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Identification and Characterization of *Stenotrophomonas rhizophila* Isolated from Rhizosphere Soil of *Tephrosia purpurea* L. (Green Manure Crop) of Tamil Nadu

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Abstract

Phosphate solubilizing bacteria plays a significant role in converting insoluble form of phosphorus into soluble form which can be utilized by the plants and possess many plants growth promoting traits. Eighty-six efficient phosphate solubilizing bacteria were isolated from the rhizosphere soil of *Tephrosia purpurea* L (A medicinal plant and a green manure crop belongs to family Fabaceae known as Sharpunkha) from different districts in Tamil Nadu. Out of 86 strains based on the highest phosphate solubilization four strains SS2, SS5, SS7 and SS11were identified and screened for multifarious PGPR traits (Phosphate solubilization, phosphate solubilizing index, determining pH change of the medium, production of siderophore, IAA, gibberellin, ammonia, HCN and Nitrogen fixation) and extracellular enzyme activities (protease, amylase, catalase, cellulase and ACC deaminase). All the four strains showed positive for the PGPR and extracellular enzyme activities. From the biochemical tests (IMViC, H_2S production, nitrate reduction, Urea, Gelatin and casein hydrolysis) and 16SrRNA sequencing the isolates were identified as *Stenotrophomonas rhizophila*_SS2, *Stenotrophomonas rhizophila*_SS5, Stenotrophomonas *rhizophila*_SS7, *Stenotrophomonas rhizophila*_SS11 (GenBank Accession no OM131765, OM131766, OM131767, OM13178) respectively. The studies on the efficient four strains conclude that these strains possess the plant growth promoting traits and produces extracellular enzymes can be used as an efficient bioinoculants.

***Key words*:** *Stenotrophomonas rhizophila, Tephrosia purpurea*, PGPR traits, Plant growth hormones, Extracellular enzymes, 16SrRNA sequencing

Plant growth-promoting rhizobacteria exhibit significant activity within soil ecosystems, particularly in relation to legumes, owing to their biotic functions. *Vuralia turcica* (Kit Tan, Vural & Kucukoduk) Uysal and Ertugrul, an indigenous legume species from Turkey, holds promising potential as both an ornamental and food crop. Nevertheless, there is a dearth of literature documenting the presence and impact of plant growth-promoting rhizobacteria within the rhizosphere of *Vuralia turcica* [1].

Phosphorus is one of the vital macronutrients of crops for energy production, growth and development of plants which were present in both organic and inorganic forms in soil. In soils the phosphorus content was about 0.05% (w/w) in average and of this 0.1% is available to plants and the remaining is present as insoluble form. In order to rectify the phosphorus deficiency, chemical fertilizers were used but the overloaded of phosphates in soil leads to eutrophication. Phosphate solubilizing bacteria plays a major role in converting the insoluble form of phosphorus into soluble form which helps in overcome these problems. Plant growth promoting bacteria (PGPR) had the ability to promote the growth and development of plant by the mechanisms such as nitrogen fixation, phosphate solubilization, phytohormones and extracellular enzymes production [2].

A total of 73 actinomycetes isolates were obtained from the compost samples. The qualitative investigation focused on the ability of these isolates to produce various extracellular enzymes. The results revealed that 68.5% of the isolates exhibited amylase activity, while all of them demonstrated cellulase activity. Additionally, 47.9% of the isolates displayed chitinase activity, 94.5% exhibited pectinase activity, and 98.6% showed protease activity. Furthermore, 96.3% of the isolates demonstrated lipase/esterase activity. To assess their antibacterial potential, the isolates were primarily tested using the cross-streak method. The findings indicated that the isolates exhibited significant antibacterial activities. Specifically, they displayed 98.6% and 84.9% inhibition against *Staphylococcus aureus* Rosenbach and *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Bäl, respectively [3]. Analyzing the 16S rRNA and 16S-23S rRNA ITS region, a total of ten bacterial strains were isolated and identified. Among these, four isolates were identified as Bacillus megaterium, three strains as *Stenotrophomonas rhizophila*, one strain as *Rhodococcus*

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erythropolis, one strain as *Xanthomonas albilineans*, and the remaining one strain as *Lysobacter enzymogenes* [1].

