Int.J.Curr.Microbiol.App.Sci (2019) *8*(1): 2633-2645

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 01 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.801.277

Identification of Plant Growth Promoting Rhizobacteria as Biofertilizer for Salt Stress Environment

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Keywords

Auxin, Hydrogen cyanide, PGPR, Phosphorus, Potassium, *Pseudomonas aeruginosa*, Siderophore

Article Info

Accepted:
17 December 2018
Available Online:
10 January 2019

A B S T R A C T

Plant growth promoting rhizobacteria are found in the rhizosphere of plants worldwide and enhance their growth and development under unfavorable environmental condition. In the present investigation, twelve plant growth promoting rhizobacteria were isolated from the rhizosphere of different plants. Among these isolated rhizobacteria, identified one out of twelve, which showed best biofertilizer activity efficiently and also performing well under salt stress environment. Such biofertilizer activities were observed as solubilization of phosphorus and potassium, production of iron sequester complex-siderophore, production of auxin (a plant growth regulator) and production of hydrogen cyanide (an anti-microbial compound). The best performing rhizobacterial isolate, which showed best biofertilizer activity, was confirmed as *Pseudomonas aeruginosa* based on 16S rRNA sequencing under salt stress conditions.

Introduction

Rhizosphere is the most important ecological niche comprising different types of soil surrounding plant root zone with maximum beneficial bacterial population that are influenced by root exudates. Rhizobacterial population in the rhizospheric root zone are 100–1,000 times higher than in bulk soil and occupy metabolic flexibility to modify the soil composition efficiently by utilizing the root exudates (Jha *et al.*, 2010; Govindasamy *et al.*, 2011). Plant roots secreted photosynthates in the soil about 5 to 30% in form of different sugars that is utilized by microbial populations (Glick, 2014). Subsequent metabolic activities of these rhizobacteria stimulate plant growth and development by solubilising mineral nutrient present in the insoluble form in the soil (Glick, 1995). Plant Growth Promoting Rhizobacteria (PGPR) is beneficial bacteria to the plant growth under both biotic and abiotic stress environment. PGPR have ability to colonize around the root zone and encourage plants for their growth and development through either direct or indirect mechanisms. In the direct mechanisms, which can be

correlated with their capability to produce iron chelator compound siderophore, indole acetic acid (IAA), solubilise phosphorus, potassium, exo-polysaccharide and ACC deaminase activity directly help the plant in several ways: (1) Provide nutrient availability to the plant by solubilizing unavailable or fixed nutrients in the soil (2) Enhance plant growth by producing phytohormone (3) Encourage plant growth from abiotic stresses i.e. drought, salinity and water logging through reducing the production of ethylene by ACC deaminase activity and (4) Check the entry of Na^+ salt into the plant cell through formation of exopolysaccharide (EPS) around the root surface. Indirectly, by releasing anti-fungal and anti- bacterial compounds i.e. hydrogen cyanide and ammonia that reduce the pathogenic microbial population. PGPR include bacteria that reside in the rhizosphere and improve plant health ultimately boosting up the plant growth.

Majority of PGPR belongs to genera *Acinetobacter*, *Agrobacterium*, *Arthobacter*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Frankia*, *Serratia*, *Thiobacillus*, *Pseudomonads* and *Bacillus* (Vessey, 2003; Dobbelaere *et al.*, 2003; Sheng, 2005; Han *et al.*, 2006; Spaepen *et al.*, 2007; Hayat *et al.*, 2010; Mishra *et al.*, 2010; Park *et al.*, 2010; Tallapragada, 2010; Yousefi *et al.*, 2011; Liu *et al.*, 2012; Ahmed and Holmstrom, 2014; Retha *et al.*, 2014). Other than PGPR, plant growth promoting fungi (PGPF) are also present in the soil around the root zone that produced beneficial effects in terms of plant growth promotion and biological control resulting in enhanced plant growth and development (Hoitink *et al.*, 2006; Mathys *et al.*, 2012; Singh *et al.*, 2015; Singh and Dwivedi, 2018a). A specific PGPF like *Trichoderma* have been shown to have ameliorated the hostile effects on plants, increasing their growth potential, nutrient uptake, rate of seed germination and stimulation of plant defence against biotic and abiotic damages (Shoresh *et al.*,2010; Singh *et al.*, 2015; Singh and Dwivedi, 2018a; Singh and Dwivedi, 2018b).

