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Diversity of Plant Growth Promoting *Pseudomonads* from Banana Rhizosphere

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A sample of 12 *Fluorescent pseudomonads* isolated from the rhizosphere of banana during the cultivation season and shown to be closely related on the basis of whole cell protein analysis was subjected to detailed phenotypic and genotypic characterization. Phenotypic traits were assessed on the basis of biochemical properties, assimilation of sole carbon sources. The studies of protein electrophoregram for elucidating relationships among *Fluorescent pseudomonads* by UPGMA cluster analysis based on similarity index indicated that the isolates were mainly distinguished into five major clusters representing different biovars. With the exception of total cellular protein profiles analysis of the data revealed five main clusters.

Key words: Banana rhizosphere, *Pseudomonas*, Gel electrophoresis, whole cell proteins.

Plant growth-promoting rhizobacteria (PGPR) affect plant growth directly or indirectly. PGPR produces plant growth regulators that directly enhance the plant growth of the plants[1]. or assist uptake of nutrients from the soil by producing siderophores etc.[2]. The genus *Pseudomonas* includes species that are pathogens with wide functionality, where some species are pathogenic for plants and human[3], while others show plant growth promotion activity. Some species have pathogen-suppressing potential that are now used as biological control agents[4]. Some species or strains are well known for their metabolic flexibility with diverse functions. Thus this diversity of the species makes them an ideal candidate for study the variation by phenotypic, metabolic as well genotypic methods[5].

Fluorescent pseudomonas strains have been reported to control several diseases caused by soil borne pathogens[6] and are known to survive in the rhizosphere. Biological control of plant diseases using antagonistic microorganisms offers a highly effective, economical and environmental friendly alternative to the use of synthetic pesticides[7]. Bacteria found in banana fields produced fluorescent and non-fluorescent pigments on King's B medium were shown to be antagonistic to *Fusarium oxysporum* causing wilt [8,9].

Proteins comprise 55% of the dry mass in bacterial cells[10] and can be separated by electrophoretic techniques such as polyacrylamide gel electrophoresis (PAGE) of whole-cell soluble proteins to achieve a protein electrophoregram. The electrophoretic protein patterns can be used to assess similarity among strains at species and subspecies levels. Also, protein profiles combined

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with computer-aided analysis have potential in phylogenetic and taxonomic studies. Separation of cellular proteins by electrophoresis is a sensitive technique that can provide information of strains at the sub species level[11]. Total cell protein separation by SDS-PAGE was used in bacterial identification[12,13]. The cell envelope protein fraction had been used to characterize *Pseudomonas* spp.[14-16] and to screen the rhizobacteria from different soil types and locations[17].

This study was aimed to examine the degree of phenotypic and genotypic diversity within a selection of closely related pseudomonad isolates sampled from banana crop during growing season.

MATERIAL AND METHODS

Isolation of plant growth promoting rhizobacteria

Rhizobacteria were isolated from the rhizosphere of banana plants, roots were collected and shaken gently to remove the adhering soil. One gram of soil was suspended in 100 ml sterile distilled water test and shaken at 120 rpm for 30 min. The suspension from all samples were vortexed and serial dilutions were made and dilutions of 10^{-6} with three replications for each sample. One mL of this dilution was pour plated on Pseudomonas Isolation Agar for fluorescein and pyocyanin production. 0.1ml of each dilution was spread on Luria Bertani plates and plates were incubated at 27 ±2°C until colony development was observed.

Pseudomonas was identified on the basis of cell morphology, colony morphology, and gram staining. A total of fifteen bacterial strains were isolated on the basis of colony morphology from the rhizosphere of diseased and healthy roots of Banana plants colleted form different sites of Maharashtra, India.

Biochemical tests and Characterization

Bacteriological characteristics of the isolates were examined by using the methods described by Palleroni[18]. Gram stain, colony color, LOPAT test (Levan, Oxidase, Pectate liquefaction, Arginine hydrolysis, Tobacco hypersensitivity) Fluorescent pigments, Aesculin hydrolysis, Nitrate reduction, Gelatin hydrolysis, Sucrose Utilization, Inositol utilization, L-Arabinose utilization, Tyrosinase, fluorescent pigment on King's medium B. The results of all these tests were recorded as either positive or negative for distinguishing into different biovars[19,20].

SDS-PAGE of whole cell proteins

The whole-cell protein SDS– PAGE electrophoresis was performed as described by Shanmugam *et al.*[16]. The gels were destained then photographed and readings of the gels were taken.

Determination of phylogenetic relationships

The strains were compared on the basis of presence or absence of protein bands in the gel. The presence or absence of band was used as 1 or 0 for preparing binary matrix and used for analysis using NTSYS. The cluster analysis was performed by unweighted pair group method using arithmetic averages (UPGMA) and the dendrogram was generated with the NTSYS-PC to show the similarity coefficient between the genotypes.