Phosphate solubilizing bacteria also involved in biodegradation, controlling environmental stress tolerance and as a biological control agent in addition to the production of siderophore and organic acids. *Tephrosia purpurea*, a medicinal plant belongs to family Fabaceae commonly known as Sharpunkha occurs naturally in the waste places along the road sides and it prefers to grow in dry, gravelly or rocky and sandy soil used as a folk medicine distributed among India, Australia, China and Sri Lanka up to 400 m to 1300 m altitude. Traditionally agriculture fields were applied with this plant as a source for nitrogen at the time of ploughing which helps in fixation of nitrogen for crops and helps in soil fertility. The present work was to identify the efficient phosphate solubilizing bacteria isolated from rhizosphere soil of *Tephrosia purpurea* L. and to screened for multifarious PGPR and extracellular enzyme activities.

MATERIALS AND METHODS

Sample collection

Thirty rhizosphere soil samples of *Tephrosia purpurea* L plants were collected from different districts in Tamil Nadu. The rhizoshere soil samples which adhere to the root hairs and about 0-15 cm below the surface soil of *Tephrosia purpurea* L plants. The samples were collected randomly from the agricultural fields and bushy areas within 1-2cm interval between the same samples and air dried, sieved and stored in sterile plastic containers. All the soil samples were tested for the physical and chemical parameters in the Soil testing Lab, Department of Agriculture, Government of Tamil znadu, Mannarpuram, Trichy-20, TamilNadu.

Isolation of phosphate solubilizing bacteria

One gram (1g) of each soil sample was measured and transferred to 9 ml of sterile distilled water to form 10-1 dilution and from this 1 ml of sample was serially diluted up to 10-9.0.1 ml from the dilutions 10-4 to 10-9 was spreaded over the sterile Pikovskaya medium and incubated at 37°C for 2-7 days. Triplicates were done and the uninoculated plate served as control [4].

Characterization of isolated bacterial strains

From the collected soil samples, four efficient phosphate solubilizing bacteria were isolated and selected on the basis of zone formation and identified as SS2, SS5, SS7 and SS11. Morphological characteristics of each isolated colony were examined on pikovskaya plates. After 7 days of incubation, different characteristics of colonies such as size, shape, elevation, margin, opacity, pigmentation, etc. were recorded [5].

Biochemical characterization of selected bacterial strains

The isolated efficient strains SS2, SS5, SS7 and SS11 were identified using standard biochemical tests as in the Bergey's Manual of Determinative Bacteriology [5].

PGPR activity of isolated bacteria

Phosphate solubilization

Phosphate solubilizing ability of all PGPR isolates was checked on to the Pikovskya's agar medium [6]. All the isolates were streaked on to the Pikovskya's agar plate and incubated at room temperature for 5 to 6 days. Phosphate solubilization was detected by clear halo zones around the colony.

Determination of phosphate solubilizing activity

Phosphate solubilizing activity was measured quantitatively by the change in the pH of broth media. For this the identified efficient phosphate solubilizing bacterial cultures was inoculated in Pikovskaya broth and incubated for 7 days. The initial pH and the change in pH were checked and recorded from Day 1 to Day 7 of incubation and further reduction of pH were also noted for up to 14 days [4].

Siderophore production

Siderophore production ability of the all PGPR isolates was carried out on to the CAS agar medium (Chrome Azurol S medium). All the isolates were streaked on to the CAS agar plate and incubated at room temperature for 24 to 72 hours. Siderophore productions were detected with orange halos around the colonies [7].

HCN production

HCN production was evaluated by streaking the bacterial isolates on King's B agar medium supplemented with glycine. Whatman No.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. The plates were then sealed airtight with parafilm and incubated at 30°C for 48 h. A colour change of the filter paper from deep yellow to reddish- brown colour was considered as an indication of HCN production [8].

Ammonia production

This test is based on the production of urease which break urea into ammonia and which in turn increase the pH of the medium. Freshly grown cultures of bacterial isolates were inoculated into urea broth containing peptone and incubated at 37°C for 24 hrs. Bacterial cultures were then centrifuged.1 ml of Nessler's reagent was added to the supernatant and change in color yellow to brown was a positive test for ammonia production [9].

Nitrogen fixation

Nitrogen fixation ability was evaluating by growing on N- free LGI medium. Bacterial isolate was streaked on LGI medium and incubated at 28 °C for 7 days. Observation of bacterial growth on plate was observed as qualitative evidence of atmospheric nitrogen fixation [10].

IAA (Indole-3-acetic acid) production

IAA production of PGPR isolates was carried out by using Salkowaski's method. All the isolates were enriched in to peptone broth containing tryptophan for 24 hours. Then, the culture medium was centrifuged at 9000x rpm for 10-15 minutes to separate bacterial cells from the culture medium. Further, Salkowaski's reagent (4.5 grams of Fecl3 per liter in 10.8 M H_2SO_4) were mixed with the supernatant and incubated in dark at room temperature for 30-40 minutes. Absorbance was taken at 530 nm [11].