Materials and Methods

Isolation and purification of isolated rhizobacteria

Rhizobacteria were isolated from the rhizosphere of *Solanum melongena, Capsicum annuum and Solanum lycopersicum* from different districts of Uttar Pradesh (Varanasi, Mirzapur and Sonbhadra) and Uttarakhand (Pant Nagar), India. Isolated rhizobacteria were purified by dissolving 1g of rhizospheric soil in 10 mL of distilled water. Using this as stock, 100 µl of soil solution was taken from test tube containing 10^{-6} and 10^{-7} concentration in autoclaved plates of nutrient media containing 5 g peptone, 3 g beef extract, 5 g sodium chloride, 15 g agar, 1L distilled water and pH 6.8 ± 0.5. Soil solution was spread with the help of spreader and finally incubated at temperature 28^{o}C for 48-72 h. Single bacterial colony was isolated from incubated plates after 48-72 h and, for purification, inoculated single bacterial colony on fresh media and then performing several biofertilizer tests with these single bacterial colonies.

Phosphate solubilization efficiency

The phosphate solubilization abilities of different isolated rhizobacteria were evaluated qualitatively according to the methods of Mehta and Nautiyal (2001).

This method is based on the decolourization of bromophenol blue (BPB) following a decrease in pH of the culture medium. All the rhizobacteria were inoculated separately in the plates of NBRI-BPB medium. This media containing 10 g of sucrose, 5 g of $Ca_3(PO_4)_2$,

5g of $MgCl_2.6H_2O$, 0.25 g of $MgSO_4.7H_2O$, 0.2 g of KCl, 0.1 g of $(NH_4)_2SO_4$ and 0.025 g of BPB were dissolved in 1000 mL of distilled water. Isolated pure cultures of rhizobacteria was inoculated on previously poured NBRI-BPB media, and transferred to the incubator at temperature 26±2°C. Finally, data were recorded at 5, 10 and 15 days after inoculation, if blue colour on media disappeared, and calculated phosphorus solubilization efficiency (PSE) using the formula given below:

$$PSE = \frac{\text{Zone of decolourization (cm)}}{\text{Bacterial growth (cm)}} \times 100$$

Potassium solubilization efficiency

Potassium solubilizing capabilities of purified isolated rhizobacteria from different rhizospheres of plants were evaluated qualitatively on Aleksandraov medium (Hu *et al.,* 2006) with some modifications. This media contained 5.0 g of Glucose, 0.005 g of $MgSO_4.7H_2O$, 0.1 g of $FeCl_3$, 2.0 g of $CaCO_3$, 3.0 g of potassium mineral (mica), 2.0g of $Ca_3(PO_4)_2$, 20.0 g agar and 1.0 L of distilled water and recorded potassium solubilization efficiency (PSE) at 5, 10 and 15 days after inoculation by using the same formula, which is given in phosphate solubilization test.

Siderophore production

Evaluation of siderophore production abilities of isolated rhizobacteria was done qualitatively by the methods of Schwyn and Neilands (1987). Freshly prepared chrome azurole S mixture containing 60.5 mg of chrome azurole S in 50 ml water, 10 mM of $FeCl_3.6H_2O$ in 10 ml of 10 mM HCl and 72.9 mg of HDTMA in 40 ml of distilled water were prepared separately and mixed in the ratio of 5:1:4, respectively, and the final azurole S mixture solution was mixed with nutrient media in 1:3 ratio. Siderophore production was observed at 5, 10 and 15 days after inoculation by measuring dark red zone appeared on inoculated plates, and calculated siderophore production efficiency by using the same formula, which is given above.