RESULTS AND DISCUSSION

A total of 187 strains from 12 different banana growing field were isolated. These stains were subjected to screening of their growth promoting potential on banana plants. The result thus obtained were recorded and of these 187 strain 12 strains with relatively better growth promoting activity were selected. The selection was done such that one strain from each location is represented.

Pseudomonas fluorescens is a physiologically diverse species of opportunistic bacteria (gamma-proteobacteria) found throughout terrestrial habitats. The species contributes greatly to the turnover of organic matter and, while present in soil, is abundant on the surfaces of plant roots and leaves. Of the plant-colonizing strains, some, such as isolates SBW25 and Pf-5, positively affect plant health and nutrition[21-23]. The mechanistic bases of these effects remain unclear, but are known to include the production of plant growth hormones, the suppression of pathogens harmful to plant health, and the direct elicitation of plant defense responses[24]. It has been argued that exploitation of these plant growth promoting bacteria in agriculture requires an improved understanding

of the determinants of ecological performance[25].

The biochemical and physiological tests identified different strains within the genus *Pseudomonas* based on the different biochemical and physiological tests. The identification of a small group of strains could not be confirmed using the biochemical and physiological analysis. In the present study, most of the species were identified as *P. fluorescens.* The results of the biochemical and physiological tests were considered individually for each strain to compare the phenotypic diversity present within them. All the strains were positive for oxidase and arginine hydrolysis. Three of the strains were negative for sucrose utilization, levan production and inositol utilization. The biochemical and physiological tests identified different strains within the genus *Pseudomonas.* The substrate utilization pattern of the strains within each site is given in Table 1.

The strains fell into five groups based on analysis of the patterns of total proteins following SDS-PAGE. These groups were identical to those indicated by biochemical tests. Protein profiles comprised 23 to 26 reproducible bands, ranging from about 4 kDa to more than 97 kDa. The protein profile of all the strains appeared to be similar, there was variation in the protein profile.

Although strains in each group appeared to be homogeneous in protein profiles but differed with respect to low molecular weight proteins, about 3.5 - 20 kDa, in that three bands were apparent whereas three different bands were apparent. Furthermore, an intense band was present at more than 20 kDa in all lane (Fig. 1). UPGMA analysis performed on SDS–PAGE profiles of protein extracts revealed the existence of five different groups of strains. Strain membership to the grouping identified by whole-cell protein analysis showed a high degree of similarity. The isolates were mainly distinguished into five major clusters representing five different biovars. The cluster I representing biovars IV, cluster II representing biovar III, cluster III representing biovar I, cluster IV representing biovars II and cluster V representing biovars V. Thus, the isolates belonging to rhizosphere of same crop from different geographic location were distributed into different phenotypic clusters (Fig. 2).

Table 1. Biochemical characters of fluorescent *Pseudomonas* species used for differentiating various strains

Biochemical Characteristic	Pymb 1	Pymb 2	Pymb 3	Pymb 4	Pymb 5	Pymb 6	Pymb 7	Pymb 8	Pymb 9	Pymb 10	Pymb 11	Pymb 12
Levan	-	+	+	-	+	+	+	+	-	-	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Pectate hydrolysis	+	+	+	+	+	+	+	-	+	-	+	+
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Tobacco hypersensitivity	-	+	-	+	+	+	-	-	+	-	+	+
Fluorescent pigments	-	+	+	+	+	+	+	+	+	-	+	+
Nitrate reduction	+	-	+	-	+	+	+	-	-	+	+	+
Gelatin hydrolysis	-	+	+	+	+	+	+	-	+	-	+	+
Sucrose Utilization	-	-	+	-	+	+	+	+	+	-	+	+
Inositol utilization	-	-	+	-	+	+	+	+	+	-	+	+
L-Arabinose utilization	+	-	+	-	+	+	+	+	+	+	+	+
Tyrosinase	+	-	-	-	+	+	-	+	+	+	+	+
Lecithinase	+	-	+	-	+	+	+	+	+	-	+	+
D-Xylose utilization	+	+	+	+	-	-	+	+	+	+	-	-
D-Galactose	-	+	+	+	+	+	+	+	+	+	-	-
D-Mannose	-	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	+	-	-	+	+	-	+	+	-	+	+
L-Tartarate utilization	-	+	-	+	-	-	-	-	-	-	-	-
BIOVAR	III	V	I	BV5	II	II	I	IV	II	III	II	II

P. fluorescens is an opportunistic species long recognized for its genetic, physiological and functional diversity[19]. The previously sequenced genome of isolate Pf-5 offered a glimpse of genome content and organization, but in the absence of comparative data sheds little insight into the extent of genomic diversity. The importance of pseudomonads in agriculture has already been well established in non-problematic soils with good irrigation sources. a comprehensive collection of *Pseudomonas* strains was isolated from twelve different banana rhizosphere populations. Banana was the chosen crop as it is the most widely cultivated crop covering more than 2 million hectares in the state of Maharastra alone.

