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Prevalence of *Bacillus subtilis* and *Pseudomonas fluorescens* of Paddy rhizosphere soil in Tirupattur, Tamilnadu, India

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Abstract

The paddy rhizosphere soil sample was collected from ten different locations in Tirupattur district of Tamil Nadu. The *Pseudomonas fluorescens* and *Bacillus subtilis* isolates were 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. The *Bacillus subtilis* population was designated as BS - 1 to BS - 10. 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. Plant growth-promoting rhizobacteria (PGPR) such as *Bacillus* and *Pseudomonas* have drawn broad attention and interest due to their agricultural benefits.

Key words : Paddy, Rhizosphere soil, PGPR, *Pseudomonas fluorescens* and *Bacillus subtilis*.

Free-living bacteria known as plant growth-promoting rhizobacteria (PGPR) invade plant roots and stimulate plant development[2]. By using their own metabolism (fixing nitrogen, generating hormones, or solubilizing phosphates), directly influencing the plant metabolism (increasing the uptake of water and minerals), improving root development, raising the plant's enzymatic activity, other advantageous microbes to increase their action on the plant, or suppressing plant pathogens, PGPR may encourage plant growth[6]. The ability of several microorganisms to stimulate plant development has led to the commercialisation of microbial products that improve plant health and growth[4]. It has been shown that bacteria from the plant rhizosphere positively impact roots and overall plant development. Plant-growth-promoting rhizobacteria (PGPR) are the name given to these kinds of bacteria[8]. These rhizobacteria have a significant positive impact on plant development through direct and indirect processes. Creating substances that promote plant development and reduce

stress is one of the direct approaches[5].

The PGPR activity of several strains of *Bacillus*, the most prevalent species in the rhizosphere, has long been recognized, leading to a thorough understanding of the processes at play. The metabolites that these strains emit can significantly impact the environment by making plants more nutrient-available[6]. *Bacillus subtilis* can develop taller plants by keeping a steady touch with them. By producing plant growth hormones, *Bacillus* species utilized as biofertilizers most likely directly affect plant development[7]. Through improved Phosphorus feeding, which increases the absorption of nitrogen, phosphorus, potassium, and iron, phosphate-solubilizing *Bacillus species* promote plant development[1]. By improving the effectiveness of nitrogen fixation by bacteria and the availability of iron (Fe) and zinc (Zn) through the synthesis of chemicals that promote plant development, phosphorus biofertilizers may help increase the availability of phosphates that have accumulated in the soil and may also improve plant growth[3].

Details of the Locations :

The survey was conducted at ten locations Tirupattur district of Tamil Nadu comprising Andiyapanur, Kurisilapattu, Kudapattu, Madapalli, Madavalam, Vengalapuram, Somalapuram, Kathirampatti, Pichanur and Asiriyar Nagar.

Collection of Paddy rhizosphere from different locations :

In each and every location of the survey area, a field which has been under long-term monoculture practice was selected. The locations of rhizosphere samples were made at different locations of the paddy field. In each and every location of the survey area, a field which has been under long-term monoculture practice was selected. The locations of rhizosphere samples were made at different locations of the paddy field. The collected soil samples were brought to the laboratory for further analysis.

Isolation and enumeration of Pseudomonas fluorescens and Bacillus subtilis population:

The paddy rhizosphere soil samples collected from ten paddy fields 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 up to 10^{-6} dilution. One ml of suspension from 10^{-4} and 10^{-5} dilution was aseptically transferred to sterile Petri plates and 10 – 20 ml of selective King's B medium and Nutrient agar medium was added and incubated at 37 °C for 24 hours. Three replications were maintained for each dilution. The colonies were counted by using the Quebec 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 the 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 were obtained from the rhizosphere of paddy grown at ten different locations in Tirupattur

District and designated as PF. The *Bacillus subtilis* isolates were designated as BS. Both 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; Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase; Plating on Selective media and By performing biochemical tests.

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 occurrence of *Pseudomonas fluorescens* and *Bacillus subtilis* population in the rhizosphere of paddy grown at ten selected locations was studied and the results were shown in Table - 1. The location, namely Kathirampatti, recorded maximum community population of *Pseudomonas fluorescens* (7.71 cfu × 106 g^{-1}) and *Bacillus subtilis* (5.60 cfu × 106 g^{-1}), while Asiriyar Nagar recorded least population of 7.21 cfu × 10^6 g^{-1} (*Pseudomonas fluorescens*) and 5.10 cfu × 10^6 g^{-1} (*Bacillus subtilis*) in the rhizosphere. All other locations recorded the community population of *Pseudomonas fluorescens* and *Bacillus subtilis*.

Table-1. Occurrence of Community *Pseudomonas fluorescens* population from Rhizosphere of Rice at Tirupattur District

Rhizosphere soil sample	*Pseudomonas fluore-scens* cfu×10^6 g^{-1}	*Bacillus subtilis* cfu × 10^6 g^{-1}
Andiyapanur	7.65	5.54
Kurisilapattu	7.66	5.33
Kudapattu	7.55	5.45
Madapalli	7.60	5.50
Madavalam	7.36	5.25
Vengalapuram	7.63	5.52
Somalapuram	7.66	5.33
Kathirampatti	7.71	5.60
Pichanur	7.31	5.20
Asiriyar nagar	7.21	5.10

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

Rhizosphere soil sample	*Pseudomonas fluorescens* Designation	*Bacillus subtilis* Designation
Andiyapanur	PF - 1	BS - 1
Kurisilapattu	PF - 2	BS - 2
Kudapattu	PF - 3	BS - 3
Madapalli	PF - 4	BS - 4
Madavalam	PF - 5	BS - 5
Vengalapuram	PF - 6	BS - 6
Somalapuram	PF - 7	BS - 7
Kathirampatti	PF - 8	BS - 8
Pichanur	PF - 9	BS - 9
Asiriyar nagar	PF - 10	BS - 10

Table-3. Characterization of *Pseudomonas fluorescens* from the Rhizosphere Rice Soil

Characters studied	*Pseudomonas fluorescens* (PF) isolates									
	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	-	-	-	-	-	-	-	-	-	-

Table-4. Characterization of *Bacillus subtilis* from the Rhizosphere Rice Soil

Characters studied	*Bacillus subtilis* (BS) isolates									
	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	BS-7	BS-8	BS-9	BS-10
Gram staining	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+
Endospore	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	-	-	-
VP	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
O-F Test	+	+	+	+	+	+	+	+	+	+

Ten strains of *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated from various areas from Tirupattur District. The *Pseudomonas fluorescens* isolates were designated as "PF" series and numbered randomly. The *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. The characteristics of *Pseudomonas fluorescens* were shown in Table-3 and the characteristics of Bacillus subtilis isolates were shown in Table-4.

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	+

The ten *Pseudomonas fluorescens* and *Bacillus subtilis* isolates obtained from the rhizosphere of paddy were tested for their Phosphate solubilization efficiency. 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).

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

From the present research, it was concluded that the Plant Growth Promoting Rhizobacteria (PGPR) isolates *Pseudomonas fluorescens* and *Bacillus subtilis* can produce plant growth promoting substances and the isolates PF – 8 and BS - 8 isolated from Kathirampatti village was highly effective in the production of plant growth promoting substances when compared to other *Pseudomonas fluorescens* and *Bacillus subtilis* isolates. 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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