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Research Article

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Efficiency of plant growth promoting rhizobacteria for the enhancement of *Vigna mungo* growth

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ABSTRACT

The rhizosphere soil of vigna mungo was collected and physicochemical parameter of the soil was studied. The plant growth promoting rhizobacteria were isolated and identified based on morphological and biochemical characteristics.The bacterial species were Pseudomonas fluorescens and Bacillus subtilis. The isolates were screened for phosphate solubilization and IAA producting ability was also measured. The PGP also characteristic such as catalase and siderosphore production was determined. The pot culture experiment was performed with PGPRs. From this,the phytochemical compounds such as chlorophyll, carbohydrate, flavonoids and phenol contents were determined from the leaves of Vigna mungo at 15d, 30d, 45d and 60 days interval.The role of PGPR as biofertilizers that exert beneficial effect on plant growth and development.

Key words: Rhizobacteria, Phosphate solubilization, IAA production, catalase, siderosphore production and phytochemical analysis.

INTRODUCTION

Microorganisms play an important role in the availability of soil phosphorous to plant roots, and increasing P-mobilization in soil. The ability of soil microorganisms to convert insoluble forms of phosphorus into a soluble form is an important trait in plant growth-promoting bacteria for increasing plant yields[1]. The main advantage of using Rhizobia as P-solubilizing microorganism will be their dual beneficial nutritional effect resulting both from phosphorous mobilization, N_2-fixation [2] and their well-documented synergistic interactions with Arbuscular Mycorrhizal fungi[3]. Many P-solubilizing bacteria belong to the *Pseudomonas*, *Bacillus*, *Enterobacter*, *Serratia*, *Pantoea*, *Rhizobium*, *Flavobacterium* and fungal genera such as *Aspergillus* and *Penicillium*[4]. Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers, forcing the search for alternative ways to improve a more sustainable agriculture[5]. Beneficial free-living soil bacteria isolated from the rhizosphere, which have been shown to improve plant health or increase yield, are usually referred to as plant growth-promoting rhizobacteria (PGPR).

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as Plant Growth-Promoting Rhizobacteria (PGPR). In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue. PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized[6]. A PGPR *Pseudomonas fluorescens* B16 isolated from the roots of graminaceous plants has been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit

weight of tomato plants[7]. Plant growth promoting rhizobacteria (PGPR) are often novel and potential tools to provide substantial benefits to agriculture.

The main group of phytohormones is auxin, cytokinin, gibberellin, and ethylene like substances. One of the phytohormones produced by soil microorganisms is indole-3 acetic acid (IAA) which is an important hormone for plant growth and development. Many investigations have focused on the close relationship between plants and plant growth-promoting rhizobacteria (PGPR). Some rhizobacteria can reduce the toxicity of heavy metals, resulting in the stimulation of plant growth[8].

Hence, the present study was carried out with the Collection of soil sample form the rhizosphere of *Vigna mungo.* Analysis of physicochemical parameters of the soil. Isolation and identification of plant growth promoting Rhizobacteria from the *Vigna mungo* rhizosphere soil. Effect of PGPRs on the growth and photochemical constituents of *Vigna mungo* was determined by pot culturing method.

EXPERIMENTAL SECTION

Sample collection

Soil samples were collected from the rhizosphere region of black gram (*vigna mungo*) in Thanjavur district, Tamil Nadu, South India. The collected soil samples were taken in a sterile container and were transferred to the laboratory for microbiological analysis.

Analysis of physicochemical properties

Test plant rhizosphere soil samples were collected separately from study site, and a portion of soil was analysis for texture, pH, conductivity, total organic matter and total soluble sugar at the soil testing Laboratory, Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu by the following standard methods [9] and [10].

Isolation and identification of plant growth promoting bacteria

Serial dilution and plating method used for isolation and characterization the organisms. The morphological and biochemical tests were done by the methods described in experiments in microbiology, plant pathology and Biotechnology [11].

Plant growth promoting activities

Phosphate solubilization activities

All bacterial isolates were screened for inorganic phosphate solubilization. A loopfull of fresh bacterial cultures were streaked on to National Botanical Research Institute's Phosphate Growth Medium containing inorganic phosphate and plates were incubated at 28±2^{o}C for 3 days. After 3 days, the colonies showing the clear halo zone around them indicated solubilization of mineral phosphate. Phosphate solubilization activities were screened by measuring the clearing zone surrounding the developed bacterial colony via calculation of phosphate solubilization index [12].

