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ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM COASTAL REGION AND THEIR EFFECT ON DIFFERENT VEGETABLES

Avishek Pahari[1], Alisha Pradhan[1], Sujata Priyadarshini[1], Suraja Kumar Nayak[2] and B.B. Mishra[1]*

[1]Department of Microbiology, College of Basic Science & Humanities,Orissa University of Agriculture and Technology, Bhubaneswar-751003, India

[2]Department of Biotechnology, College of Engineering and Technology, BijuPatnaik University of Technology, Bhubaneswar-751029, India

E-mail: bb_mishra58@yahoo.com (**Corresponding Author*)

Abstract: In view of increasing environmental contamination with the deterioration of soil health and due to use of chemical fertilizers for increasing crop productivity, this present study is conducted using bacterial isolates from coastal region of Odisha as plant growth promoter and an alternative to chemical fertilizer. A total number of 11 bacteria were isolated from the Chilika region and among them two bacteria PGPR-2 and PGPR-8 showed excellent plant growth promoting activities such as indole acetic acid (IAA), ammonia production, nitrate reduction, siderophore production, phosphate solubilization and biological nitrogen fixation. PGPR-2 and PGPR-8 were further characterized for their biochemical and extracellular enzymatic activities. The potential isolates also showed antagonistic activities against different phytopathogens. From biochemical and enzymatic characterization, it was apparent that these two bacteria belonged to the genus of *Bacillus*. The potential isolates were further tested with different vegetables to study the germination percentage, root length and shoot length using Roll towel method. A significant increase in various parameters of vegetables was observed which was also statistically significant.

Keywords: Chemical fertilizer, PGPR, Phytopathogen, *Bacillus,* germination.

Introduction

Indiscriminate use of chemical fertilizer and pesticides over the last few decades has not one just resulted in the contamination of environment, but also reduced soil fertility in general and soil microbial in particular. Due to the decreasing soil health, emphasis has been given to organic farming and the application of microbial formulations for increasing crop productivity with concomitant decrease in application of the chemical fertilizer.

Plant growth promoting rhizobacteria (PGPR) are the bacteria that colonize in the rhizosphere region of plant roots and enhance plant growth by different mechanisms (Schroth and Hancock, 1982). Root colonization is influenced by biotic factors such as genetic traits of host plant, the colonizing organism as well as abiotic factors such as soil humidity, soil pH, temperature etc. PGPR enhances plant growth by phosphate solubilization, IAA production,

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siderophore, production, biological nitrogen-fixation, HCN production, cytokinins and gibberellic acid production (Glick, 1995). Ramamoorthy *et al*., (2001) opined that PGPR increase germination percentage, seedling vigor, root and shoot growth, total biomass of plants, seed weight, early flowering grains, fodder and fruit yields, that can serve as a source as 'bio' fertilizers (Boddey and Dobereiner, 2000). One of the mechanism used PGPR affect growth is antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), which are low molecular weight phenolic compound or citric acid derivatives that chelates to iron, preventing the growth of soil borne fungal pathogens.

Microbial diversity in coastal region requires greater emphasize for isolation and characterization as they are acclimated to adverse conditions. Such organisms having plant growth promoting characters can be of immense benefit under various environmental conditions. In this regard, an attempt has been made to isolate and characterize plant growth promoting rhizobacteria from coastal rhizopheric soil of rice plant of Chilika, Odisha and examine their antagonistic effect against different phytopathogens under *in vitro* condition. Besides their effects on germination percentage, root length, shoot length and other growth related characters in different vegetables under *in vitro* conditions are also studied

Materials and methods

Sample collection and bacterial isolation

Soil sample was collected from the rhizosphere region of rice plant from Barakul, Chilika and intact root system was dug out and the rhizospheric soil sample was carefully collected in plastic bags under aseptic condition. The soil sample was air dried and subjected to the isolation of bacteria by serial dilution method. A total of 11 bacterial isolates were isolated from the rhizospheric soil sample by spread plate technique and named as PGPR-1 to PGPR-11.