Determination of gibberellins production

The gibberellins were estimated calorimetrically by the method of Holbrook *et al*. [12]. 15 ml of supernatant, 2 ml of zinc acetate reagent (21.9 g zinc acetate + 1 ml of glacial acetic acid and volume was made up to 100 ml with distilled water) was added. 2 ml of potassium ferrocyanide (10.6% in distilled water) was added after 2 minutes and was centrifuged at 2000 rpm for 15 minutes. To 5ml of supernatant 5 ml of 30 per cent HCI was added and mixture was incubated at 200C for 75 min. Absorbance was read at 254 nm and concentration of gibberellins was calculated by preparing standard curve by using gibberellic acid as standard (10-100 µg/ml).

Extracellular enzyme activities of PGPR

Catalase activity

Catalase test was performed by taking a 3-4 drops of hydrogen peroxide (3% H_2O_2) was added to 48 h old bacterial colony which is grown on nutrient agar medium. The release of gas bubbles indicated a positive test for catalase activity [13].

Protease activity

Protease production assay was carried on sterile skim milk agar plate (Pancreatic digest of casein 5.0 g, Yeast extract 2.5 g, Glucose 1.0 g, Agar 15.0 g, distilled water 1000 ml, and skim milk 7% was added as inducer). All the PGPR isolates were streaked on to the sterile skim milk agar plates and incubated at room temperature for 72 hours. Protease production was detected by a clear zone around the colony [14].

Amylase activity

The bacterial isolates were spot inoculated on starch agar medium plates and incubated at 28°C for 48 h. After incubation, plates were flooded with iodine solution. Yellow zones against a blue background around the colony indicated production of amylase [15].

Chitinase production

Chitinase production was determined on nutrient agar plates containing colloidal chitin. Bacterial cultures were streaked on nutrient agar plates and incubated for 7 days at 30°C. The ability of chitinase production was shown by a clear halo around bacterial colonies [16].

Cellulase enzyme production

Carboxy Methyl Cellulose (CMC) agar plates were prepared by screening for cellulose enzyme production according to the method by Samira et al., 2011 [17]. A sterile paper disc was dipped into microbial culture and transferred onto the CMC agar plates. The plates were incubated overnight at 33°C. After incubation, the plates were flooded with Congo red solution for 15 min; followed by de-staining with the salt solution for 15 min. Unstained areas indicate where the CMC has been degraded due to production of cellulose by the bacterial strain.

Pectinase enzyme production

Pectinase agar plates were prepared to screen for pectinase enzymes production, according to the method by Yogesh *et al.* [18]. A sterile paper disc was dipped into microbial culture and transferred onto the pectinase agar plates. The plates were incubated overnight at 33°C. After incubation, the plates were flooded with 50 mM iodine solution for 15 min to observe the halo zone which indicates the ability of the strain to produce pectinase enzyme.

Bacterial identification using 16S rRNA gene sequence

The selected strains were identified by partial sequencing of the 16S rRNA gene. Genomic DNA were isolated from the bacterial culture by using EX pure Microbial DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd. 16S rRNA gene was amplified using universal primers forward (5´-'AGAGTTTGATCTGGCTCAG-3') and reverse (5´-ACGGTACCTTGTTACGACTT-3'). 50µL reaction mixture were prepared containing 5 µL of DNA template, 5µL 10X PCR Reaction Buffer,0.75 µL 10 µM of each primer, 1 µL 10 µM dNTPs mix, 0.5 µL 5µ/µ L Taq polymerase, 3µL 25 µM MgCl2 and 34µL ultra-pure water. PCR reactions were carried out in a thermal cycler. The PCR product was sequenced using the primers. Sequencing reactions were performed using an ABI PRISM® Big Dye TM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems) using cycles as follows: 5 min at 95°C, 30 sec at 95°C, 30 sec at 50°C, 1 min at 72°C and final extension for 5 min at 72°C. The amplified 16S rRNA gene was purified with a Gel/PCR DNA Fragments Extraction Kit. The sequence data was aligned and analyzed to identify the bacterium and its closest neighbors by using BLAST (NCBI, USA).16sRNA gene sequencing were carried out in Yaazh Xenomics, Coimbatore.