Phosphate solubilization Index = A/B×100.

A = total diameter (colony + halo zone).
B = diameter of colony.

Quantitative estimation of phosphate

Quantitative estimation of inorganic phosphate solubilization was done as per methodology described by Nautiyal and Jackson, 2001. Bacterial isolates were grown in National Botanical Research Institute's phosphate (NBRIP) broth containing 0.5% tricalcium phosphate (TCP). The absorbance of the resultant colour was read after 10 min at 430 nm in UV/Visible spectrophotometer.

IAA Production

A single colony of bacterial culture was grown on LB liquid medium. The cultures in the flask shows dense milky white growth were tested for purity [13].

Catalase activity
Catalase test was performed by taking a drop of 3% hydrogen peroxide was added to 48 hours old bacterial colony on a clean glass slide and mixed using a sterile tooth – pick. The effervescence indicated catalase activity.

Siderophore test
Thin layer of silica gel G60 slurry was prepared on glass plate and spotted with the isolated bacterium supernatant on thin layer plate, development of the chromatogram in the solvent system of methanol: chloroform (1:9). Appearance of reddish brown colour indicating the presence of siderophore [14].

Pot culture experiment
The seedlings of *vigna mungo* were transplanted in four pots of equal size. Soil samples were collected at, Okkanadu keeliyur, Thanjavur district. Soil was used as the culture medium. The pots were provided with water facilities. Pot culture experiment was conducted at PG and Research Department of Microbiology, STET, Women's college, Mannargudi, Tamil Nadu.

There were four treatments were performed. The three replication for each treatment were performed. All the pots were arranged in a randomized design. The pots were maintained in the open shade at the temperature 27C°- 30°C

Morphological parameter
Following morphological parameters were studied They were height of the plant (in cm), number of leaves (per plant), number of roots, (per plant), shoot length (in cm), root length (in cm), yield (seed in gm), root nodules (per plant) and number of flowers (per plant).

Phytochemical analysis
Chlorophyll Estimation [15]
Chlorophyll content was calculated by following standard formula ,

$$\text{mg of chlorophyll 'a'/g of Fresh leaves} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg of chlorophyll 'b'/g of Fresh leaves} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg of total chlorophyll/g of fresh leaves} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

Where,
A = absorbance at specific wave lengths.
V = final volume of chlorophyll extract in 80% acetone
W = fresh weight of tissue extracted

Carbohydrate estimation by Anthrone method [16]
Amount of carbohydrate present in 100 mg of the sample, was calculated by

$$= \frac{\text{Mg of glucose}}{\text{Volume of the sample}} \times 100$$

Test for flavonoids – Shinoda's test [17]
Methanolic extract with few ml of alcohol was heated with magnesium and concentrated HCl was added under cooling. Appearance of pink colour indicates the presence of Flavonoids.

Test for phenols [18]
Three drops of methanol extract was taken in a spot plate and then a drop of neutral ferric chloride was added. Appearance of purple colour showed the presence of phenolic compounds.

Statistical analysis
Random sampling was used for the entire test. The data of all the parameters were statistically analyzed and expressed as mean ± S.D by using the formula given [19].

$$\text{Mean} = \overline{X} = \frac{\sum X}{N}$$

ΣX = sum of all the values of variable
N = Number of observation

S.D

$$= \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Where,
$\sum (x - \bar{x})^2$ = The sum of the square of the deviation of each value from the mean
N = Number of observation.

RESULTS AND DISCUSSION

Sample collection
In the present study, the rhizosphere soil of *vigna mungo* was collected and physicochemical parameters were studied.

Physicochemical properties
The organic content in soil samples was considered as one of the key determinants driving the microbial community structure [20].The physicochemical properties of soil such as soil texture, pH, Electrical conductivity, total soluble salt and organic matter content were studied. The soil texture was clay loam soil and showed the pH 7. The total organic content was 1.06%. It showed 0.33 µm/cm^{-1} conductivity. The soluble sugar content of soil was 12.4%.

Isolation and characterization of organism
Serial dilution and plating method used for isolation and characterization of the organisms. Hence, the isolated organisms were confirmed as, *Bacillus subtilis* and *Psudomonas fluorescens.* The bacterial colony was compared with Bergey's manual of systematic Bacteriology.