In vitro screening of isolates for different plant growth promoting characters

All rhizobacterial isolates obtained were screened for different plant growth promoting traits. Each culture was placed on modified Pikovskaya agar (Pikovaskya *et al*.,1948) with insoluble tricalcium phosphate (TCP) and incubated at 30±0.1°C for 5 days to check the phosphate solubilization. IAA production was assayed using qualitative method developed by Bric *et al*., (1991). Bacterial cultures were inoculated in nutrient broth with tryptophan (1mg/ml) incubated at 35 ± 2°C for 7 days. Cultures were centrifuged at 3000 rpm for 30 min. 2 ml of supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5 $FeCl_3$). The development of a pink

colour indicated IAA production (Loper and Schroth, 1986). Meanwhile, siderophore production by the isolates was checked on solid CAS universal blue agar plates. Actively growing cultures were spot inoculated on the CAS blue agar plate and incubated at 35 ± 2ºC for 72 h. Formation of yellow-orange halo zone around the colony indicated production and release of the siderophores on the agar plate (Schwyn and Neilands, 1986). After that bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48 h at 35 ± 2°C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour observed was a positive test for ammonia production (Cappuccino and Sherman, 1992). Isolates were further screened for HCN production. Bacterial cultures were streaked on nutrient agar medium containing 4.4 gm/l of glycine. 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 35 ± 2ºC for 4 days (Castric *et al*.,1975).For nitrate to nitrite reduction was detected through the test. Bacteria were inoculated into nitrate broth, incubation at 30 ±0.1^{0}C for 96 h. After inoculation sulphanillic acid and α-naphthyl amine mixture (1:1) was added. The appearance of deep pink colour indicated a positive result. N_2 fixation ability of the isolates were checked by the using of nitrogen free agar based Jensen (1951) agar media and incubated for 72 h at 30±1^{0}C.

Identification, biochemical characterization & enzymatic activities of bacterial isolates

The potential isolates were further characterized on the basis of their staining characteristics and further investigated in terms of biochemical properties like indole, catalase, urease, citrate, ammonia, nitrate producing abilities and enzymatic activities like amylase, cellulase, gelatinase, caesinase and fermentation of various sugars, which helped in identifying the bacteria up to the genus level (Gupta *et al*.,2000) by Bergey's manual of Determinative bacteriology (Holt *et al.,*1994) and ABIS online software.

***In vitro* antagonism against *Fusarium* sp. and *Rhizoctonia* sp.**

Two isolates namely PGPR-2 & PGPR-8 were tested for antibiosis by duel culture method against two common plant pathogen namely *Fusarium* sp. (ITCC 578) and *Rhizoctonia* sp. (ITCC 186). Spores of fungal cultures grown on potato dextrose agar medium (PDA). A 5mm diameter mycelial agar disc was cut from the margin of 7-day-old fungus culture and placed on one side of a 9 cm petri dish containing PDA medium and test bacteria was streaked on the other end of the petri dish. Plates were incubated at 28±2°C for 5 to 8 days. Dishes inoculated only with test pathogens served as controls. The percent of inhibition

for each fungus was measured using the formula (Vincent, 1927; Pahari *et al*., 2017): Inhibition percentage (%) = (R1-R2) / R1 X 100 where R1 is redial growth of mycelia in control and R2 represent the redial growth of mycelia in treatment.

Trial with Seed germination

Among the 11 bacterial isolates, PGPR-2 and PGPR-8 are found with more potential and tested for seed germination under lab condition. Brinjal (*Solanum melongena* L.), Okra (*Abelmoschus esculentus* L.) and tomato (*Solanum lycopersicum* L.) seeds were collected from Dept. of Vegetable science, OUAT and were surface sterilized with 0.1% $HgCl_2$ for 2 min and rinsed with sterile distilled water for 10 times. Bacterial isolates were grown in respective broth on shaking incubator (180 rpm) at 28 ± 2°C for 24 h. Cell densities in the suspension were adjusted to a final density of approximately 10^8CFU $seed^{-1}$.The surface sterilized seeds were inoculated in broth culture for 30 minutes (ISTA, 1993). Germination tests were carried out by using the paper towel method. Treated seeds and control were seeded onto paper towels. Germination percentage was measured with the following formula: Germination percentage = Number of germinated seeds / Number of seeds in sample × 100. Root length and shoot length of individual was measured.