RESULTS AND DISCUSSION

A total of 86 efficient phosphate solubilizing bacterial isolates were obtained from rhizosphere soil samples collected from *Tephrosia purpurea* (Linn.) plants in different districts of Tamil Nadu. Among these isolates, four strains (SS2 from Samayapuram in Trichy district, SS5 from Karur district, SS7 from Ramanathapuram district, and SS11 from Nachipatti in Dindigul district) were found to be highly efficient. All four strains exhibited similar morphological characteristics, being of moderate size and irregular shape with an entire, umbonate elevation. They had a smooth texture and produced a pale-yellow pigment, except for SS5 which produced a pale white pigment (Table 1-2, Fig 1).

Table 1 Sample collection sites

S. No	Sample collected districts	Place of sample collected	Type of soil	Number of samples collected	Number of isolates
1.	Pudukottai	Viralimalai	Red sandy soil	3	3
2.	Pudukottai	Pudukottai	Sandy clay loam soil	2	2
3.	Tiruchirappalli	Samayapuram	Loamy soil	3	3
4.	Tiruchirappalli	Trichy	Sandy alluvial soil	5	5
5.	Tiruchirappalli	Thuraiyur	Red loamy soil	2	2
6.	Ariyalur	Ariyalur	Red loamy soil	2	3
7.	Perambalur	Perambalur	Red loamy soil	2	2
8.	Karur	Karur	Red loamy soil	5	4
9.	Karur	Vennaimalai	Sandy loam soil	2	2
10.	Karur	Kulithalai	Red alluvial soil	2	2
11.	Thanjavur	Thanjavur	Alluvial soil	5	2
12.	Thanjavur	Kumbakonam	Alluvial soil	2	2
13.	Thanjavur	Pattukkottai	Sandy loam soil	1	2
14.	Thiruvarur	Thiruvarur	Alluvial soil	2	2
15.	Kallakurichi	Kallakurichi	Black soil	1	2
16.	Dindigul	Nachipatti	Red soil	3	3
17.	Dindigul	Nilakottai	Red soil	1	1

18.	Madurai	Madurai	Red loamy soil	2	2
19.	Theni	Theni	Alluvial soil	3	3
20.	Sivagangai	Sivagangai	Alluvial soil	3	3
21.	Virudunagar	Virudunagar	Alluvial soil	2	2
22.	Thenkasi	Thenkasi	Red soil	2	2
23.	Namakkal	Namakkal	Red soil	2	2
24.	Salem	Salem	Red loamy soil	2	2
25.	Dharmapuri	Dharmapuri	Black soil	2	2
26.	Krishnagiri	Krishnagiri	Red sandy soil	2	2
27.	Erode	Erode	Red sandy soil	2	2
28.	Tirupur	Tirupur	Alluvial soil	2	2
29.	Tiruvannamalai	Tiruvannamalai	Red soil	2	2
30.	Vellore	Vellore	Red loamy soil	2	2
31.	Ramanathapuram	Ramanathapuram	Red soil clay	3	3
32.	Ramanathapuram	Paramakudi	clay loam soil	2	3
33.	Mayiladuthurai	Mayiladuthurai	Red loamy soil	2	2
34.	Tirunelveli	Tirunelveli	clay loam soil	3	3
35.	Nagapattinam	Nagapattinam	Sandy coastal alluvium soil	3	3
36.	*Kanyakumari*	Nagerkovil	Deep Red Loam soil	2	2
	Total number of samples collected			80	86

Table 2 Identification of bacterial strains on the basis of external colony morphology

Strain	Size	Shape	Margin	Elevation	Surface texture	Consistency	Optical Character	Pigmentation
SS2	Moderate	Irregular	Entire	umbonate	Smooth	Butyrous	Opaque	Pale yellow
SS5	Moderate	Irregular	Entire	umbonate	Smooth	Butyrous	Opaque	Pale yellow
SS7	Moderate	Irregular	Entire	Lobate	Smooth	Butyrous	Opaque	Pale yellow
SS11	Moderate	Irregular	Entire	umbonate	Smooth	Butyrous	Opaque	Pale yellow

