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RESEARCH ARTICLE

ADAPTABLE BENEFITS OF SELECTED RHIZOSPHERIC ISOLATES FROM RICE AND LEGUMES

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ABSTRACT

Biofertilizer is a relatively safer, environmentally friendly and cost-effective approach as an alternative to reduce chemical fertilizer usage. The selection of bacterial strains with multiple beneficial characteristics are important to maximize the effectiveness on the host plant. Due to a fore mentioned interest, several Plant Growth-Promoting Rhizobacterial (PGPR) and rhizobial strains were isolated from rice and legume roots. Six bacterial strains namely TS1, TS2, TP3, TP4, TR5, TR6 were obtained from surface sterilized healthy roots of rice and root nodules from groundnut. The isolates were tested for morphological and biochemical characteristics. The selected isolates were then tested for IAA production and other biochemical tests such as potassium solubilization, hydrolyzing enzymes (cellulase and pectinase) HCN and iron siderophore productions. The results of in vitro assays showed that all isolates can produce IAA, while three isolates (TS1, TS2 and TR5) fix N2 and (TP3 and TR5) solubilize phosphate and potassium; two isolates were positive for both tests. This study has successfully demonstrated the effectiveness of locally isolated PGPR and rhizobial strains with multiple beneficial characteristics. The result proved to be a vital information in the development of a liquid biofertilizer for rice and leguminous plants.

INTRODUCTION

Different bacterial genera are vital components of soils. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production (Ahemad *et al.,* 2009). They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediating the polluted soils by sequestering toxic heavy metal species and degrading xenobiotic compounds (like pesticides) (Ahemad, 2012; Ahemad and Malik (2011); Rajkumar *et al.,* 2010; Braud *et al.,* 2009). Indeed, the bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilizing the nutrients compared to those from bulk soils (Hayat *et al.,* 2010). Therefore, the rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility (Glick, 2012). Currently, the biological approaches for improving crop production are gaining strong status among agronomists and environmentalists following integrated plant nutrient management system. In this paper we focus on the bacteria that are derived from root and exert beneficial effect on the root. Such bacteria generally designated as Plant Growth Promoting Rhizobacteria (PGPR). Research on PGPR with non-legumes such as rice have shown beneficial effects through biological N2 fixation (Malik *et al.,* 1997), increased root growth (Mia *et al.,* 2012) with enhanced nutrient uptake (Yanni *et al.,* 1997), phytohormone production (Chabot *et al.,* 1996), plant growth enhancement stimulation by other beneficial bacteria and fungi (Saharan and Nehra, 2011) and disease control (Ramamoorthy *et al.,* 2001). Peng *et al.* (2002), however, reported that rhizobial inoculation known for their symbiotic relationship with legumes, could also increase rice grain yield, but little is known about the mechanism(s) involved. The beneficial effects of the selected rhizobial isolates could be due to their plant growth-promoting abilities namely biological N2 fixation, phosphate solubilization and plant growthregulator/phytohormone and siderophore production, similar to the known beneficial effects of PGPR (Boddey *et al.,* 1997; Verma *et al.,* 2001; Araujo *et al.,* 2013; Kloepper *et al.,* 1980). Yanni *et al*. (1997) have also shown beneficial interactions of rhizobial isolate son growth of rice, which was believed to be due to increased root efficiency in water and nutrient uptake. Micro-organisms mineralize organic phosphorus in soil by solubilizing complex-Micro-organisms mineralize organic phosphorus in soil by solubilizing complex- structured phosphates viz. tricalcium phosphate, rock phosphate, aluminum phosphate, etc. which turns organic phosphorous to inorganic form ultimately aiding the phosphate availability to plants. These phosphate-solubilizing bacteria use different mechanism(s) to solubilize

