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Production of Plant Growth Promoting Substance by *Pseudomonas fluorescens* and *Bacillus subtilis* Isolates from Paddy Rhizosphere Soil of Cuddalore District, Tamil Nadu, India

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Abstract: Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which enhance the growth of the plant either directly or indirectly. In the present study, *Pseudomonas fluorescens* and *Bacillus subtilis* isolates were isolated and characterized by Gram staining, motility test, plating on selective medium and performing biochemical tests. The *Pseudomonas fluorescens* isolates were designated as PF -1 to PF – 10 and *Bacillus subtilis* population was designated as BS - 1 to BS - 10. The efficiency of *Pseudomonas fluorescens* and *Bacillus subtilis* for IAA production, Gibberellic acid production, Siderophore production and Phosphate solubilization was estimated. The maximum Indole acetic acid (IAA) and Gibberellic acid (GA_3) production by *Pseudomonas fluorescens* was recorded by the isolate PF - 8 and the minimum production was observed in PF - 4 isolate. The IAA production, Gibberellic acid production and siderophore production by *Bacillus subtilis* was low when compared to *Pseudomonas fluorescens*. The maximum phosphate solubilization was recorded by the isolate BS - 8 and the phosphate solubilization by *Pseudomonas fluorescens* was very low.

Key words: Paddy · Rhizosphere Soil · PGPR · *Pseudomonas fluorescens* and *Bacillus subtilis*

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly [1]. Many soil microorganisms possess multiple beneficial traits of nutrient mobilization, production of plant growth promoting substances and biocontrol ability [2]. Amongst the PGPRs, *Pseudomonas fluorescens* and *Bacillus subtilis* have emerged as the largest and potentially the most promising group of PGPR with their rapid growth, simple nutritional requirements, ability to utilize diverse organic substrates and mobility [3].

Bacillus is the most abundant genus in the rhizosphere and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved. There are a number of metabolites that are released by these strains [4], which strongly affect the environment by increasing nutrient availability of the plants. *Bacillus subtilis* is able to maintain stable contact with higher plants and promote their growth [5]. Phosphate solubilizing *Bacillus* spp. stimulates plant growth through enhanced P nutrition increasing the uptake of nitrogen, phosphorous, potassium and iron [6].

Pseudomonas sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been Fluorescent *Pseudomonas* spp. [7]. Specific strains of the *Pseudomonas fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields [8]. *Pseudomonas* spp. are important plant growth promoting rhizobacteria used as biofertilizers and are able to enhance crop yield by direct and indirect mechanisms [9]. Some strains of *Pseudomonas* produce chelating agents called siderophores with high affinity for iron absorption. Microbial siderophores can enhance plant growth through increasing iron solubility in the plant rhizosphere.

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MATERIALS AND METHODS

Details of the Locations: The survey was conducted at ten locations in Cuddalore district of Tamil Nadu comprising Annamalai Nagar, Bhuvanagiri, Kurinjipadi, Vadalur, Neyveli, Keelamoongiladi, Vayalore, Sivayam, Kannangudi and Mangalam.

Collection of Paddy Rhizosphere from Different Locations: In each and every location of the survey area a field which has been under long behind mono culture practice was selected. The locations of rhizosphere samples were made at different location of the paddy field. In each and every location of the survey area a field which has been under long behind mono culture practice was selected. The locations of rhizosphere samples were made at different location of the paddy field. The collected soil samples were brought to laboratory for further analysis.

Isolation and Enumeration of *Pseudomonas fluorescens* and *Bacillus subtilis* Population: The paddy rhizosphere soil samples collected from ten paddy field of a particular location, were pooled and one ml of paddy rhizosphere soil sample was transferred to 100 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotator shaker (100 rpm) for 30 minutes at ambient temperature. The well mixed suspension was then diluted appropriately upto 10^{-6} dilution. One ml of suspension from 10^{-4} and 10^{-5} dilution was aseptically transferred to sterile petriplates and 10 – 20 ml of selective King's B medium and Nutrient agar medium was added and incubated at 37ºC for 24 hours. Three replications were maintained for each dilution. The colonies were counted by using Qubec colony counter. The total number of colonies in the original samples was expressed as cfu g^{-1}. All the ten *Pseudomonas fluorescens* and *Bacillus subtilis* isolates were purified by Streak plate method using King's B medium and Nutrient agar medium frequently.