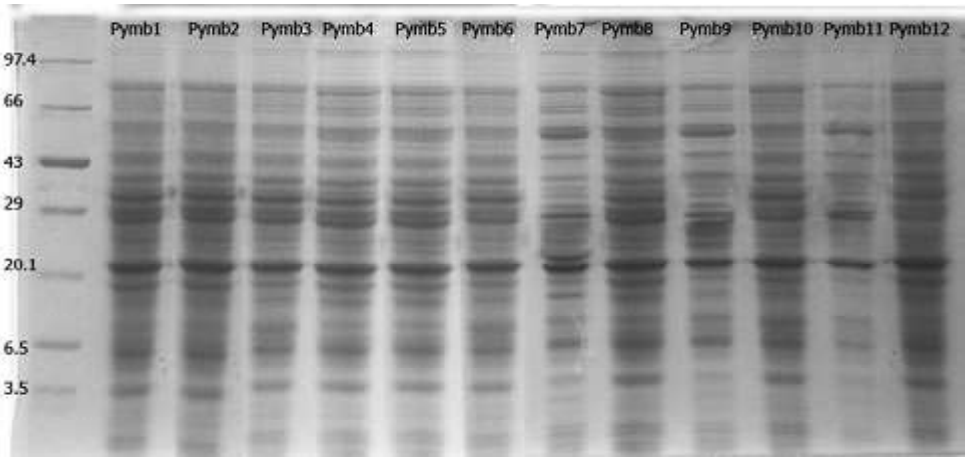

Fig. 1. SDS-PAGE analysis of 12 representative *Fluorescent pseudomonads*

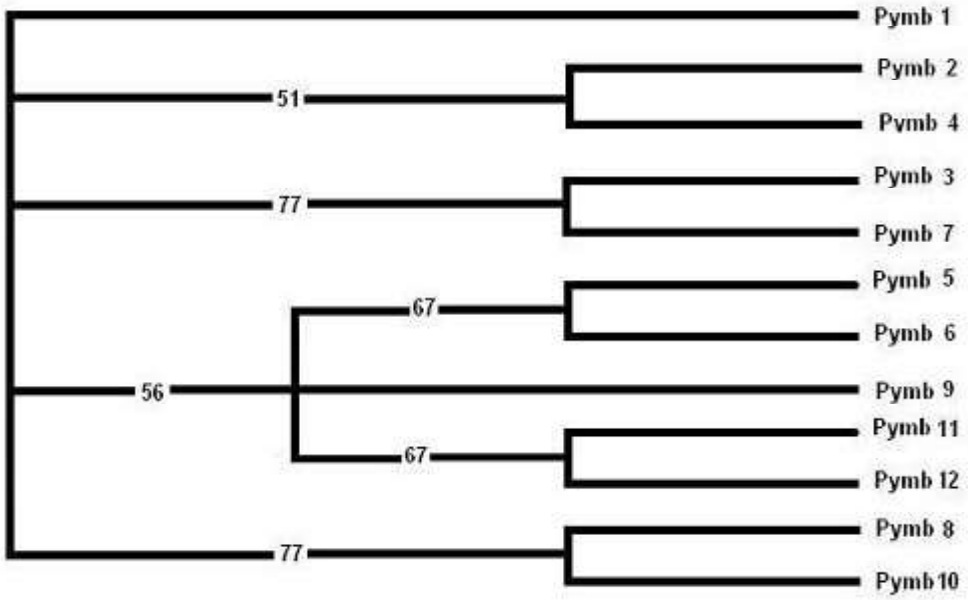

Fig. 2. Dendrogram representing the relationship between 12 representative *Fluorescent pseudomonas* isolates based on total cellular proteins resolved by SDS-PAGE

The genus *Pseudomonas* comprises of a diverse group bacteria with high levels of physiological and genetic diversity within species. This is evident from the facts that they can colonize a wide range of habitats. Diversity arises and is maintained through the interaction between ecological and genetic factors. Genetic factors are the ultimate determinants of patterns of diversity but the products of these genetic factors – the protein are the ultimate expression of these factor. Physiological state of the cell is reflected from the proteins present in the cell, this is expressed in the form of variation in banding pattern of protein bands (Glynn and Reid, 1969).

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