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the insoluble forms of the phosphate. The primary mechanism of phosphate solubilization is based on organic acid secretion by microbes because of sugar metabolism. Organisms residing in the rhizosphere utilize sugars from root exudates; metabolize it to produce organic acids (Goswami, Dhandhukia, Patel, & Thakker, 2014; Goswami, Pithwa, Dhandhukia, & Thakker, 2014). Auxin, indole-3-acetic acid (IAA), is an important phytohormone produced by several strains of PGPR and it is well-known that treatment of IAA-producing rhizobacteria increases the plant growth (Amara, Khalid, & Hayat, 2015; Kaymak, 2011; Vessey, 2003). IAA released by rhizobacteria mainly affect the root system by increasing its size and weight, branching number, and the surface area in contact with soil. All these changes lead to an increase in its ability to probe the soil for nutrient exchange, therefore improving plant's nutrition pool and growth capacity (Gutierrez-Manero *et al.,* 2001; Ramos-Solano *et al.,* 2008). IAA also drives the differentiation of adventitious roots from stem as auxins induce stem tissues to redifferentiate as root tissue. Etesami, Alikhani, and Hosseini (2015) reported that the PGPRs residing in rhizosphere, rhizoplane, and endophytic niches can produce IAA and support plant growth. Plant growth promoting rhizobacteria is a promising sustainable and environmentally friendly approach to obtain sustainable fertility of the soil and plant growth indirectly. This approach takes inspire a wide range of exploitation of plant growth promoting rhizobacteria led to reducing the need for agrochemicals (fertilizers and pesticides) for improve soil fertility by a variety of mechanisms that via production of antibiotics, siderophores, HCN, hydrolytic enzymes etc (Miransari and Smith, 2014). Thus, it is essential to isolate native rhizobia and PGPR from rice grown fields for multiple beneficial effects on the crops and for potential biofertilizer development. In the present study, isolation and screening of local rhizobia and PGPR with multiple plant growth-promoting abilities were conducted to identify the potential PGPR for liquid formulation of biofertilizers.

MATERIALS AND METHODS

Sampling of Rice and Legume Roots for Bacterial Isolation

At every location, eight sampling points, each point approximately 50 m apart, were randomly selected. One healthy rice plant per point was chosen for bacterial isolation. The roots were washed twice with clean water to remove the adhering soil particles. Three fresh and whitish root tips, each approximately 2.5 cm long, were cut and placed into a test tube containing 9 mL sterile distilled water. The test tubes were shaken manually to remove soil particles and other debris on the roots and the water was replaced with new sterile distilled water. The samples were taken to the laboratory in an ice box, the ice replaced regularly every 6 h. Volunteer leguminous plant, (*Mimosa pudica*) in the rice field and *Arachis hypogaea* were collected randomly and the nodules from each plant were removed and placed in McCartney bottles containing 15 mL sterile distilled water. The bottles were stored in ice box and taken to the laboratory (Tan *et al.,* 2014).

Isolation of Rhizobia/PGPR

The rice root samples were transferred into McCartney bottles containing 15 mL sterile distilled water using sterile forceps. The bottles were shaken vigorously with a vortex mixer for 10 sec. The solutions were used to isolate rhizospheric bacteria. The roots were taken out and surface sterilized by soaking in 95% ethanol for 10 sec, followed by 3% sodium hypochlorite for 1 min. 6 times washed with sterile distilled water. Then, the roots were cut longitudinally using sterile blade, followed by vertical sections into 5 pieces. These dissected root pieces were transferred into another McCartney bottle containing 15 mL sterile distilled water and the bottle shaken vigorously with a vortex mixer for 10 sec. These solutions were used to isolate for endophytic bacteria. The above procedures were repeated for all rice plants from each sampling location. 0.1 mL of the solution from each bottle was transferred onto Tryptic Soy Agar (TSA) plates and spread evenly using a hockey stick (Davis *et al.,* 2005). The plates were covered with parafilm, inverted and placed in the incubator for 24 h at 33°C. Similarly, nodules from the leguminous plants were surface sterilized, dissected and crushed using a sterile pestle. It was transferred into a test tube containing 9 mL sterile distilled water and shaken using a vortex mixer. 0.1 mL of the solution from each tube was pipetted into Yeast Mannitol Agar (YMA) plate and spread evenly using a hockey stick. The plates were covered with parafilm, inverted and placed in the incubator for 24 h at 33°C. After 24 h, all the plates were observed for different types of bacterial growth, based on colony morphology such as color and shape. Different colonies were subcultured into new TSA/YMA several times to get the pure, single colony. Each of the isolates was transferred onto agar slant (TSA/YMA) and kept in the refrigerator at -3°C for the next subsequent experiment.