Designation of *Pseudomonas fluorescens* and *Bacillus subtilis* Isolates: The *Pseudomonas fluorescens* isolates, obtained from the rhizosphere of paddy grown at ten different location of Cuddalore District and were designated as PF. The *Bacillus subtilis* isolates were designated as BS. Both the bacterial isolates were numbered randomly from 1 to 10.

Characterization of *Pseudomonas fluorescens* and *Bacillus subtilis* Isolates: Identification of the *Pseudomonas fluorescens* and *Bacillus subtilis* were carried out by the routine bacteriological methods i.e., by the colony morphology, staining techniques, plating on selective media and by performing biochemical tests.

Estimation of Indole Acetic Acid (IAA): The Indole acetic acid produced by *Pseudomonas fluorescens* and *Bacillus subtilis* was determined by using the method of Gorden and Paleg [10].

Estimation of Gibberellic Acid (GA3): The gibberellic acid production by *Pseudomonas fluorescens* and *Bacillus subtilis* was determined by following the method of Borrow *et al.* [11].

Estimation of Siderophore Production: Siderophore production by the plant growth promoting rhizobacteria isolates *Pseudomonas fluorescens* and *Bacillus subtilis* was estimated by the method described by Reeves *et al.* [12].

Screening of *Pseudomonas fluorescens* and *Bacillus subtilis* for Phosphate Solubilization: The plates were prepared with Pikovskya's medium. The culture of ten isolates of *Pseudomonas fluorescens* (PF-1 to PF-10) and *Bacillus subtilis* (BS-1 to BS -10) were streaked on the plates and incubated in an incubator at 28°C for 7 days. The plates were then examined and data were recorded [13].

RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria may promote growth directly by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus and potassium, production of siderophore that solublize and sequester iron, or production of plant growth regulators [14, 15]. The occurrence of *Pseudomonas fluorescens* and *Bacillus subtilis* population in the rhizosphere of paddy grown at ten selected locations was studied and the results were showed in Table 1. The location, namely Sivayam recorded maximum community population of *Pseudomonas fluorescens* (7.71 cfu x 106 g^{-1}) and *Bacillus subtilis* (5.60 cfu x 106 g^{-1}) from Mangalam recorded least population of 7.21 cfu x 106 g^{-1} (*Pseudomonas fluorescens*) and 5.10 cfu x 106 g^{-1}

Table 1: Occurrence of community *Pseudomonas fluorescens* population from rhizosphere of paddy

Rhizosphere soil sample	*Pseudomonas fluorescens* cfu x 10^6 g^{-1}	*Bacillus subtilis* cfu x 10^6 g^{-1}
Annamalai Nagar	7.65	5.54
Kurinjipadi	7.66	5.33
Bhuvanagiri	7.55	5.45
Vadalur	7.60	5.50
Neyveli	7.36	5.25
Keelamoongiladi	7.63	5.52
Vayalore	7.66	5.33
Sivayam	7.71	5.60
Kannangudi	7.31	5.20
Mangalam	7.21	5.10

Table 2: Designation of *Pseudomonas fluorescens* isolates from ten locations of Cuddalore District

Rhizosphere soil sample	*Pseudomonas fluorescens* Designation	*Bacillus subtilis* Designation
Annamalai Nagar	PF – 1	BS – 1
Kurinjipadi	PF – 2	BS – 2
Bhuvanagiri	PF – 3	BS – 3
Vadalur	PF – 4	BS – 4
Neyveli	PF – 5	BS – 5
Keelamoongiladi	PF – 6	BS – 6
Vayalore	PF – 7	BS – 7
Sivayam	PF – 8	BS – 8
Kannangudi	PF – 9	BS – 9
Mangalam	PF – 10	BS – 10