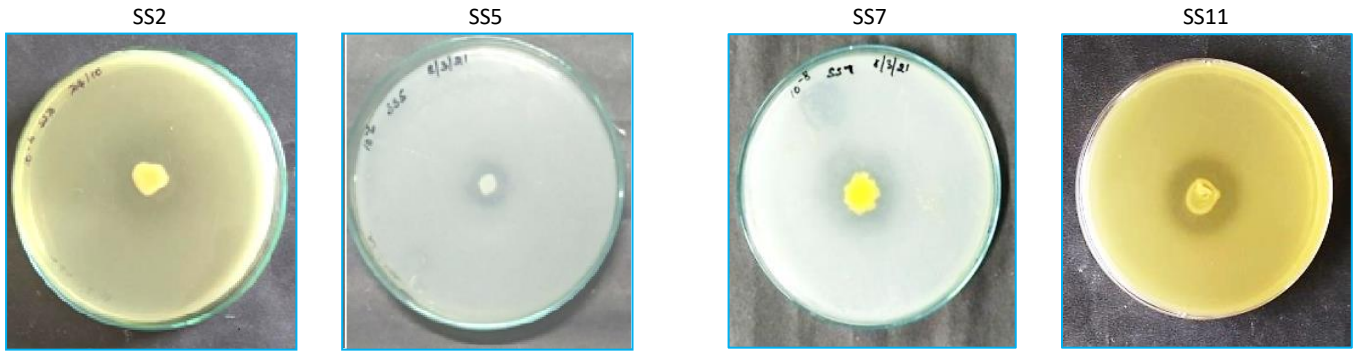

Fig 2 Isolated bacterial strains on Pikovskaya medium

Table 3 Biochemical characterization of selected efficient bacterial strains

Tests	SS2	SS5	SS7	SS11
Gram staining	Gram negative	Gram negative	Gram negative	Gram negative
Motility	Positive	Positive	Positive	Positive
Indole production	Negative	Negative	Negative	Negative
Methyl red	Positive	Positive	Positive	Positive
Voges roskauer	Negative	Negative	Negative	Negative
Catalase	Positive	Positive	Positive	Positive
Oxidase	Positive	Positive	Positive	Positive
Urease	Positive	Positive	Positive	Positive
Citrate utilization	Positive	Positive	Positive	Positive
Starch hydrolysis	Positive	Positive	Positive	Positive
Casein hydrolysis	Positive	Positive	Positive	Positive
Gelatin hydrolysis	Positive	Positive	Positive	Positive
H_2S production	Positive	Positive	Positive	Positive
Nitrate reduction	Positive	Positive	Positive	Positive
Identified isolates	*Stenotrophomonas rhizophila*_SS2	*S. rhizophila*_SS5	*S. rhizophila*_SS7	*S. rhizophila*_SS11

The strains SS2, SS5, SS7 and SS11 were subjected to biochemical characterization where all the four strains showed similar results of Gram negative, motile and showed positive for Methyl Red, Catalase, Oxidase, Urease, Citrate Utilization, Starch, Casein and Gelatin Hydrolysis. The strains also had the ability of H_2S Production and Nitrate Reduction whereas negative for Indole and Voges Proskauer (Table 3).

The isolates SS2, SS5, SS7 and SS11 showed maximum zone formation in Pikovskaya medium were SS11 showed the highest solubilization index of 60.1 followed by SS2 and SS7 with 58.8, 57.5 respectively whereas SS5 showed 52.4 of solubilization index at the incubation period of 7 days after seven days of incubation no reduction changes in zone diameter (Table 4). The data of Table-5 shows the quantitative estimation of isolates on PKV broth with initial pH 7.0. The maximum reduction in pH was noted in 7th day of incubation which falls down to 2.5 and 2.3 respectively. The strain SS2 and SS11 showed maximum reduction in pH 7.0 to 2.3 whereas SS5 and SS7 reduce pH 7.0 to 2.5 (Table 5).

Table 4 Efficiency of phosphate solubilization of isolated bacterial strains

Strain	Colony diameter (mm)	Halozone diameter (mm)	Solubilization index (mm)
SS2	1.2	69.1	58.8
SS5	1.4	71.4	52.4
SS7	1.2	67.6	57.5
SS11	1.1	64.9	60.1

All the four isolates had shown the ability for siderophore production on CAS medium with the observation of bright yellowish fluorescent color zone in the culture plate which proved the production of siderophore. The ability for hydrogen cyanide synthesis were observed for selected isolates of four strains (SS2, SS5, SS7, SS11). The increased production of HCN by the efficient strains were noted by the change in colour of the filter paper from deep yellow to reddish-brown cooler. All the four isolates had the ability of production of ammonia which was observed in by the change of colour of the supernatant from yellow to brown on the addition of Nessler's reagent. The isolates were also screened for the nitrogen fixation ability in LGI medium. The strains exhibit good growth in the nitrogen free LGI medium was observed as qualitative evidence of atmospheric nitrogen fixation (Table 6).