Determination of Nitrogen Fixation

The nitrogen-fixing ability of the organisms was determined in a solution buffered at pH 7.2 by 1.8 g K_2HP04 and 0.7 g KH_2P04 per liter of 2% mannitol solution plus mineral salts. Two milliliters of a washed resting suspension of cells, adjusted to a concentration which gave a reading of 15% transmittance at 585 mμ on a "Spectronic 20" colorimeter, were added to 5 ml of mannitol solution. The controls immediately received 1.5 ml of concentrated H_2S04. The cultures were incubated at 25ºC for 48 hours in a constant temperature room equipped with a reciprocating shaker. The determination of total nitrogen employed the principles of a method developed by Polly (1958). The cultures after incubation were digested with 1.5 ml concentrated H_2S04, 0.05 ml of 10% HgS04, and 0.5 g K_2S04. After 30 minutes' digestion, the solution was cooled and five drops of 30%hydrogen peroxide were added. Then digestion was continued for another 10 minutes. The flasks were allowed to cool, the mercury precipitated with sodium sulphide and aliquots of the supernatant diluted for Nesslerization.

Evalution of phosphate solubilizing ability of bacterial isolates On agar plates

Bacterial isolates were screened for their phosphate solubilizing ability on NBRIP and PVK media, respectively. These media were supplemented with insoluble phosphate i.e. tri-calcium phosphate $Ca3(PO_4)_2$ at final concentration of 0.5%. The solubilization zone and colony diameters were measured after 120 h of incubation at 30 °C. The results were expressed according to Nguyen *et al.* (1992) as follows:

$$SE = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

Quantitative assessment of phosphate solubilization in liquid culture

Erlenmeyer flasks (100 ml) contained 20 ml of NBRIP broth medium (Nautiyal, 1999) were inoculated in triplicate with an inoculum of (350×10^5 CFU/ml) and then incubated at 28 °C for 7 days. The cultures were harvested by centrifuging at 5000 rpm for 20 min. and the phosphorous content in culture supernatant was determined by the para molybdenum blue method (Olsen and Sommers, 1982). The resulted color was estimated by spectrophotometer at 600 nm (Naik *et al.,* 2008). For quantitative determination, standard solution of potassium monohydrogen phosphate (KH_2PO_4) was used for drawing a standard curve.

Phytohormone Production

Fully grown bacterial cultures were inoculated in 100 mL Tryptic Soy Broth (TSB) and shaken on an orbital shaker for 24 h. 1mL of the bacterial culture was transferred into a new 100 mL TSB with the addition of 5 mL L-Tryptophan as the precursor of Indole-3-acetic Acid. TSB without bacterial inoculation served as the control. 1.5 mL of the bacterial culture were transferred into sterile Eppendorf tube and centrifuged at 7000 rpm for 7 min. The supernatant (1mL) was mixed with 2mL of Salkowsky reagent (2% of 0.5 M FeCl3 in 35% perchloric acid) according to the method by Gordon and Weber (1951). The solution was allowed to settle for 25 min and development of a pink color would indicate IAA production. The absorbance values were determined using a spectrophotometer at 535 nm and compared to the standard curve to determine the IAA concentration. The IAA standard curve was prepared using pure IAA at 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 µg mL−1 of IAA. Supernatants of uninoculated test tubes were used as control, where no visible color was observed.

Potassium Solubilization Ability

The selected isolates were grown on a modified Aleksandrov agar medium to determine the ability to solubilize potassium (Hu *et al.,* 2006). About 10 µL of bacterial suspension (containing approximately 108 CFUmL−1) were streaked onto the media, incubated at 33oC and observed at 5 and 10 Days After Incubation (DAI). The ability to solubilize muscovite mica as a source of insoluble form of potassium was derived by the formation of clear halo zone around the colony. The quantitative estimation of potassium solubilization rate was examined based on the bacterial abilities to release K from media supplemented with muscovite mica as an insoluble source of potassium. One mL of overnight culture of each isolate was inoculated to 100 mL of Aleksandrov broth and the amount of K released in the broth was estimated at 5, 10 and 15 DAI from triplicate flasks, in comparison with a set of uninoculated control. The broth cultures were centrifuged at 10,000 rpm for 10 min to separate the supernatant from the bacterial cells and insoluble potassium. The available K content in the supernatant was determined by Atomic Absorption Spectrometer (AAS) (ELICO 680). One mL of the supernatant was taken in a 50 mL volumetric flask and the volume was made to 50 mL with distilled water and mixed thoroughly. Then, the solution was fed to AAS for K content determination.

Iron Sequestration

(Siderophore Production) Chrome Azurol S (CAS) agar similar to that described by Schwyn and Neilands (1987) was prepared to detect the siderophore production by the bacteria. CAS agar plates were spot inoculated with each of the bacterial strain and incubated for 48 h at 33°C. Development of an orange halo zone around the colony (10 µL inoculum, 1×108 CFU mL−1) indicates a positive result as the siderophore removes Fe from the Fe-CAS dye complex and gives the medium its characteristic blue color.