Table 3: Characterization of *Pseudomonas fluorescens* from the rhizosphere rice soil

	Pseudomonas fluorescens (PF) isolates									
Characters studied	PF-1	PF-2	PF-3	PF-4	PF-5	PF-6	PF-7	PF-8	PF-9	PF-10
Gram staining	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Fluorescent pigment	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-	-	-

(*Bacillus subtilis*) in the rhizosphere. All other locations recorded the community population of *Pseudomonas fluorescens* and *Bacillus subtilis*.

Ten strains of *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated from various areas from Cuddalore District. The *Pseudomonas fluorescens* isolates were designated as "PF" series and numbered randomly. They *Bacillus subtilis* isolates were designated as "BS" series and numbered randomly. The details of designation of the isolates their rise of collection are presents in Table 2. The *Pseudomonas fluorescens* and *Bacillus subtilis* isolates were characterized by Gram staining, motility test, plating on selective medium and by performing biochemical tests (Table 3 and Table 4). Saranraj *et al*. [16] also isolated the *Pseudomonas fluorescens* from ten different locations in Cuddalore district.

Indole-3-acetic acid (IAA) is a member of the auxin family of phytohormones that influence many cellular functions in plants and therefore are important regulators of plant growth and development. In addition to production in plant tissues, IAA synthesis is widespread

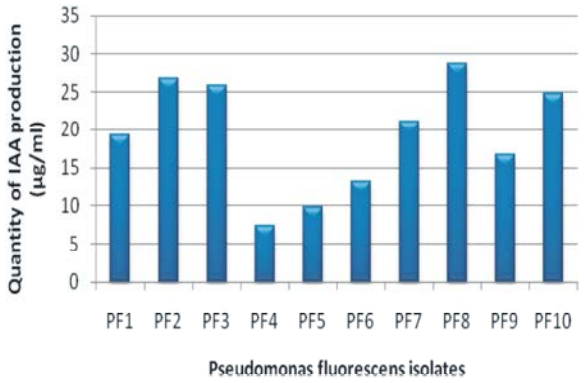

Fig. 1: Indole acetic acid production by *Pseudomonas fluorescens* isolates

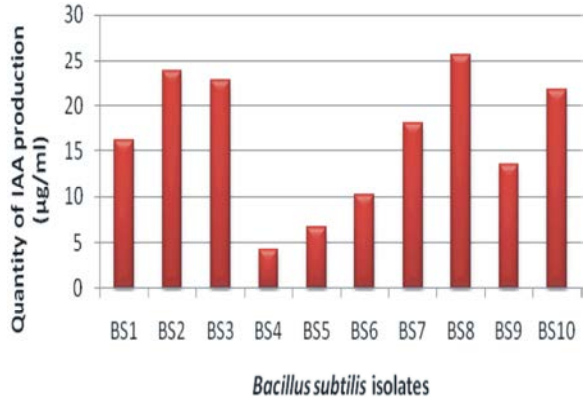

Fig. 2: Indole acetic acid production by *Bacillus subtilis* isolates

among plant-associated bacteria and provides bacteria with a mechanism to influence plant growth [17]. IAA is the member of the group of phytohormones and is generally considered the most important native Auxin [18]. It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division and differentiation and gene regulation [19]. The ten *Pseudomonas fluorescens* and *Bacillus subtilis* isolates obtained from the rhizosphere of paddy were tested for their efficiency of IAA production. All the above 10 isolates taken from the study showed positive results producing IAA. The amount of IAA produced expressed in µg/ml of culture filtrate. The maximum IAA production by *Pseudomonas fluorescens* was recorded by the isolate PF - 8 (28.80 µg/ml). The minimum production of IAA was found in PF - 4 (07.36 µg/ml) isolates. The IAA production by *Bacillus subtilis* was relatively low when compared to *Pseudomonas fluorescens* (Figure 1 and Figure 2).

