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Research Paper

Isolation and Identification of soil isolates of Pseudomonas species via FAME analysis

Microbiology

KEYWORDS : Pseudomonas, FAME, Identification, Characterization

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ABSTRACT

Pseudomonas is a genus that contains over 40 species of bacteria; the genus is divided into five groups of classification. It is important genus of bacteria from clinical as well as environmental perspective. Most of the species of the genus harbour one or multiple plasmids. These plasmid impart various characteristics to the organisms like antibiotic resistance, catabolism of complex organic compound etc. The genus is also known for its lateral gene transfer and hence very important clinically. In present study soil samples were collected from the soil near to cotton industry effluent treatment, sugar mill and soil from automobile garage. Samples were screened for presence of Pseudomonas sp. on Cetrimide agar media. Gram staining, Biochemical tests and FAME analysis were carried out to confirm findings. The study gives insight into vast potential of Pseudomonas sp. in the field of biotechnology. It can be exploited for its diverse plasmid composition. These soil isolates posses ecological importance also, which can be further explored.

INTRODUCTION

The genus *Pseudomonas* is the most heterogenous and ecologically significant group of known bacteria, and includes Gram-negative motile aerobic rods that are wide-spread in all natural environments with some forming associations with plants and animals (Palleroni, 1993; Thornley, 1967). The nutritional requirements of *Pseudomonas* spp. are very simple, and the genus is found in natural habitats like soil, fresh water, marine environments (Palleroni, 1984; Molin, 1986a; Molin, 1986b; Gyllenberg, 1963; Gyllenberg, 1966) but it has also been isolated from clinical instruments, aseptic solutions, cosmetics and medical products (Rainey,2000). Certain members of the genus *Pseudomonas* are considered to be important phyto-pathogens and agents or carriers of human infections (Oliver, 2000; Eissa, 2010), whereas its other strains and species exhibit activities of bioremediation and biocontrol (Krieg, 1984). The genetic diversity found within *pseudomonads* gives rise to a wide range of phenotypes (Ginard,1997; Haubold, 1996). Increasing evidence suggests that the diversity of genome architecture (chromosomes and accessory genetic elements) is of particular importance (Anzai, 2000; Yamamoto, 2000). Examination of the deoxyribonucleic acid (DNA) fingerprints of *Pseudomonas* sp. reveals a high level of polymorphism among strains of a species and among strains highly related on a phenotypic basis (Rainey,1994).

In present study eight different isolates of *Pseudomonas* were extracted from soil. Biochemical tests were performed for preliminary identification and confirmative identification was done by GC-FAME analysis. Presence of *Pseudomonas* in waste area revealed their contribution in Bioremediation.

MATERIALS AND METHOD

Sample collection

Bacteria studied were isolated from soil collected from three locations 1. cotton (gene) industry at Himmatnagar, 2. Garage near Bhavsar Hostel, Ahmedabad and 3. Near a sugar mill where sugar wastes were dumped. Samples were collected aseptically and transported to the laboratory, where they were processed for isolation of bacteria within 8h of collection.

Isolation

Soil samples were appropriately diluted in sterilized water and plated on cetrimide agar which is selective medium for *Pseudomonas* sp. The isolates were further subcultured and maintained on nutrient agar media. Gram staining was performed to check the purity of culture.

Morphological and Biochemical Characterization

Colony characteristics of the organism were studied. Colonies were observed for texture, colour, margin, shape and size, pigmentation and elevation. Gram staining was performed for all isolates.

All strains were characterized according to, Carbohydrate Fermentation Test, Methyl Red test, Voges-Proskauer test(v-p), Triple sugar iron test, Catalase test, Citrate test, Urea hydrolysis test, Hydrogen sulphide production test, Indole production test, Gelatin hydrolysis test(tube test), Oxidase test, Nitrate reduction test, Starch hydrolysis test, F-agar for detection of fluorescence.

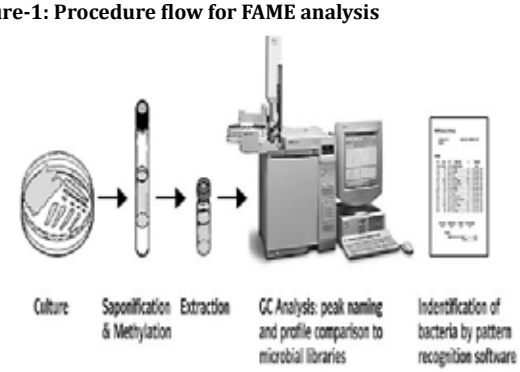
Identification through FAME

Preparation and analysis of FAMEs from whole-cell fatty acids of the bacterial strains were prepared and analyzed according to the method described by the MIS manufacturer (MIDI, Newark, DE) on Agilent 6890N Network GC system. All strains were grown on Difco Trypticase Soy Broth Agar.