Statistical analysis

All the experiment was done in triplicate and the data was analyzed statistically by one way ANOVA at $p < 0.05$ significant level.

Results and discussion

Isolation, identification and screening of plant growth promoting activities of the bacterial isolates

A total of 11 bacterial isolates were isolated from the rhizospheric region of rice plant from Barkul, Odisha. The bacterial isolates PGPR-2, PGPR-3, PGPR-7 and PGPR-8 produced plant growth promoting hormone including IAA. IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant (Arshad and Frankenberger, 1991; Pradhan and Mishra, 2015). On Pikovskaya medium, PGPR-1, PGPR-2, PGPR-4 and PGPR-6 showed a development of sharp halo zones and among them. PGPR-2 showed maximum zone (Table 1). Similar observations has been reported by Ngomle *et al*., (2014), stating that microorganisms capable of producing a clear zone due to P solubilization in the surrounding medium were selected as potential phosphate solubilizers where clear zones around the colonies indicated the capacity of phosphate solubilization on Pikovskaya medium. Five bacterial isolates were also found with the ability to produce

siderophore and PGPR-8 showed the highest activity (Table 1). Similar Loper and Henkels (1997) have observed that iron is an essential growth element for all living organisms including plant pathogens. Furthermore seven bacterial isolates also exhibited strong production of ammonia from peptone water (Table 1) which is another important trait of PGPR and taken up by plants as a source of nitrogen for their growth (Ahmad *et al*., 2008). None of the isolates were positive for HCN production. Out of 11, two bacterial isolates i.e. PGPR-2 and PGPR-8 showed good plant growth promoting activities and were further characterized for biochemical and enzymatic activities.

Biochemical characterization and Identification

Biochemical tests such as oxidase test, nitrate reduction, catalase, carbohydrate utilization, citrate utilization etc. were carried out for phenotypic identification of isolates (Holt *et al.,*1994). PGPR-2 & PGPR-8 were examined for catalase, oxidase and urease test. Biochemical characterization of all isolates and enzymatic activities of the isolates were tabulated in Table 2 & 3. Briefly, all rod shaped isolates were positive for oxidase, catalase, lactose, maltose, fructose, dextrose, galactose and salicin utilization (Table 4). The bacterial isolate were characterized by biochemical attributes and were identified as PGPR-2 *Bacillus* sp. on the basis of ABIS online software (Table 6).

Table 1: Plant growth promoting activities by the bacterial isolates from Chilika

Isolate No.	Test					
	IAA Production	Phosphate solubilization	HCN Production	NH_3 production	N_2 fixation	Siderophore production
PGPR-1	-	+	-	-	-	+
PGPR-2	+	+	-	+	+	+
PGPR-3	+	-	-	+	+	-
PGPR-4	-	+	-	-	-	-
PGPR-5	-	-	-	+	+	-
PGPR-6	-	+	-	+	+	-
PGPR-7	+	-	-	-	-	-
PGPR-8	+	-	-	+	+	+
PGPR-9	-	-	-	+	+	+
PGPR-10	-	-	-	+	+	-
PHPR-11	-	-	-	-	-	+

Table 2: Physiological and biochemical properties of potential plant growth promoting rhizobacterial isolates

Test	PGPR-2	PGPR-8
Gram staining	+	+
Endospore staining	+	+
Catalase	+	+
H_2S production	-	-
Indole	+	+
Methyl red test	+	+
VP	-	-
Nitrate reduction	+	+
Urease production	+	+
Citrate utilization	+	+
Oxidase	-	+
Mannitol	+	+
Aesculin hydrolysis	+	+
Anaerobic growth	+	+

Table 3: Extracellular enzymatic activities of the plant growth promoting rhizobacterial isolates