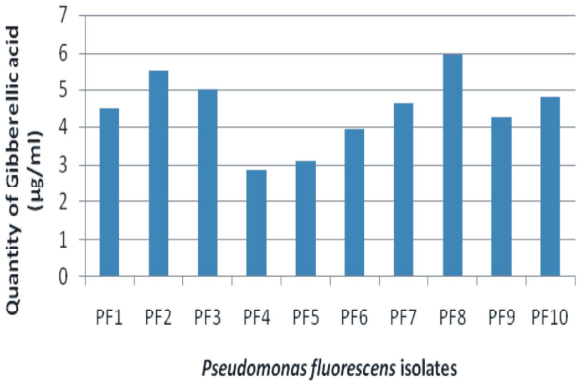

Fig. 3: Gibberellic acid production by *Pseudomonas fluorescens* isolates

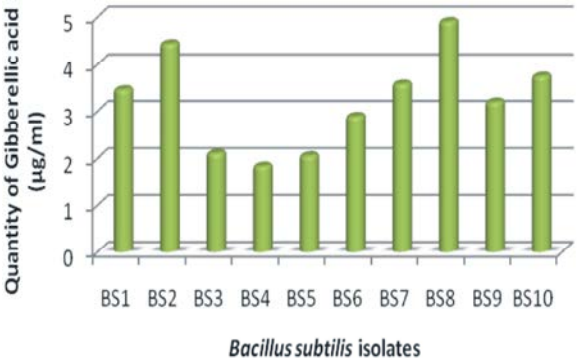

Fig. 4: Gibberellic acid production by *Bacillus subtilis* isolates

Mandira Kochar *et al*. [20] analyzed the biocontrol strain *Pseudomonas fluorescens* Psd for indole-3-acetic acid (IAA) biosynthesis and studied the effect of its consequent manipulation on its plant-growth-promoting (PGP) potential. While the indole pyruvic acid (IPyA) pathway commonly associated with PGP bacteria was lacking, the indole acetamide (IAM) pathway generally observed in phytopathogens was expressed in strain Psd. Over expression of IAM pathway genes *iaaM-iaaH*, from *Pseudomonas syringae* subsp. *savastanoi* drastically increased IAA levels and showed a detrimental effect on sorghum root development.

The Gibberellic acid produced *Pseudomonas fluorescens* and *Bacillus subtilis* was estimated and the results were showed in Figure 3 and Figure 4. The isolate *Pseudomonas fluorescens* (PS – 8) showed maximum Gibberellic acid production (5.96 µg/ml) and least Gibberellic acid production was showed by PS- 4 (2.89 µg/ml). The Gibberellic acid production by *Bacillus subtilis* was low when compared to *Pseudomonas fluorescens*.