Auxin production

Auxin production abilities of different isolated rhizobacteria were evaluated qualitatively by freshly prepared NATD media containing 35 g of nutrient agar, 0.9 g of tryptophane, 0.6g of SDS and 10 mL of glycerol dissolved in 1000 mL of distilled water under aseptic condition, and put the sterilized filter paper over the inoculated rhizobacteria.

Then, transferred inoculated plates into BOD for overnight at 26±2°C. Removed the filter paper next day, and treated with salkowaski reagent, prepared by the methods of Ehmann (1977). If bacteria produced auxin, it formed red or pink halo within the membrane surrounding the colony. Measured the diameter of red or pink halo appeared on filter paper.

Hydrogen cyanide (HCN) production

Screening of purified rhizobacteria isolated from different plants was evaluated for HCN production by methods of Castric (1975). Bacterial cultures were streaked on nutrient medium containing 20 g of peptone, 15 ml of glycerol, 1.5 g of K_2HPO_4, 1.5 g of $MgSO_4.7H_2O$, 20 g of Agar and 1L of distilled water.

A Whatman filter paper No. 1 soaked in 0.5% picric acid solution (in 2% sodium carbonate) was placed inside the lid of a plate. Plates were sealed with parafilm and incubated at 26±2°C. Development of light brown to dark brown color indicated HCN production.

Colony forming unit (CFU mL^{-1}) of

isolated rhizobacteria under 2% and 5% NaCl solution

Finally, among isolated rhizobacterial strains, selected single rhizobacteria that showed the best PGPR activities in several tests and well survived under salty conditions (2% and 5% NaCl) was selected for identification by 16S r RNA gene sequencing. These identified selected single rhizobacteria were used for seed treatments under salt stress conditions (data not shown here).

Identification of bacterial strain using 16S rRNA gene

The bacterial strain was identified using standard method of 16S rRNA gene sequencing. DNA template was prepared by picking individual colony of each strain and amplification of 16S rRNA gene was carried out by PCR. PCR amplification of DNA was done using universal primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTT ACGAC TT-3'). PCR reaction mixture (20 µL) prepared for full-length 16S rRNA gene amplification was initially denatured at 94°C for 3 min, followed by 30 cycles consisting of denaturation at 94°C for 30 sec, primer annealing at 50°C for 60 sec followed by 72^0C for 60 sec and primer extension at 72°C for 10 min in a thermocycler. Unincorporated PCR primers and dNTPs were removed from PCR products using Montage PCR Clean up kit (Millipore).

The PCR product was sequenced using the 27F/1492R primers. Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

The purified PCR product samples were sequenced using DNA sequencing service of TRIYAT SCIENTIFIC CO., Nagpur, Maharashtra, India (https://www.indiamart.com/triyat-scientific/#) using universal 16S rRNA gene sequencing primers (27F/1492R). The sequence results were blast through NCBI (https://blast.ncbi.nlm.nih.gov) and sequence of all the related species were retrieved to get the exact nomenclature of the isolates. Phylogenetic analyses were performed using bioinformatics software PhyML 3.0 aLRT and HKY85 as Substitution model. Other software MUSCLE 3.7 was used for sequence alignments (Edgar, 2004) and aligned sequences were cured using the program Gblocks 0.91b (Talavera and Castrresana, 2007).

Statistical analysis

All data were presented as Mean ± SEM of three replicates and analyzed using a statistical package, SPSS (Version 16.0). One-way ANOVA (analysis of variance) was employed followed by Duncan's multiple range tests to determine the significant difference among means of the treatment at $P \leq 0.05$.

Results and Discussion

Phosphorus solubilization efficiency

Observation related to phosphorus solubilization efficiency was recorded qualitatively, from different isolated rhizobacterial strains at 5, 10 and 15 days after inoculation (Table 1). There was a significant difference observed among isolated rhizobacteria. The maximum solubilization efficiency was recorded in CH red strain (348.08%) at 5 days after inoculation (DAI), followed by BP red (339.05%) and T1 (309.44%) strain, but at 10 and 15 DAI, rhizobacteria BP red (607.78% and 621.43%), showed maximum efficiency

followed by CH red (545.56% and 548.41%) and T3 white (350.40% and 375.93%, respectively). Some rhizobacteria did not solubilise tri-calcium phosphate i.e., CH yellow, BP white, CH white and T3 white.