Production of HCN

All the isolates were screened for the production of hydrogen cyanide by performing the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g glycine/l and bacteria were streaked on modified agar plate. A Whatman filter paper No. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 36±2 °C for 4 days. Development of orange to red colour indicated HCN production.

Cellulase Enzyme Production

Carboxy Methyl Cellulose (CMC) agar plates were prepared by screening for cellulose enzyme production according to the method by Kasana et al. (2008). 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 degradeddue 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. (2009). 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.

RESULTS

Characterization of bacterial isolates

A total of 30 bacterial isolates were obtained from three locations in Thiruvarur Distict. Among these, six bacterial isolates namely TS1, TS2, TP3, TP4, TR5 and TR6 were identified. The data presented in Table 1 showed some characteristics of the obtained bacterial isolates. Bacterial morphotypes were selected on the basis of colony morphological characteristics viz., colony shape and colour. According to morphological characteristics, three bacterial isolates were short rods and Gram −ve, while two isolates were medium and one curved rods.

Table 1. Morphological characteristics of bacterial isolates from the root nodules and rice rhizosphere from three locations in Thiruvarur (Dt), Tamil Nadu

Strains	Isolated from	Colony size and shape	Colony color	Cell motility	Cell shape	Gram reaction	Catalase
TS1	Sundarakkottai	Circular	Light pink	Highly motile	Short rods	-	+
TS2		Round	Whitish pink	Motile	Short rods	-	+
TP3	Paravakkottai	Elevation	Shiny mucoid	Highly motile	Medium rods	+	+
TP4		Medium, Wavy	Off-white	Non motile	Medium rods	-	+
TR5	Rishiyur	Round	Light pink	Highly motile	Curved rods	-	+
TR6		Large, flat	Greenish	Highly motile	Short rods	-	+

Table 2 Assessment of siderophore and hydrolyzing enzyme production of selected isolates

S.No	Rhiizosphere isolates	Siderophore production	Cellulase production	HCN Production
1.	TS1	++	+	-
2.	TS2	++	-	-
3.	TP3	++	++	+
4.	TP4	+	-	+
5.	TR5	++	-	-
6.	TR6	++	++	++

Table 3. Quantitative determination phosphate solubilization efficiency of plant growth promoting bacteria isolated from rice and leguminous plants

Bacterial isolates	Phosphate solubilization efficiency (%)[a]	Phosphate concentration (µg/ml) [b]
TS1	ND	105.7
TS2	ND	101.1
TP3	350	354.3
TP4	ND	110.6
TR5	108.3	240.6
TR6	250.9	293.4

ND: not detectable; a PVK agar media; b NBRIP broth media.

Table 4. Atmospheric nitrogen fixed by selected bacterial isolates during 48 hours' incubation in 5 ml of 2% mannitol solution

Location of soils from which organisms were isolated	Selected isolates	Nitrogen content		Nitrogen fixed	
		Initial	After incubation	µg/ml	mg/g of mannitol
Sundarakkottai	TS1	0.18 (0.13-0.25) *	1.24 (1.19-1.35) *	212	10.6
	TS2	0.17 (0.17-0.18)	1.45 (1.19-1.58)	256	12.8
Paravakkottai	TP3	.158 (.14-.17)	.212 (.18-.23)	11	0.55
	TP4	.218 (.10-.13)	.270 (. 11-. 17)	6	0.30
Rishiyur	TR5	0.19 (0.17-0.18)	1.35 (1.25-2.41)*	258	13.0.
	TR6	0.19 (0.17-0.18)	0.35 (0.27-0.41)	35	1.65

*Range of four determinations.

One isolates, namely TP3 was Gram positive bacteria. All isolated bacteria produced catalase enzyme which used by all aerobic microorganisms. All the isolates were motile except isolates TP4.