Characterization of rhizobacteria for PGP traits
Plant growth promoting rhizobacteria (PGPR) colonizing the surface or inner part of roots play an important positive role that directly or indirectly influences plant growth and development [21]. The plant growth promoting activity of rhizobacteria were determined and the results were indicated.

Phosphate solubilization
In the concentration of phosphate released in to the medium varied from strain to strain which would be a consequence phosphate precipitation of organic metabolites as reported earlies[22]. The maximum phosphate solubilization was measured in *Bacillus subtilis* (36.8mm) when compared with *pseudomonas fluorescens* (34.6 mm).

Quantification estimation of phosphate
The maximum phosphate solubilization was noticed in *B. subtilis* 562. 34 µg/ml and in *Pseudomonas fluorescens* (482.62 µg/ml).

Indole Acidic Acid Production

The ability of bacteria to produce IAA in the rhizosphere dependents on the availability of precursors and uptake of microbial IAA by plant. Growth promotion may be attributed to other mechanism such as production of plant growth promoting hormones in the rhizosphere and other PGP activities [23 and 24]. IAA production was shown higher in *Pseudomonas fluorescens* (5.3 µg) followed by *Bacillus subtilis* (3.8µg).

Catalase activity

Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. Some of the above tested isolate could exhibit more than two or three PGP traits, which many promote plant growth directly or indirectly synergistically. Similar to our findings of multiple PGP activities among PGPR have been reported by some other workers while such finding on indigenous isolates of India are less commonly explored [25].

Catalase activity was detected in *Pseudomonas fluorescens* and *Bacillus subtilis*. Evolution of gas bubbles from the H_2O_2 solution showed positive for the presence of catalase enzyme by the organisms.

Siderophore production

Production of siderophores was detected less frequently than other PGP characteristic. The isolates of *Pseudomonas* species were strong siderophore production while few isolates of *Bacillus* were able to produce siderophore.

Siderophores may directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria. Antibiotics and siderophores may further function as stress factors or singles including local and systematic host resistance [26].

Pot culture experiment

The PGPRS were used for Pot culture experiment. The parameters were analysed at 15d, 30d, 45d and 60 days interval.

Morphological parameter

Morphological parameters such as height of the plant, number of leaves , number of root, root length, shoot length, chlorophyll analysis, carbohydrate analysis, test for flavonoids and test for phenols were also determined (Table-1 to Table-6).

Table – 1 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on different morphological parameters in *Vigna mungo* (15th Days)

Treatments	Height of the plant (cm)	Number of leaves / plant	Shoot length (cm)	Root length (cm)	Number of roots/plant
T1	12.5±4.00	8±2.64	7.5±0.70	3.2±1.12	9±1.00
T2	10.4±3.54	7±2.24	6.4±1.22	2.4±1.9	8±1.11
T3	9.7±3.61	6±2.00	5.8±1.72	1.8±0.63	7±2.00
Control	7.6±1.68	4±2.00	4.0±1.95	1.8±0.63	5±2.00

Values are triplicate, mean± standard deviation
T1 = *Pseudomonas fluorescence + Bacillus subtilis*
T2 = *Pseudomonas fluorescence*
T3 = *Bacillus subtilis*

Table – 2 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on different morphological parameters in *Vigna mungo* (30th Days)

Treatments	Height of the plant (cm)	Number of leaves / plant	Shoot length (cm)	Root length (cm)	Number of roots/plant
T1	17.83±2.21	15±2.52	10±3.70	3.0±0.41	13±0.89
T2	15.9±2.70	13±2.60	9.4±1.54	3.5±0.54	12±0.72
T3	14.7±2.25	12±1.14	8.1±2.12	2.4±0.15	11±1.07
Control	13.8±2.25	11±1.73	7.9±1.80	2.5±0.43	8±0.81

Values are triplicate, mean± standard deviation
T1 = Pseudomonas fluorescence + Bacillus subtilis
T2 = Pseudomonas fluorescence T3 = Bacillus subtilis

Table- 3 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on different morphological parameters in Vigna mungo 45th days

Treatment	Height of the plant(cm)	Number of leaves / plant	Shoot length (cm)	Root length (cm)	Number of roots /plant
T1	20.4±2.85	19±2.00	11.2±0.38	3.2±0.38	15±0.96
T2	19.7±2.21	16±2.64	10.2±1.82	2.8±0.30	13±0.89
T3	18.2±3.10	15±2.32	10.0±1.66	2.7±0.29	12±0.83
control	17.5±2.55	14±2.29	9.44±2.17	2.5±0.45	11±0.54