Table 5 Determination of phosphate solubilizing activity

DAY	SS2	SS5	SS7	SS11
1	7.0	7.0	7.0	7.0
2	5.6	5.8	5.9	5.5
3	5.5	5.5	4.6	4.2
4	4.6	4.7	4.0	3.8
5	4.0	4.1	3.5	3.4
6	3.4	3.6	3.1	2.8
7	2.3	2.5	2.5	2.3
8	2.3	2.5	2.5	2.3
9	2.3	2.5	2.5	2.3
10	2.3	2.5	2.5	2.3
11	2.3	2.5	2.5	2.3
12	2.3	2.5	2.5	2.3
13	2.3	2.5	2.5	2.3
14	2.3	2.5	2.5	2.3

The production of phytohormones IAA (Indole-3-acetic acid) and gibberellins by the isolates were estimated quantitatively. The isolate SS11 produces the highest amount of IAA (Indole-3-acetic acid) (11.752 µg/ml) and of gibberellins 46.84 µg/ml. The strains SS2, SS5 and SS7 produce IAA (Indole-3-acetic acid) 8.234 µg/ml, 6.854 µg/ml and 7.548 µg/ml whereas gibberellins 46.14 µg/ml, 43.89 µg/ml and 45.97 µg/ml respectively (Fig 1-2).

Table 6 PGPR activity of isolated bacterial strains

PGPR activity	SS2	SS5	SS7	SS11
Phosphate solubilization	+++	+++	+++	+++
Siderophore production	++	++	++	++
HCN production	+++	+++	+++	+++
Ammonia production	+++	+++	+++	+++
Nitrogen fixation	+++	+++	+++	+++

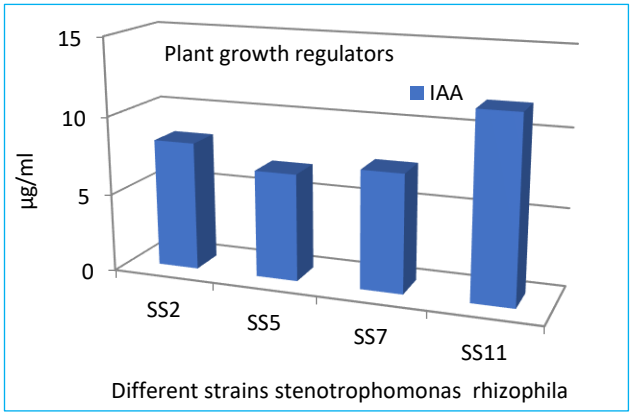

Fig 1 Production of Plant growth hormones IAA by isolates

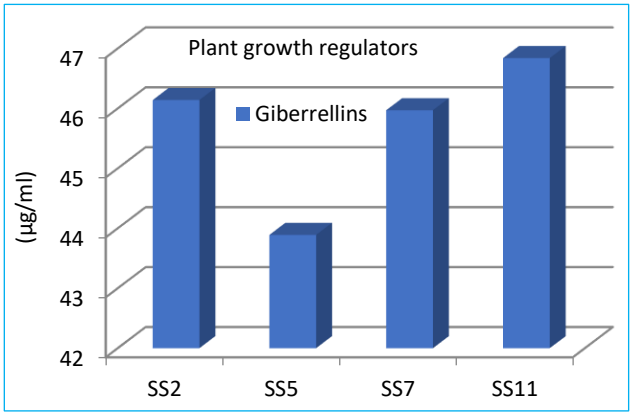

Fig 2 Production of Plant growth hormones gibberellins by isolates

The extracellular enzyme activities of catalase, protease, amylase, chitinase, cellulase and pectinase were screened for the bacterial isolates. The positive results were observed in the efficient strains of SS2, SS5, SS7 and SS11 for all the extracellular enzyme activities (Table 7).

The bacterial isolates SS2, SS5, SS7 and SS11 were identified using 16S rRNA gene amplification as *Stenotrophomonas rhizophila*_SS2, *Stenotrophomonas rhizophila*_SS5, *Stenotrophomonas rhizophila*_SS7, *Stenotrophomonas rhizophila*_SS11 from isolated from the

rhizosphere soil samples of *Tephrosia purpurea* (linn.) (Table 8, Fig 3). The sequence of SS2, SS5, SS7 and SS11 was submitted to NCBI GenBank and were assigned Accession number OM131765, OM131766, OM131767, OM13178 respectively.