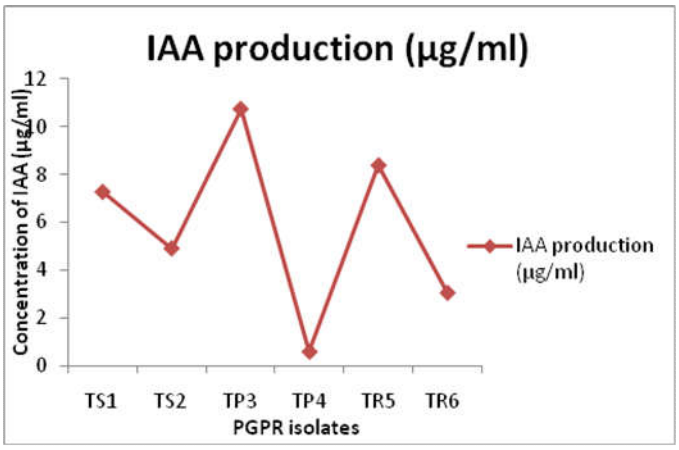
Plant growth promoting activity of selected bacterial isolates

Six bacterial isolates namely TS1, TS2, TP3, TP4, TR5 and TR6 were subjected to screening for plant growth promoting activities. Appearance of clear zone indicated the positive result for phosphate solublization and siderophore production was indicated by orange halos around the colonies. All the six isolates were positive for siderophore production. Appearance of reddish brown zone around the inoculated colony shows positive result for hydrogen cyanide production. Two of them were positive for HCN production (Table – 2).

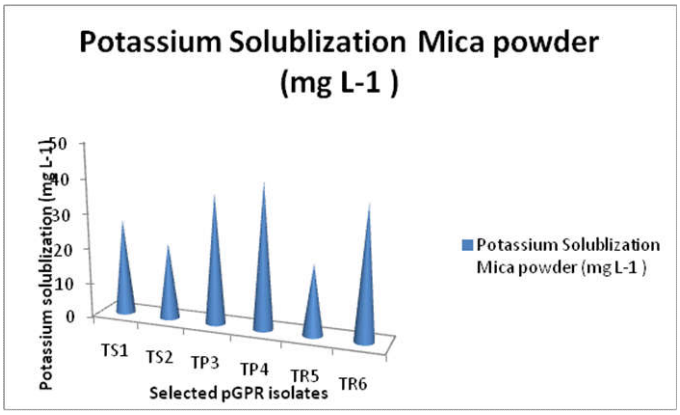
The results revealed that all the bacterial isolates produce IAA except TP4. The produced amounts of IAA ranged from 10.73 to 0.6 µg/ml (Table 3). The highest production was observed by TP3,TR5 and TR6 while the lowest production was detected by TS2. Furthermore, data in Table 3 clearly indicated that the isolates i.e. TP3, TR5, TR6, solubilize rock phosphate on PVK agar medium, TP3 showed the highest phosphate solubilization efficiency which was 350%, and this isolate also showed the highest amount of soluble P (354.3 µg/ml) after 6 days of incubation in liquid medium. . Maximum K solubilization was observed with bacterial isolates TP4 followed by isolates TR6. The data obtained from the micro-Kjeldahl determination of the amount of atmospheric nitrogen fixed by isolates from three locations soils are summarized in Table 4.

This is demonstrated by the variation in the nitrogen content of the different isolates shown in the first column of Table 4. The inocula for all the isolates had been adjusted to 15% transmittance at the maximum adsorption wave length of 585nm. The results in Table - 4 show that the nitrogen fixed in 5 ml of 2% mannitol varied from 6-212 µg/ml. Maximum nitrogen fixation observed in TS1(10.6mg/g of mannitol), TS2 and TR5(13.5mg/g). The fixation per gram of mannitol therefore ranged from 0.2 to 3.9 mg N per gram of mannitol. Fixation was statistically significant at or above the 5% level of significance for all the cultures tested.

Quantitatitive determination of IAA production by selected PGPR isolates



Quantitative determination of potassium solublization by selected PGPR isolates



DISCUSSION

The present study selected six bacterial isolates namely TS1, TS2, TP3, TP4, TR5 and TR6 based on their morphological and biochemical characteristics. The motility of these plant associated bacteria may confer an advantage for intercellular ingress and spreading as endophytes within the host plant (Elbeltagy *et al.,* 2000). Growth enhancement through enzymatic activity is another mechanism used by plant growth promoting rhizobacteria. Plant growth promoting rhizobacterial strains can produce certain enzymes such as chitinases, dehydrogenase, β-glucanase, lipases, phosphatases, proteases etc. (Joshi, 2012 and Ahemad, 2010) exhibit hyperparasitic activity, attacking pathogens by excreting cell wall hydrolases. Through the activity of these enzymes, plant growth promoting rhizobacteria play a very significant role in plant growth promotion particularly to protectthem from biotic and abiotic stresses by suppression of pathogenic fungi including *Botrytis cinerea, Sclerotium rolfsii, Fusarium oxysporum, Phytophthora* sp*., Rhizoctonia solani,* and *Pythium ultimum* (Nadeem,2013 and Upadyay, 2012). All the six isolate exhibited siderophore production. Siderophore-producing rhizobacteria improve plant health at various levels: they improve iron nutrition, inhibit the growth of other micro-organisms with the release of their antibiotic molecule, and hinder the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron–siderophore complex (Shen, Hu, Peng,Wang, & Zhang, 2013).