Test	PGPR-2	PGPR-8
Gelatinase	+	+
Casein hydrolysis	+	-
Tributyrin	+	+
Amylase	+	-
Cellulase	+	-
Chitin hydrolysis	-	-
Pectin hydrolysis	+	+
DNase	-	-
Lecithinase	-	-

Table 4: Sugar utilization by the potential plant growth promoting rhizobacterial isolates from Chilika

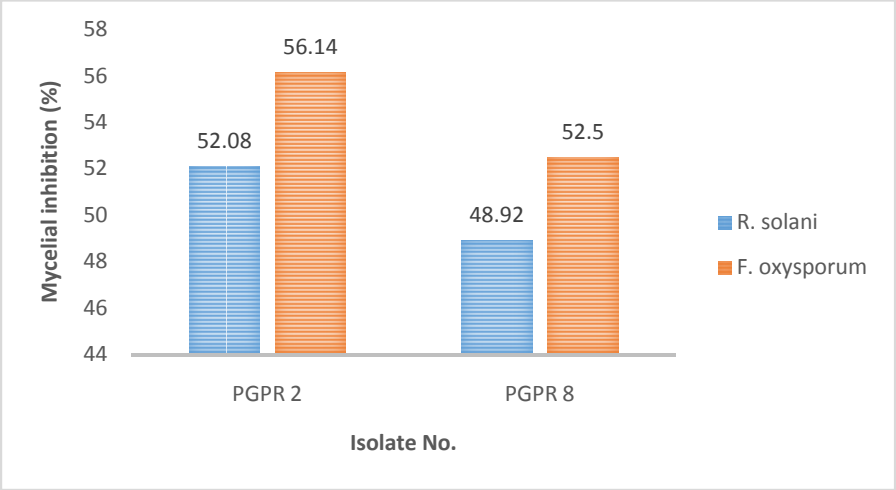
Isolate No.	Tre	De	Du	Sa	Ga	Ino	Me	So	Ma	Su	La	Rh	Mn	Ce	Glu
PGPR-2	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+
PGPR-8	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+

Tre: Trehalose, De: Dextrose, Du: Dulcitol, Sa:Salicin, Ga: Galactose, Ino: Inositol, Me: Melibiose, So: Sorbitol, Ma: Maltose, Su: Sucrose, La: Lactos, Rh: Rahmmose, Mn: Mannose, Ce: Cellobiose, Glu:Glucose

In vitro antagonistic effect on mycelial growth of phytopathogen

PGPR-2 and PGPR-8 were tested for *in vitro* antagonism against *Rhizoctonia* sp. and *Fusarium* sp. depicting positive result (Fig 1). PGPR-2 showed the highest percentage of mycelial inhibition against (56.14%) *Fusarium* sp.

Fig 1: *In vitro* antagonistic effect of PGPR on mycelial growth of different plant pathogens



Seed germination test

In this study, an increase in plant growth by seed bacterization has been demonstrated. It is well established that plant growth promoting rhizobacteria increased the synthesis of gibberellins, which would have triggered the activity of specific enzymes including amylase to the promote early germination, which brought an increase in availability of starch assimilation (Bharathi *et al.,* 2004). In the present study, it was found that PGPR-2 and PGPR-8 significantly increased the germination percentage, root and shoot length of brinjal, okra and tomato, over control (Table 5). Highest root (11.19 cm), shoot elongation (12.98 cm) and germination (79.58%) was recorded when okra seeds were pre-treated with PGPR-8.

Table 5: Effect of Plant growth promoting rhizobacteria on germination percentage, root length and shoot length of different vegetables in germination paper

Isolate No.	Brinjal			Okra			Tomato		
	Root length (cm)	Shoot length (cm)	Germination (%)	Root length (cm)	Shoot length (cm)	Germination (%)	Root length (cm)	Shoot length (cm)	Germination (%)
Control	4.21 ± 0.67	6.54 ± 0.64	45.26 ± 0.35	6.55 ± 0.22	8.31 ± 1.20	60.45 ± 1.19	4.79 ± 0.45	8.72 ± 0.64	50.20 ±1.40
PGPR-2	6.34 ± 1.09	08.59 ± 1.23	69.18 ± 1.39	10.18 ± 0.21	12.02 ± 0.35	75.26 ± 1.54	7.25 ± 0.65	11.46 ± 1.10	70.20 ± 0.67