Approximately 40 mg of living cells from each sample were harvested and transferred to 13 x 100 mm glass tubes fitted with Teflon-lined screw caps and fatty acids were extracted using method described in MIS manual. There are four basic steps in the preparation of GC-ready extracts from cell harvest for fatty acid composition analysis (Figure-1).

(1) Saponification: lysis of the cells to liberate fatty acids from the cellular lipids,
(2) Methylation: formation of fatty acid methyl esters (FAME)
(3) Extraction: transfer of the FAMEs from the aqueous phase to the organic phase
(4) Base Wash: aqueous wash of the organic extract.
2 µl of sample extract (for each sample) were injected to get chromatographic FAME profile of each bacterial strain. Sherlock software automatically compares FAME profile to stored data base and gives identification.

Figure-1: Procedure flow for FAME analysis



RESULTS AND DISCUSSION

A total of 9 *Pseudomonas* strains were isolated on Cetrimide Agar. The strains were identified initially based on their colo-

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nial morphology on plates. Different isolates showed various pigmentation in colonies that include red, yellow, colorless and transparent colonies (Figure-2). All the isolates were Gram negative short rods. Isolated organisms were identified by gas chromatographic (GC) analysis of extracted microbial fatty acid methyl esters (FAMEs). Eight strains were identified by Sherlock MIDI software out of which two strains were similar. Biochemical Characterization of identified strains confirmed *Pseudomonas* species (Table-1).

Figure-2: Growth morphology of *Pseudomonas* species on Cetrimide media

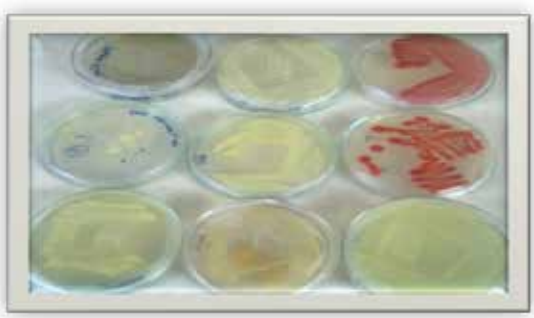

Table-1: Biochemical characteristics that differentiate Pseudomonas strains

Sr. No.	Characteristic	1	2	3	4	5	6	7
1	Carbohydrate Fermentation							
	Gas production	+	+	+	+	+	+	+
	Acid production	-	-	-	-	-	-	-
2	Methyl Red	-	-	-	-	-	-	-
3	Voges-Proskauer	-	-	-	-	-	-	-
4	Triple sugar iron							
	Gas production	-	-	-	-	-	-	-
	Acid production	+	+	+	+	+	+	+
5	Motility	+	+	+	+	+	+	+
6	Catalase	+	+	+	+	+	+	+
7	Citrate	+	+	+	+	+	+	+
8	Urea hydrolysis	-	-	-	-	-	-	-
9	Hydrogen sulphide production	-	-	-	-	-	-	-
10	Indole production	-	-	-	-	-	-	-
11	Gelatin hydrolysis	+	+	+	+	+	+	+
12	Oxidase	+	+	+	+	+	+	+
13	Nitrate reduction	-	-	-	-	-	-	-
14	Starch hydrolysis	-	-	-	-	-	-	-
15	F-Agar for detecting florescence							
	Florescence	+	+	+	+	+	+	+
	Pigmentation	+	-	-	-	-	-	-
	Growth	+	+	+	+	+	+	+

(**+** = Test Positive, **-** = Test Negative)

(1-*Pseudomonas aeruginosa*, 2-*P. aureofaciens*, 3-*P. balearia*, 4-*P. corrugate*, 5-*P. flectens*,

6-*P. gladioli*, 7-*P. pertucinogen*

CONCLUSION

In this study, *Pseudomonas* species isolated from soil were characterized by morphological and Biochemical analysis (Table-1). Subsequently FAME was carried out to confirm the identity of isolated species as FAME identification is definitely a step higher in identification and authenticity in microbiological systems (Surve, 2012). Seven strains were identified by SHERLOCK MIDI i.e. *Pseudomonas aeruginosa, P. aureofaciens, P. balearia, P. corrugata, P. flectens, P. gladioli,* and. *P. pertucinogena.* These identified species are known for various properties like, *P. pertucinogena* has known property to produce Bacteriocin.*P.aureofaciens* carries Phenazine antibiotic producing properties and play very important role in rhizosphere ecology and pathogen suppression. *P. flectens* is a plant pathogen.

