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DETERMINATION OF TOTAL CELL PROTEIN PROFILES OF *Streptomyces* SPECIES

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ABSTRACT

Present study has been conducted for finding out the total protein profile of bacterial strain *Streptomyces* sps by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Total 139 isolates of *Streptomyces* have been isolated from the soil. Amongst all isolated strain, total 20 isolates were used for getting protein profile by SDS PAGE. Amongst all isolates, 20 isolates were selected for protein profiling and these were divided in two groups. Two strains of *Streptomyces* i.e. *S. violaceus* and *S. albidoflavus* were selected as a reference strain for both groups. Band profile were analyzed and assessed by computer added program BioRad Quantity with the use of Unweighted Pair Group Method of Analysis (UPGMA). As a result of this computer assisted numeric analysis study, approximately 40 different types of protein bands were reported between 10 or 100 kD molecular weight. Analysis of acquired dendogram on the basis of similarities ratios, all 40 proteins can be divided in to 7 groups. In addition, the isolates A4B3G, D145B, S5036.6 and reference isolate *S. violaceus* were available in the same group, while 805A, C804B, F1705 isolates and reference sample *S. albidoflavus* were detected in the same group. The test organisms which were similar to each other in terms of morphological and biochemical characters delivered the same protein bands. SDS-PAGE method is an effective method in terms of determining taxonomical relations between the various species of genus *Streptomyces*.

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1 Introduction

Soil is the most frequent habitat for the members of the genus *Streptomyces* and the presence of this genus was reported from the all types of soils (Williams et al., 1989). The members of this genus have substantial role in decomposing of various components in the soil and are dependent on plant wastes or fungus mycelium (Kutzner 1986; Goodfellow and Simpson 1987). These are reported from the all habitats those are rich of organic substances (Hagedorn 1976). Amongst all available soil microflora, up to 20% generally belongs to *Streptomyces*. Amongst all isolated actinomyecete from soil, 64-97% were belongs to the Streptomyecetes (Kutzner 1986; Xu et al., 1996; Wang et al., 1999). The enzymes produced by the members of genus *Streptomyces* have degradation capacity to cellulose, silicon, cotton, PVC plastic, wool, wood, hay, cereals, jute fibrile and natural rubber materials and various other substances (Morosoli et al., 1997; Jendrossek et al., 1997). Jendrossek et al. (1997) have reported that amongst 50 discovered rubber decomposer 33 were belongs to the genus *Streptomyces*.

Protein profiling is an effective method for identifying the various species of genus *Streptomyces* and it will help to get information regarding the phylogenetic relationship. For protein profiling dissolution of cell protein is an important step. As compared to the one way electrophoresis of total cell proteins, two-way electrophoresis were giving better results (O'Farrell 1975). Recently, cellular protein profiles acquired with SDS-PAGE method and non-cell protein profiles have been successfully employed for differentiating the various types and subtypes of microorganisms. In the diagnosis and classification of various bacteria, cellular protein profiling with SDS-PAGE were used affectively (Berber et al., 2003, Berber et al., 2004; Berber and Yenidünya 2005). Protein profile and SDS-PAGE enable to Berber and Cokmus (2001) to differentiated *Bacillus sphearicus* strains into six subtypes. Manchaster et al. (1990) analyzed the cellular proteins of 37 strains belongs to *S. albidoflavus* 1A, *S. anulatus* 1B; *S. cyaneus* 18; *S. rimosus* 42 and *S. griseocarneium* 55 taxons. On the behalf of results acquired, different species of taxons *S. albidoflavus* and *S. anulatus* formed (Manchester et al., 1990). Information regarding the use of total cellular protein profile in establishment the phylogenetic relationship between various species of genus *Streptomyces* is in scarcity. Therefore, present investigation had been undertaken for the finding out the importance of SDS-PAGE based protein profile in establishment of phylogenetic relationship between various species of genus *Streptomyces*.