Values are triplicate, mean± standard deviation
T1 = Pseudomonas fluorescence + Bacillus subtilis
T2 = Pseudomonas fluorescence
T3 = Bacillus subtilis

Table- 4 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on different morphological p parameters in *Vigna mungo* 60th days

Treatment	Height of the plant(cm)	Number of leaves / plant	Shoot length (cm)	Root length (cm)	Number of roots /plant
T1	22.7± 2.98	25±2.51	12.6±2.57	3.5±0.2	16.3±1.52
T2	21.4±2.39	23±2.00	12.1±2.18	2.9±0.22	15±0.20
T3	20.5±2.23	22±2.32	12±2.18	2.8±0.15	14±0.51
control	19.5±2.32	21±2.64	11.7±2.02	2.7±0.38	14±1.00

Values are triplicate, mean± standard deviation
T1 = Pseudomonas fluorescence + Bacillus subtilis
T2 = Pseudomonas fluorescence
T3 = Bacillus subtilis

Table – 5 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on Chlorophyll content of *Vigna mungo* (15th, 30th, 45th and 60th Days)

Treatment	Chlorophyll (mg/g)											
	15th day			30th day			45th day			60th day		
	a	b	total	a	B	Total	a	B	total	A	b	total
T1	0.107	0.070	0.177	0.158	0.135	0.293	0.190	0.172	0.362	0.181	0.168	0.349
T2	0.102	0.067	0.169	0.144	0.123	0.267	0.188	0.167	0.355	0.178	0.154	0.332
T3	0.098	0.066	0.164	0.131	0.116	0.247	0.182	0.160	0.342	0.173	0.135	0.308
Control	0.073	0.053	0.126	0.112	0.098	0.210	0.180	0.143	0.323	0.162	0.128	0.290

Values are triplicate and expressed as Mean
T1 = Pseudomonas fluorescence + Bacillus subtilis
T2 = Pseudomonas fluorescence
T3 = Bacillus subtilis

TABLE-6 Effect of *Pseudomonas fluorescens* and *Bacillus subtilis* on total carbohydrate, Flavonoid and Phenol in the leaves of *Vigna mungo* (60th day)

Treatment	Carbohytrate (mg)	Phenol (µg/g)	Flavonoid (mg/g)
T1	18.0	62.2	97.5
T2	16.7	74.5	85.7
T3	15.8	74.7	72.1
Control	9.0	43.2	62.4

Values are triplicate and expressed as Mean
T1 = Pseudomonas fluorescence + Bacillus subtilis
T2 = Pseudomonas fluorescence
T3 =Bacillus subtilis

CONCLUSION

The rhizosphere soil of *Vigna mungo* was collected and physicochemical parameters of the soil were studied. The plant growth promoting rhizobacteria were isolated and characterized from the collected soil. The PGP characteristics such as phosphate solubilization activity, quantitative estimation of phosphate, indole acidic acid production, catalase and siderosphore production were also determined.

The seedling of *Vigna mungo* were transplanted in 4 pots of equal size, which were noted as Treatment 1- *Pseudomonas fluorescens* plus *Bacillus subtilis*, Treatment 2- *Pseudomonas fluorescens*, Treatment 3- *Bacillus subtilis* and control. The day's intervals. The seeds were sowed into pots. The selected PGPRs were inoculated into each pot. Then morphological parameters such as leaf number, height, shoot length, root length, root number and chlorophyll, carbohydrate, phenol, flavonoid contents were analyzed at different intervals.

The results from this study were presented in tables . From this results, maximum number of leaf, plant height, shoot length, root length and roots were observed in the plants treated with *Pseudomonas fluorescens* plus *Bacillus subtilis* (T1) than those of other treatment (T2, T3 and control) in all intervals. The percentage increases in all the parameter found in all the inoculated plants compared with control.

The result suggests that PGPR are able to induce the production of IAA, solubilization of phosphate and resistance to pathogen and pests, thereby improving growth of plants. The use of PGPR as inoculants is an efficient approach to replace chemical fertilizers. The role of PGPR as biofertilizers that exert beneficial effects on plant growth and development.

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