC-MS.

Results: The current study revealed that majority of the actinomycetes strains (90%) was belonging to the genera Streptomyces. Among selected strains, KL14, KL21, KL29, KL32, KL37, KL41, KL63, KL72, KL73, KL81, KL93, KL96, KL98 and KL116 were found to be most biologically active as they exhibited promising activity against all the test strains of multi drug resistant pathogens. In thin layer chromatographic analysis of the crude extracts, bands of different colors were visualized under short and long UV light indicating a unique pattern of different bioactive metabolites. HPLC-UV chromatograms of each crude extract of the isolates showed a number of different peaks at various retention times, which was also an indication of the presence of many valuable antimicrobials compounds in the crude extracts.

Conclusion: Overall the study reveals that halophilic actinomycetes are a promising source of useful antimicrobial agents which can be exploited for further purification of unique secondary metabolites in drug discovery.

Keywords: Halophilic actinomycetes, Biological screening, Multi drug resistant bacteria, Secondary metabolites, Metabolite diversity.

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THE ROLE OF THE ENVIRONMENT IN THE TRANSMISSION AND CONTROL OF *MYCOBACTERIUM BOVIS* IN EUROPEAN BADGERS

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The incidence of *Mycobacterium bovis*, the causative agent of bovine tuberculosis, has been increasing in UK cattle herds for over thirty years, resulting in substantial economic losses. The European badger (*Meles meles*) is implicated as a wildlife reservoir. The most likely route of transmission to cattle is through exposure to pasture that is contaminated with infected badger urine and faeces. Whilst the environment and pasture are recognised as a source of infection, its relative importance remains unknown, in part due to the lack of information on the location and size of environmental reservoirs. Here we show we show that infected badgers are shedding large numbers of *M. bovis* cells in faeces, creating substantial, seasonally variable, environmental reservoirs of pathogens. This demonstrates that the environment is a source of infectious material with the potential to be responsible for a substantial proportion of transmission between badgers and cattle. The use of oral vaccination to reduce the environmental reservoir of *M. bovis* is being implemented in the Republic of Ireland, which has changed to distribution of faecal shedding. Our findings highlight the necessity of monitoring environmental reservoirs of *M. bovis* excreted by badgers to aid in elimination of this disease from wildlife and livestock, a component of control and spread that is currently overlooked.

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FROM MICROBIAL DIFFERENTIATION TO CHANGING SOIL WETTABILITY BEHAVIOUR: A CROSS-DISCIPLINARY SOIL PROTEOMICS, HIGH-RESOLUTION IMAGING AND MODELLING APPROACH FOR PREDICTING SWITCHES BETWEEN WETTABLE AND WATER-REPELLENT SOILS

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Water repellency in soils can reduce vegetation cover, accelerate water runoff, flooding and soil erosion. The presence and degree of this water repellency is controlled by many physico-chemical and biological factors. Ultrahydrophobic cell surface and adhesion proteins from filamentous soil microbes, e.g. chaplins and hydrophobins from respectively *Streptomyces* and fungi, are capable of assembling extremely hydrophobic surfaces in vitro. Our hypothesis is that such proteins play an important role in the in switching between wettable and repellent grassland soils, while displaying different severities of repellency. Soils were collected at 5-10 cm depths from three UK locations (Rothamsted Park Grass and the Waun Las and Gower nature reserves) during a wet and dry period. Preliminary data showed that ultrahydrophobic proteins could not be detected in wettable soils, however, they were identified in moderately and severely water repellent grassland soils, respectively, at medium to low soil moisture conditions. Extracting these hydrophobic proteins from a spiked sample of wettable soil corroborated our detection process. Additionally, our newly developed metaproteomic profiling analysis suggested that soil microbes adjust to the development of water repellency. The implications of these results will be discussed.
Combining these and traditional hydrological metadata with our other novel methods of soil analysis, e.g. high-resolution atomic force and confocal microscopy imaging and modelling the soil pore voids will reveal new insights that link nano- and microscale processes with macro and field scale soil behaviour.