Table 7 Extracellular enzyme activities

Extracellular enzyme activities	SS2	SS5	SS7	SS11
Catalase	+++	+++	+++	+++
Protease	++	++	++	++
Amylase	+++	+++	+++	+++
Chitinase	+++	+++	+++	+++
Cellulase	+++	+++	+++	+++
Pectinase	+++	+++	+++	+++

Table 8 Bacterial identification using 16S rRNA gene amplification

Strains	Isolates site	16SrRNA fragment length (bd)	Closest relatives in NCBI	NCBI accession number	Similarity
*Stenotrophomonas rhizophila*_SS2	Samayapuram	1294	AB981193.1	OM131765	100%
*Stenotrophomonas rhizophila*_SS5	Karur	1298	MT856236.1	OM131766	82%
*Stenotrophomonas rhizophila*_SS7	Ramanathapuram	1295	MTO78676.1	OM131767	64%
*Stenotrophomonas rhizophila*_SS11	Nachipatti	1298	MN561287.1	OM131768	100%

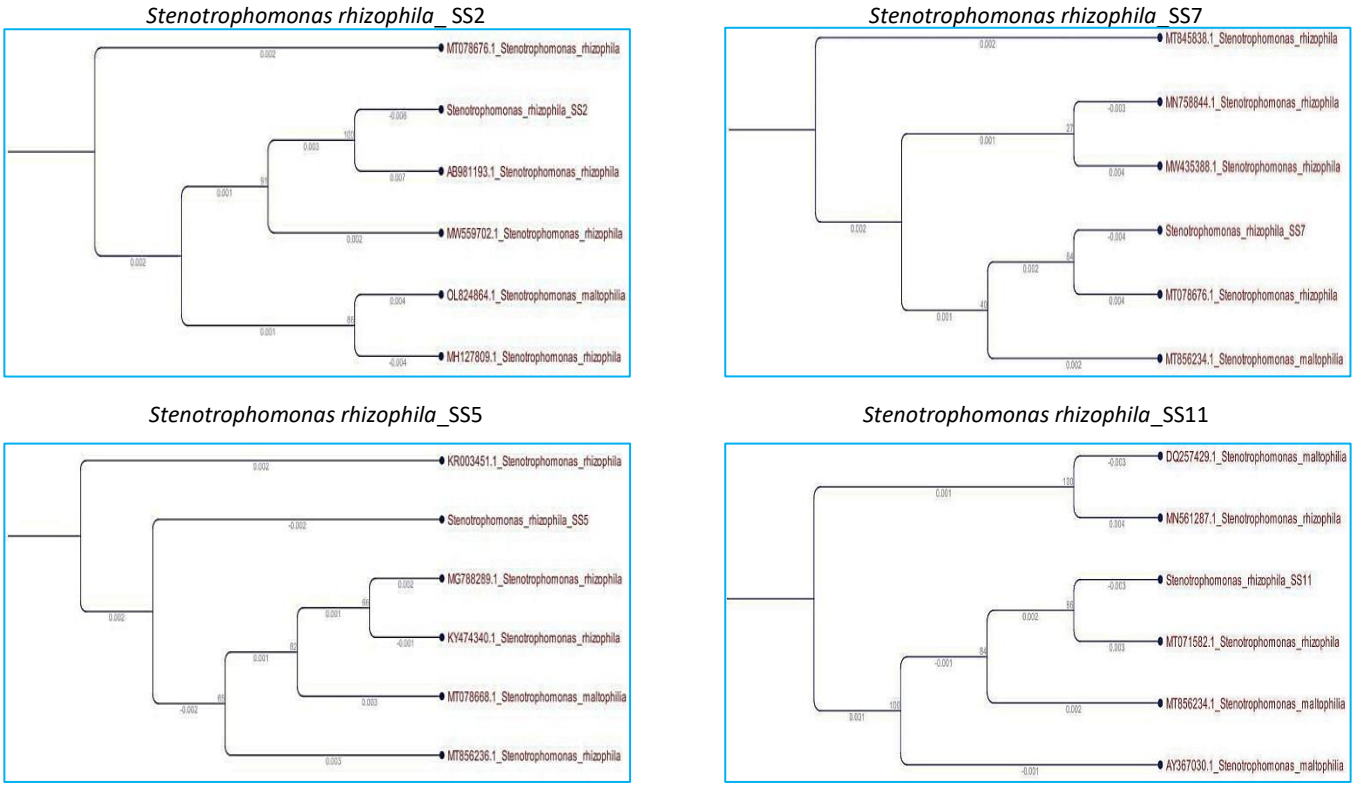

Fig 7 Bacterial identification using 16S rRNA gene amplification

Phosphate solubilizing bacteria in the rhizosphere soil of plants enhances the growth and metabolism of the plants by their PGPR and extracellular enzyme activities. [6] the pioneer in isolating phosphorite, an organism from soils capable of actively solubilizing tricalcium phosphate which was termed as 'Bacterium P'. In the previous study Reena *et al.* [19] reported that bacteria grown on Pikovskaya medium form a clear zone around the colony. The clear or halo zone was formed due to the solubilization of insoluble phosphates by bacteria isolated from different sources and Behera *et al*. [20] studied on the phosphate solubilizing bacterium PSB-37 isolated from mangrove soil of Mahanadi River delta and the strain based on the phenotypic and molecular studies was identified as species of Serratia. The strain exhibits maximum phosphate solubilizing activity of 44.84 l g/m with reduction in pH from 7.0 to 3.15. In the present study highest zone of inhibition was observed in the efficient strains SS2 (58.8), SS5 (52.4), SS7 (57.5) and SS11 (60.1). The strain SS2 and SS11 showed maximum reduction in pH of 7.0 to 2.3 whereas SS5 and SS7 reduce pH 7.0 to 2.5.

Rodríguez and Fraga [21] reported direct growth promotion due to P solubilization, N-fixation, phytohormone production, and thus making the availability of nutrients. Indirect growth promotion involves decrease or prevention of deleterious effects of pathogenic microorganisms by synthesis of antibiotics or siderophores. In previous studies Adnan *et al*. [11] collected rice rhizospheric soil samples from different field of rice from Surat region of Gujarat, India. 10 isolates in case of siderophore production, 6 isolates in case of phosphorus solubilisation, and 13 isolates in case of IAA production were