2 Materials and Methods

2.1 Isolation and culturing of bacteria

Streptomyces were isolated from the plant root and rhizosphere soils. These strains were maintained on Bennet's broth on 150 rpm at 25°C in an incubator. Afterwards, 1.3 ml sample of each strain were transferred in to eppendorf tubes and centrifuge at 1200 rpm for 3 minutes. Following to the centrifuge, cells rinse by sterilized water for 3 times. For maintaining to the cells, 25 µl SDS-sample buffer was added and later on mixed 0.06M Tris-HCl + 2.5% Glycerol + 0.5% SDS + 1.25% β mercaptoethanol. Now tube with this all mixture was boiled for 5 minutes, this process denatured the total protein available in bacterial cell (Laemmli 1970).

2.2 SDS-PAGE

Denatured proteins were exposed to SDS PAGE electrophoresis (V16-2BRL Gaithersburg MD, USA) as described by Laemmli (1970). Afterwards, bromphenol blue an indicator was processed at 30 mA current until the gel ending. Finally, gel was stained by Coomassie Brillant Blue R-250 stain for giving various colors to bacterial protein.

2.3 Analysis

The resulted gel was examined to analyze the protein profile on the basis of bands. The acquired data were transferred to electronic environment by the use of UPGMA in the BioRad Quantity One Program. The relation and similarity between the *Streptomyces* isolates and total cellular protein profiles were presented as dendogram with UPGMA.

3 Results and Discussion

The protein profile image of various isolates, acquired by polyacrylamide gel electrophoresis has been illustrated in Figure 1. Similarly, table 1 represents the similarity ratios of various isolates analyzed by UPGMA in the BioRad Quantity One Program. As illustrated in Figure 1, approximately 40 different types of protein bands were available on gel and the molecular weight of these proteins varies between 10 to 100 kD. The bands those are specific among strains were represented by arrows in figure 1.

All protein profiles of each isolate were analyzed numerically in BioRad Quantity One program, by the use of UPGMA and the acquired dendogram is illustrated in Figure 2. Results of analysis represents that 60% similarity ratio were based on the total 7 groups. Group 1st contains total 7 members including the reference strain *S. violaceus*. Other members of this group were D155D, E1105, A2B3E, A1A4C, D145B and A4B3G. Group 2nd includes the reference strain *S. albidoflavus* with C804B, E805A and F1705. 3rd group included F205B, F705A and C704B isolates while the 4th group contained only tow isolates i.e. C904H and A1A3A. Isolate D3105, C204A and C164B belongs to the group 5th.

Table 1 Similarity matrix based on SDS-PAGE protein profiles of isolates of *Streptomyces*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
F205B	100																					
E105B	44.3	100																				
D3105	65.8	56.6	100																			
D165B	54.8	63.6	59.4	100																		
A1A4C	61.0	52.7	71.1	59.0	100																	
F1705	55.6	36.8	53.3	46.5	47.7	100																
C204A	58.9	55.4	73.3	62.0	59.2	53.2	100															
A4B3G	56.1	58.7	65.4	57.3	61.7	50.3	61.6	100														
C804B	65.8	57.5	63.2	58.6	67.9	53.2	59.0	67.9	100													
E805A	67.0	50.1	64.0	54.9	67.7	62.5	60.1	58.8	85.3	100												
S5223.1A	63.0	53.8	67.8	63.7	61.8	68.5	61.2	66.5	74.6	74.3	100											
C704B	69.1	49.8	70.6	52.4	66.9	51.5	56.5	67.3	69.1	62.8	69.0	100										
F705A	58.9	46.1	56.2	43.9	68.4	48.5	48.3	68.7	73.7	63.9	64.5	64.9	100									
C904H	45.7	51.9	65.2	37.7	58.7	33.6	50.8	54.7	59.8	51.7	48.1	51.3	62.6	100								
A1A3A	41.2	33.4	33.6	50.0	32.4	33.4	34.5	38.6	39.8	33.6	39.9	39.4	39.8	22.6	100							
D145B	63.2	51.8	63.5	48.8	74.5	45.1	53.3	73.3	71.8	61.5	62.6	74.1	85.5	61.4	41.9	100						
A2B3E	61.1	57.2	66.3	52.7	71.5	40.5	52.6	67.2	73.5	64.5	60.2	66.9	77.2	72.9	41.4	78.7	100					
E1105	57.6	45.5	63.2	51.1	59.9	61.9	57.8	67.3	67.0	65.2	70.1	69.6	74.9	45.9	43.3	75.8	63.8	100				
A1A3F	52.0	50.6	45.6	41.5	45.4	42.7	45.3	59.8	55.8	48.2	48.2	50.1	56.5	51.6	46.3	57.7	58.7	52.7	100			
C164B	66.2	48.6	65.7	50.1	51.4	51.4	58.0	55.9	53.0	50.5	53.4	58.1	50.7	47.7	36.5	51.3	60.9	52.8	68.0	100		
D155D	66.6	48.2	48.2	47.9	43.8	48.0	44.6	48.7	64.5	66.0	58.0	48.2	54.7	59.5	28.0	51.0	64.2	48.5	50.5	55.3	100	
S5036.6	63.6	45.5	55.1	47.0	56.9	41.6	57.2	49.4	54.4	53.8	44.8	53.7	50.7	40.9	30.9	60.1	49.6	55.2	44.6	56.5	42.4	100

