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Characterization of Plant Growth Promoting *Pseudomonas fluorescens* From Banana Rhizosphere.

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ABSTRACT

Of the 78 strains of P. fluorescens isolated from the rhizosphere of Banana from different regions of Marathwada, eighteen strains were selected for their plant growth promoting activity. These strains were characterized by morphological and biochemical methods. They were tested for in vitro plant growth promotion activities such as phosphate solubilisation, siderophore production, IAA production and fungal cell wall degrading enzyme production. All the strains were positive for siderophore and IAA production. Ten strains were positive for phosphate solubilisation. Six strains possess the ability to produce chitinase, cellulase and protease. These strains enhanced plant growth in vitro. However when strains were inoculated in combination, there was significant increase in root and shoot length.

KEYWORDS Rhizosphere; *P. fluorescens*; plant growth promotion

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host [1] . Various species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Bacillus* and *Serratia* have been reported to enhance the plant growth [2,3] .The most effective species of *Pseudomonas* have been *Pseudomonas fluorescens* . *Pseudomonas fluorescens* helps in the maintenance of soil health and are metabolically and functionally most diverse [4,5]. The plant growth-promoting *Pseudomonas* improve plant growth either by direct or by indirect mechanism. Direct mechanism includes production of plant growth regulators in the rhizosphere [6]. The indirect mechanism involves biological control of pathogens by production of compounds like 2,4-diacetylphloroglucinol or induction of host defense mechanisms [7]. Strains of *Pseudomonas fluorescence* showed known biological control activity against certain soil-borne phytopathogenic fungi [8]. Several species of Pseudomonas and bacilli have been used as seed or root inoculants for higher growth yield of crops [9,10]. The use of *P fluorescens* as PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements.

Materials and Methods

Isolation of *Pseudomonads*

were isolated from the rhizosphere of banana plants using Kings B medium plates. Plates were incubated at 27 ± 2 °C till colonies were developed. *Pseudomonas* was identified on the basis of cell morphological characters[11]. Eighteen strains were selected for further studies representing different locations of isolation.

Identification of Pseudomonads

Pseudomonas was identified on the basis of morphological, and biochemical characteristics

Phosphate *solubilization*

Phosphate solibilization was tested qualitatively on Pikovskaya [12] medium. Bacterial culture was streaked on solidified medium. The positive reaction is indicated by zone of clearance around the colony.

Siderophore production

Amount of siderophore produced was measured by method using modified CAS solution [12] . Each strain was inoculated in 100 ml modified M9 medium and incubated at 30°C for 5 days and centrifuged at 10000 rpm. Supernatant was filtered and concentration of siderophore in filtrate was measured by mixing equal volume of CAS solution and filtrate. The solution was equilibrated and absorbance was measured at 630nm.

Cell wall degrading enzyme activity

Chitinase and cellulase activity was tested by plating on chitin agar and CMC agar respectively following Daniel et al[13]. Protease activity (casein degradation) was determined from clear zone in skimmed milk agar. The agar plates were prepared and spot inoculated with test organism and incubated at 30°C for 5 days. Development of halo/clear zone around the colony was recorded as positive for cell wall degrading enzyme production.

Determination of Indole Acetic Acid

Production of IAA was determined by a method designed by Gordon and Weber [14].

Plant growth promotion experiment

The broth containing 9 $\times 10^8$ colony forming units (CFU)per ml was used for the preparation of talc-based formulation following the method described by Saravanan et al [15]. Four hundred ml of bacterial suspension was mixed with 1 kg of the purified talc powder (sterilized at 105 °C for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC) 10 g (adhesive) under sterile conditions. After shade-drying overnight, the mixture was packed in polypropylene bag and sealed. the population of bacteria in talc formulation at the time of application was determined and found to 2.5–3 × 10^8 CFU. Greenhouse experiment was carried out in pot. Pot mixture comprised of soil, sand and compost (2:1:1; v/v) and the soil mixture. Banana suckers were dipped in 1% of the talc-based bacterial preparations and planted in pots. These pots were kept in shade house and protected with insect net.

Data were subjected to Analysis of Variance (ANOVA) depending upon experimental design following the procedure as given by Panse and Sukhatme [16].

RESULTS AND DISCUSSION

Isolation and Identification of *Pseudomonads*

Strains were gram negative short rods with fluorescent pigment. Biochemical and physiological tests identified the different strains within the genus *Pseudomonas*. All of the isolates were identified as *P.fluorescens* (Table 1).

Plant growth promoting traits of *P. fluorescens*

All the strains were positive for siderophore and IAA production.. The maximum siderophore was produced by strain Yps6 and least by strain Yps 20 (Table2). In an earlier study it was shown that siderophore producing rhizobacteria could transform pathogen supporting soil to pathogen inhibiting soil (13). The amount of IAA produced was the maximum by strain Yps20 was the least by strainYps6. It has been reported that IAA production by PGPR can vary among different species and strains, [17,18]. (Table2).