Keywords: Soil water repellency, Metaproteomics, Atomic force microscopy, Modelling, Hydrology

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BREAKING TRANSCRIPTIONAL 'LOCKS' FOR NATURAL PRODUCT DISCOVERY IN *STREPTOMYCES VENEZUELAE*

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Introduction:*Streptomyces* bacteria are prolific producers of natural products with uses including antibiotics, pesticides, immunomodulatory agents and anticancer compounds. Sequencing of Streptomyces genomes over the last decade has led to discovery of hundreds of gene clusters encoding for natural product biosynthesis whose products are unknown, and many of these cryptic biosynthetic clusters are silent in laboratory culture conditions [(1)].
Transcriptional repressor proteins such as ArpA play a vital role in regulation of secondary metabolite biosynthesis [(2)].The deletion of genes scbR2 and mmyR encoding for transcriptional repressor proteins has previously been shown to lead to overproduction of antibiotic compounds coelimycin and methylenomycin respectively, in S. *coelicolor.* However, there are currently very few examples of these strategies being employed to discover new natural products [(2,3)].
Objectives: To identify and characterise the metabolites produced in an ArpA-like deletion mutant gbnR strain of S. *venezuelae* and to investigate the biosynthesis of these compounds and the role played by the adjacent, usually silent, gbnABC cluster.
Methods: Gene deletion combined with growing S. *venezuelae* strains in a variety of culture conditions, followed by liquid-chromatography mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy.
Results: Gaburedins – a novel class of ureido-linked GABA derivatives – have been purified from Streptomyces venezuelae gbnR deletion mutant strain by comparing the metabolic profiles of the S. venezuelae wild type and a number of mutant strains via LC-MS analysis. Creation of double-deletion mutants and subsequent analysis of the metabolites produced has led to identification of a key biosynthetic enzyme, whose role has been explored in vivo by feeding with a variety of precursor molecules[4].These feeding experiments have been expanded upon further by addition of other amino acids and amines to produce a library of gaburedin analogues and drug-like molecules, indicating the potential utility of the GbnA and B enzymes as biocatalysts. Authentic standards have been synthesized in order to confirm the proposed structures and to begin biological testing.

Conclusion: A series of novel ureido-linked dipeptides has been discovered to be produced by the previously cryptic, normally silent gbnABC cluster, leading to the discovery of analogous systems across a range of bacterial genera. This is the first time that targeted deletion of an ArpA-like transcriptional repressor has led to discovery of a new natural product series in *Streptomyces*.

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NEW CULTURE MEDIUM TO EVALUATION OF PEPTIDIC ANTIMICROBIALS PRODUCED BY MARINE ACTINOMYCETE AGAINST BACTERIA B-LACTAM-RESISTANT

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MATERIALS AND METHODS: 27actinomycetes were recovered from marine invertebrates collected of the Peruvian coast (Paracas-south, Máncora and Tumbes-north) .With the actinomycetes isolated these were evaluated for their a multienzymatic activity through preparation different substrates. It was selected the best strain with antimicrobial activity through double layer's test on Marine medium and one medium new to after tackle against 16 gram-negative bacteria β-lactams resistant (*Klebsiella sp. Proteus sp. E. coli*) isolated in patients at a Health Institute of Lima.The fermentative process of the selected strain was developed on both marine broth as well as a new broth without proteins called EcoLino 322, proposed in Microbial Ecology laboratory-UNMSM,It was evaluated the antimicrobial activity of the supernatants through technique of wells. It was quantified the amount of proteins through Qubit fluorometer'test and viewed the presence of peptides by PAGE-SDS, characterizing preliminarily antimicrobial compound by HPLC.

RESULTS: The actinomycete that was able to inhibit all β-lactams resistant strain was identified genotypically as *Streptomyces californicus*. Using SDS-PAGE revealed the presence of 5 bands and confirmed using HPLC. The best results of antagonist activity were obtained in the new culture medium Ecolino 322 which helped maximize the production of these antibiotics.

CONCLUSIONS: The new culture medium maximizes the antimicrobial production in the Actinomycetes evaluated. This new medium also allows a better study of antibiotics peptidic nature.

Keywords: Actinomycetes, Peptide antibiotics, SDS-PAGE, HPLC.

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