found to be positive whereas 4 isolates shown positive HCN production and 9 isolates shown protease enzyme production and chitinase enzyme production and Singh and Jha [22] studied the plant growth promoting trait of strain AJS-15 was evaluated for the production of IAA was found to be 0.531±0.050 µg/ ml. Strain was able to solubilise inorganic phosphate 8.612±2.148 µg/ ml and able to grow on nitrogen free media, indicating nitrogen fixation ability and positive for ammonia production. Strain was found to be siderophore and Amylase negative and positive for HCN production and chitinase activity.

In the present study all the four efficient strains SS2, SS5, SS7 and SS11 showed positive for Siderophore, HCN, Ammonia production and can able to grow on nitrogen free medium as an indication of nitrogen fixation. Production of IAA by the isolate SS11 11.752 µg/ml followed by SS2 (8.234 µg/ml), SS7 (7.548 µg/ml) and SS5 (6.854 µg/ml) respectively. The efficient strains SS2, SS5, SS7 and SS11showed positive results for catalase, protease, amylases, chitinase, cellulase and pectinase activity.

In earlier studies Sharma *et al*. [23] reported that the eight isolates of fluorescent *Pseudomonas sp*. showed the production of gibberellins. The gibberellins production was found in the range of 25.92-43.32 µg/ml. The maximum gibberellins production was observed in P7 (43.32 µg/ml) followed by P12 (40.74 µg/ml). In the present study the strains SS11 and SS7 produced highest amount of gibberellins 46.84 µg/ml and 46.14 µg/ml of gibberellins respectively whereas the other two strains produced 43.37 µg/ml (SS2) and 41.89 µg/ml (SS5) of gibberellins.

In previous studies Pérez *et al*. [24] characterize and molecularly identified *Stenotrophomonas rhizophila* strains from the maize rhizosphere and reported *Stenotrophomonas* is a common bacterial genus in many soil types and rhizospheres of different crops, and presents mechanisms for growth promotion such as biological nitrogen fixation, mineral solubilization and phytohormones production. In the present study the efficient bacterial isolates SS2, SS5, SS7 and SS11 isolated from the rhizosphere soil samples of *Tephrosia purpurea* L were identified using 16S rRNA gene amplification as *Stenotrophomonas rhizophila*_SS2, *Stenotrophomonas rhizophila*_SS5, *Stenotrophomonas rhizophila*_SS7, *Stenotrophomonas rhizophila*_SS11 which enhance plant growth and crop production by direct and indirect mechanism.

CONCLUSION

Our study revealed that the use of the strains *Stenotrophomonas rhizophila*_SS2, *Stenotrophomonas rhizophila*_SS5, *Stenotrophomonas rhizophila*_SS7, *Stenotrophomonas rhizophila*_SS11 isolated from the green manure crop can be an effective biofertilizer which had PGPR activities and phytohormone production along with exopolysaccharide characteristics that solubilizes higher amount of phosphate even in dry areas when used as biofertilizer which increases the crop growth and yield along with the improvement in soil health. The continuous use of these fertilizers even had the capability of converting the infertile land into a fertile one.

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