To date, various studies have suggested that the phenotypic classification techniques are not solely adequate for identification of Pseudomonas sp, and is challenging in classification (Haynes, 1951; Juni, 1980). Thus confirmatory identification of Pseudomonads by FAME analysis is of assured aid. It is important to monitor the presence of environmental Pseudomonas species due to their potential ecological significance (Ugur, 2012). In order to make conclusions about the role of Pseudomonas spp. in the ecosystem and human health, further details about their properties, working and effects should be acquired.

REFERENCE

Anzai, Y., Kim, H., Park, J.Y., Wakabayashi, H., (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. Int J Syst Evo Microbiol. 50,1163-1589. A taxonomic study of Acinetobacter andrelated genera. J. Gen. Microbiol. 49, 211-257. | Eissa, N.M.E., Abou El-Ghiet, E.N., Shaheen, A.A., Abbass, A., (2010). Characterization of Pseudomonas Species Isolated from Tilapia Oreochromis niloticus in Qaroun and Wadi-El-Rayan Lakes, Egypt Global Veterinaria. 5(2), 116-121. | Ginard, M., Lalucat, J., Tummler, B., Romling, U.,(1997). Genome organization of Pseudomonas stutzeri and resulting taxonomic and evolutionary considerations. Int J Syst Bacteriol. 47(1), 132-143. | Gyllenberg, H., Eklund, E., Antila, M., Vartiovara, U., (1963). Contamination and deterioration of market milk V. Taxometrig classification of pseudomonas Acta. Agric. Scan. 13,158. | Gyllenberg, H., Eklund, E., Mischwissensehaft.(1966). A taxonomic survey of the psychrophilic bacteriain | Haubold, B., Rainey, P.B., (1996). Genetic and ecotypic structure of fluorescent Pseudomonas population Mol Ecol. 5, 747-761. | Haynes, W.C., (1951). Pseudomonas aeruginosa-----its characterisation and identification. J. Gem Microbiol. 5, 939. | Juni, E., Heym, G. A., (1980). Transformation assay for identification of psychrotrophic achromobacters. Appl. Environ. Microbiol. 40,1106-1114. | Krieg, N.R., Holt, J.G., (1984). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore. | milk and milk products. 21, 261. | Molin, G., Ternstrom, A., (1986a). Phenotypically based taxonomy of psychrotrophic Pseudomonas isolated from spoiled meat, water, and soil. Int. J. Syst. Bacteriol. 36, 257-274. | Molin, G., Ternstrom, A., Ursing, J., (1986b). Pseudomonaslundensis, a new bacterial species isolated from meat. Int. J.Syst. Bacteriol. Int. J.Syst. Bacteriol. 36, 339-342. | Oliver, A., Canton, R., Campo, P., Baquero, F., Blazquez, J., (2000). High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. Science. 288, 1251-1254. | Palleroni, N.J., Genus I. Pseudomonas in Krieg NR and Holt JG (ed.) (1984). Bergey's manual of systematic bacteriology. The Williams & Wilkins Co, Baltimore, vol-1, p. 141-199. | Palleroni, N.J., (1993). Pseudomonas classification: a new case history in the taxonomy of gram-negative bacteria. Antonie Leeuwenhoek. 64, 231-251. | Rainey, P.B., Bailey, M.J., Thompson IP., (1994). Phenotypic and genotypic diversity of fluorescent pseudomonads isolated from grown sugar beet. Microbiology. 140, 2315-2331 | Rainey, P.B., Buckling, A., Kassen, R. Travisano, M.(2000). The emergence and maintenance of diversity: insights from experimental bacterial populations. Trends Ecol Evol. 15, 243-247. | Surve, V.V., Patil, M.U., Dharmadhikari, S.M., (2012).FAME and 16srDNA sequence analysis of halophilic bacteria from solar salterns of Goa: A comparative study. International Journal of Scientific and Research Publication. 2(8), 01-08. | Ugur, A., Ceylan, O., Aslim, B. (2012). Characterization of Pseudomonmas spp. From seawater of the southwest coast of Turkey. J. Biol. Environ Sci. 6(16), 15-23. | Yamamoto, S., Kasai, H., Arnold, D.L., Jackson, R.W., Vivian, A., Harayama, S., (2000). Phylogeny of the genus Pseudomonas: intrageneric structure reconstructed from the nucleotide sequences of gyrB and rpoD genes. Microbiology. 146, 2385-2394. |