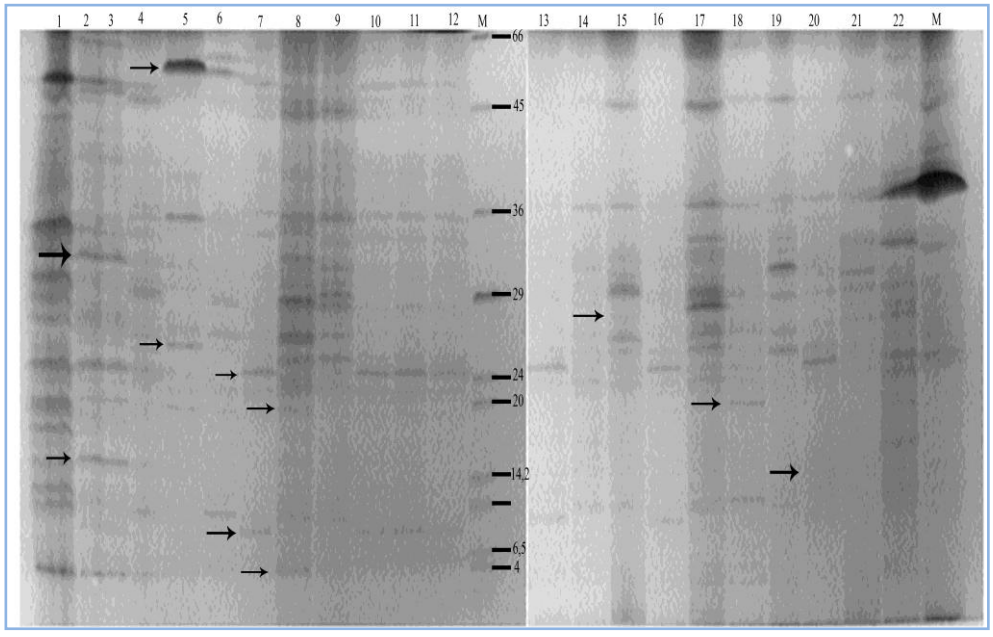

Figure 1 SDS PAGE protein profile of *Streptomyces*.

[Molecular Marker: bovine albumin 66kD; egg albumin 45 Kd, pepsin 34,7 Kd, trypsinogen 24 Kd, β-laktoglobin 18,4 Kd ve lysozyme 14,2 Kd. 1. F205B; 2. E105B; 3. D3105; 4. D165B; 5. A1A4C; 6. F1705; 7. C204A; 8. A4B3G; 9. C804B; 10. E805A; 11. S5223.1A; 12. C704B; 13. F705A; 14. C904H; 15. A1A3A; 16. D145B; 17. A2B3E; 18. E1105; 19. A1A3F; 20. C164B; 21. D155D; 22 S5036.6.]