Quantitative analysis of IAA reduction and phosphate solubilization efficiency

All the six isolates were positive for siderophore production. Microorganisms have evolved specialized mechanisms for the assimilation of iron, including the production of low molecular weight iron-chelating compounds known as siderophores, which transport this element into their cells Tariq *et al.,* 2014. Two of them were positive for HCN production. These results are in harmony with Chaiharn and Lumyong (2011) who found that 18.05% from 216 bacterial strains isolated from rice rhizospheric soils in Northern Thailand produced IAA and identified the best IAA producer by biochemical testing and 16s rDNA sequence analysis as *Klebsiella* SN 1.1. This strain produced the highest amount of IAA (291.97 ± 0.19 ppm) in culture media supplemented with L-tryptophan. Indole acetic acid affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Miransari, 2014). TP3, TR5, TR6, solubilize rock phosphate on PVK agar medium, TP3 showed the highest phosphate solubilization efficiency which was 350%, and this isolate also showed the highest amount of soluble P (354.3 µg/ml) after 6 days of incubation in liquid medium. These results are in agreement with Park *et al*. (2010) who isolated nineteen phosphate solubilizing bacteria (PSB) from various soils samples and six strains solubilized more than 250 mg/l of P from tri-calcium phosphate amended with National Botanical Research Institute's Phosphate (NBRIP) medium. Also, El-Komy (2005) found that *Pseudomonas fluorescens* and *Bacillus megaterium* strains were able to solubilize phosphate effectively and recorded higher solubilization efficiency on PVK plates up to 350 and 185, respectively. The first report appeared in 1902 and it was widely used across the globe till date (Bhattacharyya & Jha, 2012). Several strains of these genus have gained importance as along with nitrogen fixation they also enhance plant growth by producing phytohormones including indole-3-acetic acid, gibberellic acid, and cytokinins. Application of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculants in agriculture, especially in cereals has resulted in notable increases in crop yields (Oberson *et al.,* 2013).

The Kjeldahl method of determining nitrogen in media inoculated with small numbers of the organisms has, however, failed to show conclusive evidence of fixation. Ross (1958) in a series of biological studies of some Tussock grassland soils in New Zealand isolated 11 strains of Bacterium *Radiobacter* and three strains of organisms described as *Pseudomonas* capable of growth on media containing no added nitrogen. Kjeldahl determination of nitrogen content of the cultures after 28 days' incubation demonstrated that nitrogen fixation was insignificant under the conditions utilized. More refined techniques have, however, demonstrated that the ability to fix nitrogen is fairly widespread. Proctor and Wilson (1958) found that six randomly selected strains of *Pseudomonas* and eight of *Achromobacter* fixed nitrogen. An inducible enzyme system

appeared to be involved in the fixation of 1.1 to 4.3 mg N per gram of carbohydrate. Several PGPR bacterial strains are commercially available in the form of formulated products which is used as biofertilizers and biocontrol agents (Gohel, Singh, Vimal, Ashwini, & Chhatpar, 2006; Jha & Saraf, 2015; Sethi, Sahu, & Adhikary, 2014).

Conclusion

From the current study, six Plant Growth-Promoting Rhizobacteria (PGPR) were isolated and identified from the field of agricultural soils in three locations of Thiruvarur District .They promoted plant growth which could be directly attributed to the beneficial effects from biological N2 fixation and phytohormone production and indirectly to phosphate and potassium solubilizations, iron siderophore and hydrolyzing enzyme productions. These strains belong to genera *Bradyrhizobium Rhizobium, Bacillus Enterobacter, Azospirillum* and *Pseudomonas* respectively. Hence, these findings could be useful and crucial for the preliminary biofertilizer development for rice and other non-legume crops in Cauvery delta region. These initial findings are useful as a fundamental information on the effects of local beneficial strains on early plant growth, thus a subsequent glasshouse and field trials need to be undertaken to elucidate the contributions of these PGPR and rhizobia on tillering and yield of rice with reduced fertilizer-N rates.

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