Phosphate-solubilizing bacteria have ability to solubilize 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 them to produce organic acids (Goswami *et al.*, 2015). These acids released by the micro-organisms act as good chelators of divalent Ca^{2+} cations accompanying the release of phosphates from insoluble phosphatic compounds. Many of the phosphate-solubilizing microbes lower the pH of the medium by secretion of organic acids such as acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic and citric acids (Rodríguez and Fraga, 1999, Patel *et al.*, 2015) (Fig. 1).

Potassium solubilization efficiency

Data presented in Table 1 related to potassium solubilization efficiency were recorded qualitatively from different isolated rhizobacterial strains at 5, 10 and 15 DAI. Significant difference was observed among isolated rhizobacteria. There was maximum solubilization efficiency recorded in C1 (467.22%) followed by T2 brown (444.44%) and CH red (366.67%) at 5 DAI, but at 10 DAI, observed maximum solubilization efficiency in BP red (685.35%) followed by C1 (669.99%) and CH yellow (640.0%). Similarly, at 15 DAI, maximum solubilization efficiency was observed in C1 (702.38%) followed by in CH red (649.21%) and BP red (615.0%). Some rhizobacteria did not solubilise organic potassium i.e., T1, P2, CH white and T3 white. Potassium is also an essential macronutrient for the plants that helps in several metabolic processes to maintain cytosolic ions balance. Several rhizospheric bacteria have capacity to solubilise potassium by releasing organic acids (Sheng 2005; Han, 2006; Badri, 2006; Basak and Biswas, 2010; Singh *et al.*, 2010).

Siderophore production

Efficiency of siderophore production is presented in Table 2 for evaluation of different isolated rhizobacteria from rhizospheres of brinjal, chilli and tomato plants. There was a significant difference among isolated rhizobacteria. Maximum siderophore production capability was observed in T2 brown (211.52%) followed by C1 (81.30%) and C2 (76.46%). These rhizobacteria followed same trend at 10 and 15 DAI. The PGPR assay tests for all rhizobacterial strains produce siderophore that are helpful directly and/or indirectly in plant growth and development.

Siderophore-producing bacteria usually belong to the genus *Pseudomonas*, where the most studied organisms are *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* which release pyochelin and pyoverdine type of siderophores (Haas and Defago, 2005). Rhizosphere bacteria release these compounds to increase their competitive potential, since these substances have an antibiotic activity and increase availability of iron for the plant growth (Glick, 1995). Siderophore-producing rhizobacteria improve plant health at various levels: they improve iron nutrition by forming iron-siderophore complex, inhibit the growth of pathogenic micro-organisms with the release of their antibiotic molecule and inhibit the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron–siderophore complex (Shen *et al.*, 2013).

Table.1 Phosphorus and Potassium solubilization efficiency index in different bacterial strains isolated from rhizospheres of Brinjal, Chilli and Tomato plants