Production of fungal cell wall degrading enzymes is the mechanism involved indirect plant growth promotion [17]. Six isolates were positive for chitinase, seven isolates were positive for cellulase and twelve isolates were positive for protease (Table 2). Ten strains were positive for phosphate solubilisation .

Growth promoting effects of *P fluorescens*

Strains Yps6, Yps10, Yps35, were potent producers of siderophore whereas strains Yps20, Yps31, Yps65, and Yps25 produce maximum amount amount of Indole Acetic Acid. (Table3). These strains were inoculated in various combinations, each combination containing four bacterial strains of which two are direct growth promoters (IAA producers) and two are indirect growth promoters,(Siderophore, enzyme producers)..

wide range of environmental conditions.

Maximum shoot length (94cm) was observed was recorded in Yps25 and Yps72 which was as par with Yps20, Yps31, Yps35, Yps62, Yps65, Yps74 but significantly higher over others. Minimum shoot llength was recorded with control treatment (Ta- ble3). The maximum shoot length of 104cm was recorded in the treatment with the isolate combination Yps65+ Yps20+ Yps6+ Yps72 which was as par with isolate combination Yps74+ Yps35+ Yps18+ Yps44 and Yps6+ Yps10+ Yps20+ Yps31 but significantly higher over rest of isolate combination. Minimum shoot length of 60cm was recorded with control. Similar trend of observation were recorded for root length (Table4). Application of mixtures of rhizobacteria and endophytic bacterial strains exerted great effect on the morphological characteristics of the plant than the individual strains [18].

CONCLUSION

To develop future beneficial inoculants for field grown crops, one approach should consider performing inoculation assays with a consortium containing a mixture of PGPR organisms.. A consortium could contain a mixture of PGPR stimulating plant growth at different growth stages, and showing one or more of the known PGPR mechanisms of action. Multiple organisms may enhance the level and consistency of biocontrol by a more stable rhizosphere community and effectiveness over a

Table: 1. Biochemical characterization of isolated strains

Biochemical Characteristic	Yps6	Yps10	Psy15	Yps18	Yps20	Yps25	Yps31	Yps35	Yps39	Yps44	Yps51	Yps56	Yps62	Yps65	Yps72	Yps74	Yps75	Yps78
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fluorescent pigments	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aesculin hydrolysis	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
Sucrose Utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Inositol utilization	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
L-Arabinose utilization	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+
Tyrosinase	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-
D-Xylose utilization	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+
D-Galactose	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
D-Mannose	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Tartarate utilization	+	+	-	-	-	--	+	+	-	-	-	-	-	-	-	-	+	+
Pectate hydrolysis	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
BIOVAR	I	II	III	III	III	III	IV	V	III	III	III	III	III	III	III	III	VI	IV

Table: 2. Plant growth promoting attributes of selected strains of Pseudomonas

Isolate code	Siderophore (µmole/ml)	IAA (µg/ml)	Chitinase	Cellulase	Protease	Phosphate solubilization
Yps6	100	12	-	+	+	+
Yps10	106	12.1	-	+	+	+
Yps15	105.8	16.6	+	+	+	-
Yps18	80.5	23.8	-	-	-	+
Yps20	80.5	21.3	-	-	+	+
Yps25	73.3	28.4	-	-	+	+
Yps31	74.4	27.2	-	-	+	+
Yps35	76	27.8	+	+	+	-
Yps39	95.8	18.7	+	+	+	-
Yps44	82.2	22.6	-	-	-	+
Yps51	84.5	20.8	-	-	-	-
Yps56	83.9	24.5	-	-	+	+
Yps62	84.5	22.0	-	-	-	-
Yps65	78.5	26.8	-	-	-	+
Yps72	75	27.4	-	-	+	+
Yps74	87.9	19.9	+	+	+	+
Yps75	90.8	21.7	-	-	+	-
Yps78	95	20.7	+	+	+	-

Table3: Plant growth promotion experiment

Isolate code	Shoot length (cm))	Root length. (cm)
Yps6	74	76
Yps10	78	74
Yps15	81	79
Yps18	87	88
Yps20	88	90
Yps25	94	96
Yps31	92	94
Yps35	92	85
Yps39	87	85
Yps44	80	82
Yps51	82	89
Yps56	84	80
Yps62	92	89
Yps65	93	90
Yps72	94	91
Yps74	89	87
Yps75	88	80
Yps 78	86	79
Control	50	45
SE	2.09	2.33
CD	6.20	6.92

Table 4: Combined effects of strains on plant growth promotion

Isolate Code	Shoot length (cm)	Root length (cm)
Yps6+ Yps10+ Yps20+ Yps31	110	108
Yps74+ Yps35+ Yps18+ Yps44	102	102
Yps65+ Yps20+ Yps6+ Yps72	104	103
Yps39+ Yps51+ Yps62+ Yps15	84	78
Yps10+ Yps35+ Yps20+ Yps20	98	102
Control	60	64
SE	2.47	2.53
CD	7.33	7.51

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