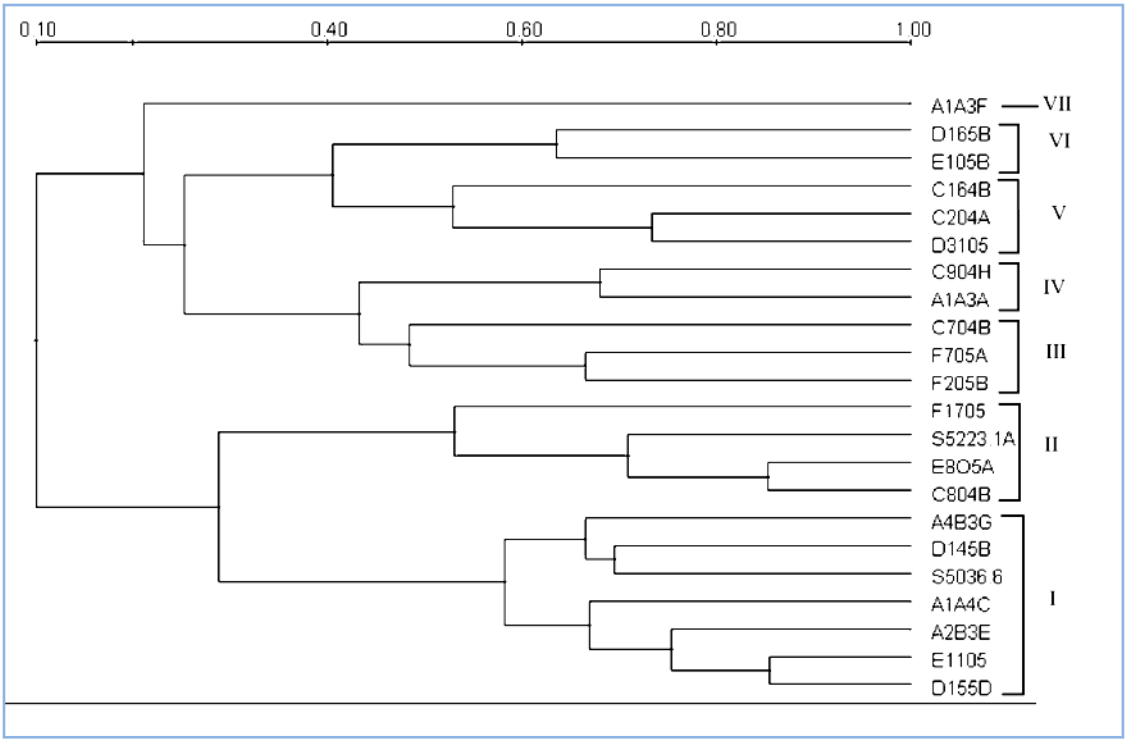

Figure 2 UPGMA dendogram SDS-PAGE protein profiles of *Streptomyces* isolates.

Similarly, isolates D165B and E105B belongs to the group 6th while group 7th have single member i.e. A1A3F. Types of amino acids available in protein and the total molecular weight of protein affect the net charge available on particular protein. So, these factors are playing an important role at the time of electrophoresis (Smithies 1955).

Although, One way protein electrophoresis is relatively simple, cheap and reliable method but two way electrophoresis is giving better results as compare to one way (Vauterin et al. 1993). Electrophoretic separation of cells and cell wall proteins generally provide sufficient information about the type and subtype of bacterial strains. Genetic relation between all cellular proteins comply the results obtained by DNA: DNA hybridization. Therefore, interspecies population shows less difference between protein profiling. Association of numeric analysis with electrophoresis profiles provides better results and taking less time than DNA:DNA hybridization studies.

Electrophoretic protein profiles of a bacterium cells can be scanned and copied to computer and following the analysis by appropriate software, can help to establish a data bank. In present study, SDS-PAGE associated protein profiles were determined for the *Streptomyces* isolated from some plant roots and rhizosphere. An appropriate distribution was reported between different isolates of *Streptomyces* and various color groups. The findings of present study are in conformity with the findings of Manchaster et al. (1990). Similarly, Boynukara et al. (2004) also prepared total cellular protein profile for bacterial strain *Aeromonas* by SDS-PAGE method and identify the similarities between various types and subtypes of bacteria (Manchester et al. 1990; Boynukara et al. 2004). In the analyzed dendogram results, 60% similarity ratio was reported from the only 7 groups. Amongst these 6 groups had numerous members only 7 group had only one member i.e. A1A3F.

The tested organisms those similar to each other in terms of morphological and biochemical characters provided the same pattern protein bands. In conclusion, it is apparent that the combinations of cellular protein profiles obtain by SDS-PAGE method with the specific software added computer analyzer helps in determining the taxonomic and phylogenetic relationship between various species of genus *Streptomyces*.

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