Parameters / Isolates	Phosphorus Solubilization Efficiency Index			Potassium Solubilization Efficiency Index		
	Inoculation after 5 days	Inoculation after 10 days	Inoculation after 15 days	Inoculation after 5 days	Inoculation after 10 days	Inoculation after 15 days
CH Yellow	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	315.00 ± 18.03^{c}	640.00 ± 23.09^{ab}	632.05 ± 16.06^{b}
T1	309.44 ± 5.80^{b}	221.98 ± 21.42^{d}	208.09 ± 11.81^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
T2 brown	149.44 ± 12.92^{e}	133.97 ± 3.31^{f}	145.83 ± 10.49^{g}	444.44 ± 18.19^{a}	449.05 ± 12.24^{c}	503.80 ± 30.51^{c}
P2	0.00 ± 0.00^{f}	154.85 ± 2.89^{f}	177.38 ± 4.29^{f}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
BP White	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	248.33 ± 16.41^{d}	281.31 ± 6.65^{d}	280.95 ± 19.05^{d}
C2	168.89 ± 11.60^{de}	176.01 ± 23.19^{ef}	195.05 ± 16.23^{ef}	230.00 ± 25.17^{d}	322.86 ± 4.36^{d}	321.43 ± 10.91^{d}
C1	181.62 ± 2.38^{d}	218.06 ± 26.61^{de}	252.98 ± 14.22^{d}	467.22 ± 4.34^{a}	669.99 ± 12.94^{a}	702.38 ± 23.13^{a}
CH White	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
CH Red	348.08 ± 17.70^{a}	545.56 ± 18.49^{b}	548.41 ± 11.03^{b}	366.67 ± 29.06^{b}	614.02 ± 36.21^{b}	649.21 ± 19.94^{ab}
BP Red	339.05 ± 19.54^{a}	607.78 ± 16.81^{a}	621.43 ± 14.87^{a}	350.00 ± 14.43^{bc}	685.35 ± 18.37^{a}	615.00 ± 35.47^{b}
CP Red	234.04 ± 8.83^{c}	350.40 ± 17.26^{c}	375.93 ± 7.28^{c}	220.00 ± 23.09^{d}	420.63 ± 21.58^{c}	551.03 ± 14.01^{c}
T3 White	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

where, CH Yellow, T1, T2 brown, P2, BP White, C2, C1, CH White, CH Red, BP Red, CP Red and T3 White are different bacterial strains isolated from rhizosphere of different plants i.e. brinjal, chilli and tomato; Data are in the form of mean ± SEM, and means followed by the same letters within the columns are not significantly different at $P \leq 0.05$ using Duncan's multiple range test

Table.2 Production of siderophore, auxin and hydrogen cyanide by different isolated rhizobacteria from brinjal, chilli and tomato plants

Parameters / Isolates	Siderophore Production			Auxin Production	HCN Production
	Inoculation after 5 days	Inoculation after 10 days	Inoculation after 15 days	Diameter (cm) of red halo on filter paper after one day	Through Visualization
CH Yellow	66.75 ± 3.42^{b}	42.84 ± 0.55^{d}	44.54 ± 1.02^{g}	0.80 ± 0.06^{c}	+++
T1	69.17 ± 4.47^{b}	78.11 ± 0.40^{b}	78.82 ± 1.82^{bc}	0.50 ± 0.06^{e}	-
T2 brown	211.52 ± 1.92^{a}	211.81 ± 8.52^{a}	200.75 ± 6.80^{a}	0.00 ± 0.00^{g}	-
P2	15.08 ± 2.21^{d}	71.81 ± 4.14^{b}	78.87 ± 5.12^{bc}	0.00 ± 0.00^{g}	-
BP White	14.91 ± 3.73^{d}	72.34 ± 5.42^{b}	83.61 ± 2.17^{b}	0.00 ± 0.00^{g}	+
C2	76.46 ± 6.99^{b}	81.20 ± 0.68^{b}	84.11 ± 0.90^{b}	0.00 ± 0.00^{g}	-
C1	81.30 ± 4.69^{b}	77.05 ± 2.60^{b}	83.78 ± 2.21^{b}	0.48 ± 0.04^{e}	-
CH White	5.81 ± 2.91^{d}	57.04 ± 3.53^{c}	78.99 ± 1.62^{bc}	0.30 ± 0.03^{f}	-
CH Red	75.27 ± 9.59^{b}	47.78 ± 2.28^{cd}	55.71 ± 2.38^{ef}	0.63 ± 0.06^{d}	++
BP Red	47.16 ± 2.60^{c}	54.66 ± 2.26^{c}	72.44 ± 5.32^{cd}	1.05 ± 0.08^{b}	+++
CP Red	66.51 ± 2.74^{b}	58.86 ± 0.41^{c}	63.23 ± 1.81^{de}	0.75 ± 0.03^{cd}	++
T3 White	44.98 ± 2.62^{c}	43.47 ± 2.17^{d}	49.29 ± 1.22^{fg}	1.70 ± 0.06^{a}	+

where, Yellow, T1, T2 brown, P2, BP White, C2, C1, CH White, CH Red BP Red CP Red and T3 White are different bacterial strains isolated from rhizosphere of different plants i.e. brinjal, chilli and tpmato, +++ = High intensity (break red), ++ = Medium intensity (break red), + = Low intensity (break red), - = No intensity (break red), Data are in the form of mean ± SEM, and means followed by the same letters within the columns are not significantly different at $P \leq 0.05$ using Duncan's multiple range test