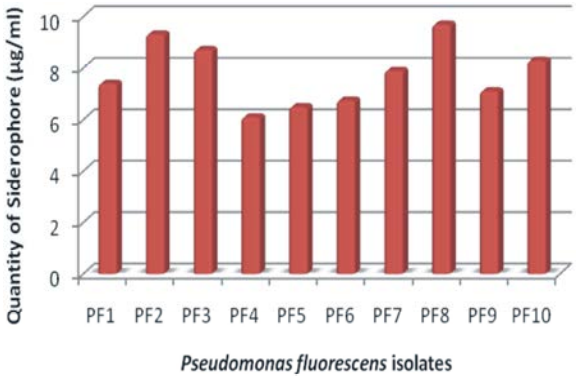

Fig. 5: Siderophore production by *Pseudomonas fluorescens* isolates

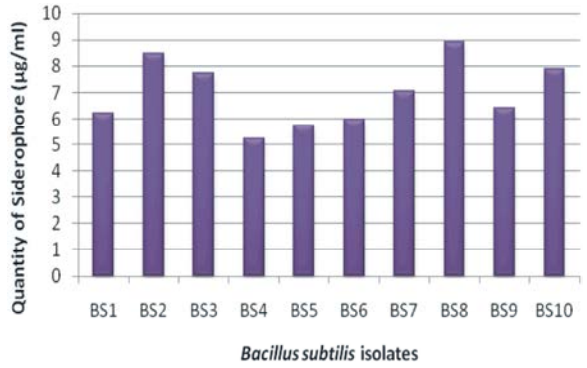

Fig. 6: Siderophore production by *Bacillus subtilis* isolates

Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi and grasses [21]. Siderophores are also important for some pathogenic bacteria for their acquisition of iron. Siderophores are amongst the strongest binders to Fe3+ known, with enterobactin being one of the strongest of these [22]. The Siderophore production *Pseudomonas fluorescens* and *Bacillus subtilis* was estimated. The isolate *Pseudomonas fluorescens* (PS – 8) showed maximum Siderophore production (8.30 µg/ml) and least Siderophore production was showed by PS- 4 (6.10 µg/ml). The Siderophore production by *Bacillus subtilis* was minimum than *Pseudomonas fluorescens* (Figure 5 and Figure 6).

The use of phosphate solubilizing bacteria as inoculants increases the phosphorus uptake by plants [23]. Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate solubilizing Microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the phosphorus demands of plants. The ten *Pseudomonas fluorescens*

Table 5: Phosphate solubilization by *Pseudomonas fluorescens* isolates

Pseudomonas fluorescens isolates	Phosphate solubilization
PF-1	+
PF-2	+
PF-3	+
PF-4	+
PF-5	+
PF-6	+
PF-7	+
PF-8	++
PF-9	+
PF-10	+

+ - Low; ++ - Medium; +++ - High

Table 6: Phosphate solubilization by *Bacillus subtilis* isolates

Bacillus subtilis isolates	Phosphate solubilization
BS-1	+
BS -2	++
BS -3	++
BS -4	+
BS -5	+
BS -6	+
BS -7	++
BS -8	+++
BS -9	++
BS -10	++

+ - Low; ++ - Medium; +++ - High

and *Bacillus subtilis* isolates obtained from the rhizosphere of paddy were tested for their efficiency of Phosphate solubilization. All the above 10 isolates taken from the study showed positive phosphate solubilization. The maximum phosphate solubilization was recorded by the isolate BS - 8 (+++). The minimum phosphate solubilization was found in BS - 1, BS - 4, BS - 5 and BS - 6 (+) isolates. The phosphate solubilization by *Pseudomonas fluorescens* was very low (Table 5 and Table 6). Anjani *et al*. [24] isolate a growth promontory fluorescent *Pseudomonas* having the potential of phosphate solubilization and siderophore production. Mishra *et al*. [25] reported higher solubilization of phosphate by *Rhizobium phaseoli* than *Pseudomonas* spp.

CONCLUSION

From the present research, it was concluded that the plant growth promoting rhizobacteria (PGPR) isolates *Pseudomonas fluorescens* and *Bacillus subtilis* have the capacity to produce plant growth promoting substances and the isolates PF – 8 and BS - 8 isolated from Sivayam village was highly effective in the production of plant growth promoting substances when compared to other

Pseudomonas fluorescens and *Bacillus subtilis* isolates. The isolate *Pseudomonas fluorescence* showed maximum production of Indole acetic acid (IAA) and Gibberellic acid (GA3) when compared to *Bacillus subtilis*. Phosphate solubilization was highly observed in *Bacillus subtilis*. In conclusion, application of the PGPR isolates *Pseudomonas fluorescens* and *Bacillus subtilis* as an individual inoculum or in combination will maximize the growth and yield of Paddy (*Oryza sativa* L.).

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