Table.3 Colony Forming Unit (CFU mL^{-1}) of different bacterial strains isolated from rhizospheres of brinjal, chilli and tomato plants under 2% and 5% NaCl solutions

Parameters / Isolates	Bacterial broth with 2% NaCl solution		Bacterial broth with 5% NaCl solution	
	CFU after 7 days	CFU after 14 days	CFU after 7 days	CFU after 14 days
CH Yellow	2.69×10^{9}	5.07×10^{9}	2.74×10^{8}	4.58×10^{7}
T1	3.48×10^{8}	1.53×10^{9}	2.09×10^{8}	1.62×10^{8}
T2 brown	3.16×10^{7}	1.35×10^{8}	4.27×10^{6}	1.94×10^{7}
P2	4.29×10^{6}	3.58×10^{7}	3.42×10^{6}	6.20×10^{5}
BP White	5.82×10^{8}	1.43×10^{9}	1.68×10^{8}	4.11×10^{8}
C2	4.64×10^{7}	2.16×10^{8}	1.58×10^{7}	7.19×10^{6}
C1	3.48×10^{8}	2.61×10^{9}	1.67×10^{8}	3.25×10^{8}
CH White	4.72×10^{10}	6.47×10^{9}	2.43×10^{9}	3.68×10^{9}
CH Red	1.61×10^{8}	2.71×10^{8}	4.54×10^{7}	4.72×10^{6}
BP Red	4.53×10^{8}	1.22×10^{9}	1.11×10^{8}	6.13×10^{7}
CP Red	5.27×10^{9}	1.26×10^{10}	2.61×10^{8}	4.69×10^{7}
T3 White	2.63×10^{9}	6.94×10^{8}	4.38×10^{8}	1.25×10^{8}

where, CH Yellow, T1, T2 brown, P2, BP White, C2, C1, CH White, CH Red, BP Red, CP Red and T3 White are different bacterial strains isolated from rhizosphere of different plants i.e. brinjal, chilli and tomato

Table.4 Bio-fertilizer activity and efficiency of different bacterial strains isolated from rhizospheres of brinjal, chilli and tomato plants

Isolates	Phosphate solubilization	Potassium solubilization	Siderophore production	Auxin production	HCN Production
CH Yellow	_	+++	+	++	+++
T1	+	_	++	+	-
T2 brown	+	++	+++	_	-
P2	+	_	++	_	-
BP White	_	+	++	_	+
C2	+	+	++	_	-
C1	+	+++	++	+	-
CH White	_	_	++	+	-
CH Red	+++	+++	+	+	+
BP Red (*Pseudomonas aeruginosa*)	+++	+++	++	++	+++
CP Red	++	+	++	+	++
T3 White	_	_	+	+++	+

where, CH Yellow, T1, T2 brown, P2, BP White, C2, C1, CH White, CH Red BP Red CP Red and T3 White are different bacterial strains isolated from rhizosphere of different plants i.e. brinjal, chilli and tomato, and +++ = High positive response, ++ = Medium positive response, + = Low positive response and - = Negative response of rhizobacterial isolates for particular PGPR assay

Fig.1 Phosphorus solubilization (1A), Potassium solubilization (1B), Siderophore production (1C), HCN production (1D) and Auxin production (1E) of isolated rhizobacteria (BP Red)

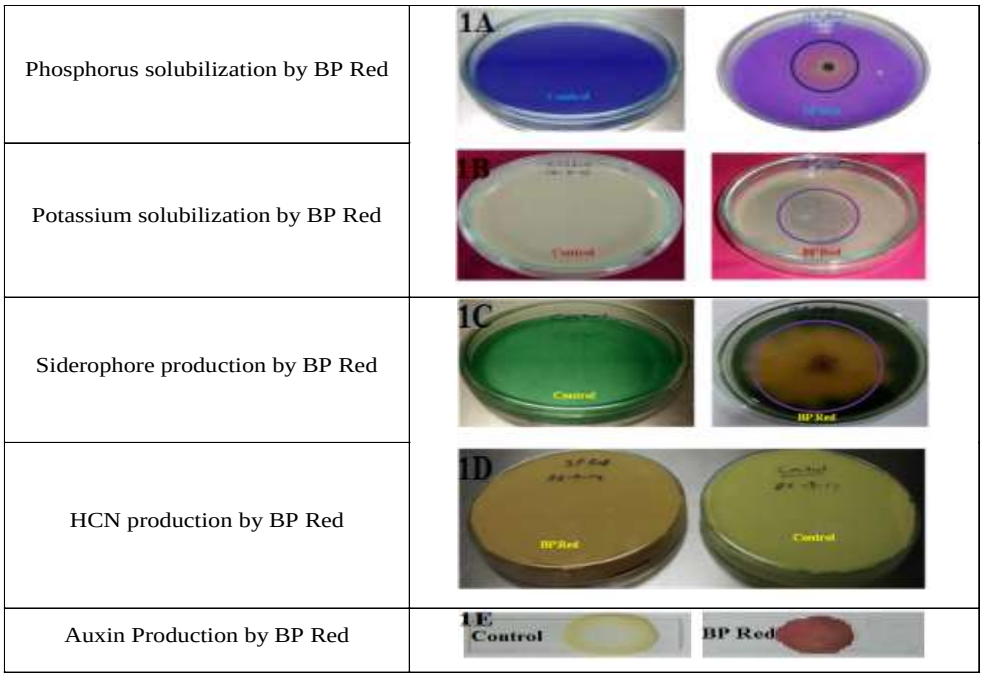
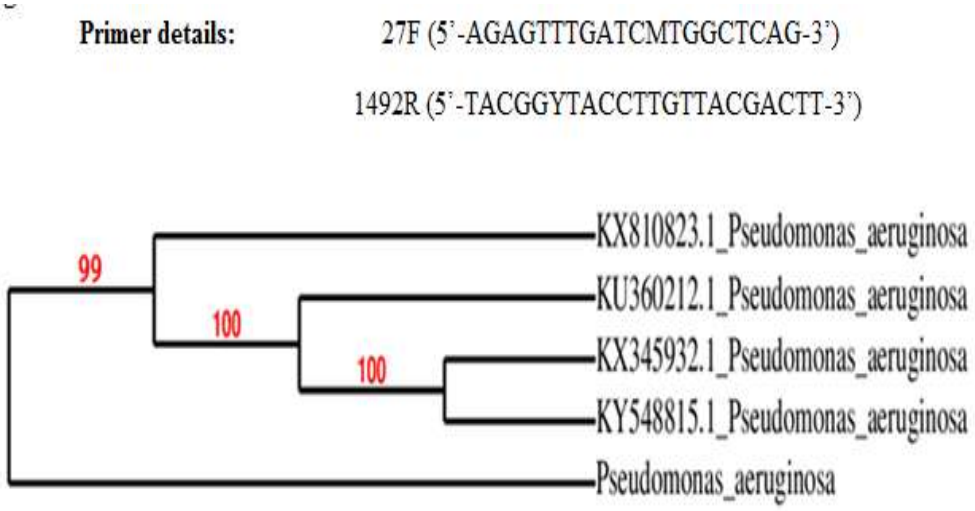


Fig.2 Phylogenic Tree of isolated rhizobacteria (BP Red- identified as *Pseudomonas aeruginosa* as per 16S rRNA sequencing



Auxin production

There was a significant difference observed among isolated rhizobacteria in respect to auxin production, quantitatively (Table 2). Ability of maximum auxin production was recorded in T3 white (1.7 cm) followed by BP red (1.05 cm) and CH yellow (0.8 cm) which developed red halo (cm in diameter) on filter paper one day after inoculation. Some rhizobacteria did not synthesise auxin i.e., T2 brown, P2, BP white and C2. Auxins are plant hormones that are essential for plant growth and development.

They have a fundamental role in coordination of many growth and physiological processes in the plant's life. Plant growth promoting bacteria exhibit a variety of characteristics responsible for influencing plant growth including the production of IAA (Fukuhara *et al.,* 1994, Patten and Glick, 2002). Indole-3-acetic acid in rhizobacteria helps loosen plant cell walls, which may facilitate rhizobacteria to absorb various substances secreted by roots (Glick, 2012). Several studies have suggested that elevated auxin levels, including IAA in host plants, are required for nodule formation (Fukuhara *et al.,* 1994, Mathesius *et al.,* 1998, Pii *et al.,* 2007).

It has been reported that IAA production by bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mandal *et al.,* 2007).

Hydrogen cyanide (HCN) production

Hydrogen cyanide produced by the plant growth promoting microorganisms helps protect plants from soil borne pathogens. Intensity of HCN production was evaluated in different rhizobacteria, isolated from rhizosphere of brinjal, chilli and tomato plants (Table 2). Significant difference was found among isolated rhizobacteria. The maximum intensity of HCN was found in BP red and CH yellow followed by CH red and CP red. However, some isolated rhizobacteria did not produce HCN (T1, T2 brown, P2, C2, C1 and CH white). HCN produced by several rhizobacteria help plants in their growth and development indirectly by suppression of plant pathogenic populations. Changing the colour of plates from yellow to brown/red

confirmed HCN production (Castric, 1975; Reetha *et al.,* 2014).

Colony forming unit (CFU) of rhizobacteria under 2% and 5% NaCl solution

Colony forming unit (CFU) of different isolated rhizobacteria was evaluated for the regeneration abilities of these rhizobacteria under 2% and 5% NaCl solution at 7 and 14 days after inoculation (Table 3).

There was a significant reduction in rhizobacterial CFU with increasing concentration of NaCl. In 2% NaCl, regeneration abilities in terms of CFU was observed maximum in CH white (4.72×10^{10}) followed by CP red (5.27×10^{9}) and CH yellow (2.69×10^{9}) at 7 days after inoculation, but after 14 days inoculation, found maximum in CP red (1.26×10^{10}) followed by CH white (6.47×10^{9}) and CH yellow (5.07×10^{9}). CFU under 5% NaCl solution was maximum in CH white (2.43×10^{9}) followed by T3 white (4.38×10^{8}) and CH yellow (2.74×10^{8}) at 7days after inoculation but after 14 days, maximum CFU was found in CH white (3.68×10^{9}) followed by BP white (4.11×10^{8}) and C1 (3.25×10^{8}) CFU mL^{-1}.

It is concluded that, among isolated rhizospheric strains, BP red performed best in terms of various PGPR assays (Table 4). BP red stain was identified by 16S rRNA sequencing and phylogenetic tree showed that the sequence had a high similarity of 99% with *Pseudomonas aeruginosa* (KX810823.1) (Fig. 2). Therefore, PGPR strain BP red was confirmed as *Pseudomonas aeruginosa* based on 16S ribosomal RNA sequencing. In the subsequent experiments under stress and ameliorative treatments, henceforth, *Pseudomonas aeruginosa* has been refered to as rhizobacteria.

Acknowledgement

Authors thank to the UGC New Delhi for providing fellowship to the first author.

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How to cite this article:

Ajay Kumar Singh and Padmanabh Dwivedi. 2019. Identification of Plant Growth Promoting Rhizobacteria as Biofertilizer for Salt Stress Environment. *Int.J.Curr.Microbiol.App.Sci.* 8(01): 2633-2645. doi: https://doi.org/10.20546/ijcmas.2019.801.277