PGPR-8	5.56 ± 0.34	08.11 ± 0.27	63.47 ± 0.54	11.19 ± 0.85	12.98 ± 0.27	79.58 ± 0.32	6.79 ± 0.54	11.25 ± 0.68	65.65 ± 1.20

Conclusion

From the present study it is evident that two Gram positive isolates obtained from the rhizosphere region of the genus *Bacillus* showed certain PGPR characteristics like phosphate solubilization, ammonia production, nitrate production, siderophore production etc. On top of that they also showed antibiosis against various plant pathogens like *Rhizoctonia* sp. and *Fusarium* sp. These two isolates increased germination percentage, root and shoot length of brinjal, okra and tomato seeds. Thus the use of PGPR as 'bio fertilizers' is a novel approach to replace chemical fertilizers and pesticides for sustainable agriculture in India. Since these organisms are acclimatized to coastal environment, the PGPR characters and antibiosis can be effective trend in various environmental conditions.

References

[1] Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizobacteria for their multiple plant growth promoting activities. Microbiol. Res. 2008; 163:173-181.

[2] Arshad M, Frankenberger WT. Microbial production of plant hormones. Plant Soil. 1991; 1(133):1-8.

[3] Bharati R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R.Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Protec. 2004; 23: 835–843.

[4] Boddey RM, Dobereiner, J. Nitrogen fixation associated with grasses and cereals. Recent Progress and Propectives for the Future Fert. Res. 2000; 42:241-250.

[5] Bric JM, Bostock RM, Silverstone SE. Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 1991; 57:535-538.

[6] Cappuccino JC, Sherman N. Microbiology. In: A Laboratory Manual (Eds.: 3rd). New york, Benjamin/cummings Pub. Co. 1992; p.125–179.

[7] Castric PA. Hydrogen cyanide, a secondary metabolite of Psuedomonas aeruginosa. Can. J. Microbiol. 1975; 21:613-618.

[8] Glick BRX. The enhancement of plant growth by free living bacteria. Can J. Microbiol. 1995;41:109–117.

[9] Gupta A, Gopal M, Tilak KV. Mechanism of plant growth promotion by rhizobacteria. Indian J Exp Biol. 2000; 38:856–862.

[10] Holt J, Krieg N, Sneath P, Staley J, Williams S. Bergey's manual of determinative bacteriology. 1994; Williamson and Wilkins.

[11] ISTA: Proceedings of the international Seed Testing Association, International Rules for Seed Testing. Seed Science Technology. 1993; 21:25–30

[12] Jensen HL. Notes on the biology of Azotobacter. Proceedings of the Societyfor Applied Bacteriology.1951.

[13] Loper JE, Henkels MD. Availability of iron to P. fluorescence in rhizospheric and bulk soil evaluated with an ice nucleation reporter gene. Appl. Env.Microbiol. 1997; 63:99-105.

[14] Loper JE, Schroth MN. Influence of bacterial source of indole-3-acetic acid of root elongation of sugar beet. Phyto pathol. 1986;**76**:386-389.

[15] Ngomle S, Bhattacharya PM, Roy A, Ambesh BS. Isolation and screening of phosphate solubilizing Bacteria from different crop rhizosphere. The Ecoscan. 2014; 6:505-509.

[16] Pahari A, Mishra BB. Antibiosis of Siderophore Producing Bacterial Isolates against Phythopathogens and Their Effect on Growth of Okra. Int.J.Curr.Microbiol.App.Sci. 2017; 6(8): 1925-1929.

[17] Pikovaskya RI. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya. 1948; 17:362-370.

[18] Pradhan A, Mishra BB. Effect of Plant Growth Promoting Rhizobacteria on germination and Growth of Rice (*Oryza Sativa* L.). The Ecoscan.2015; 9(1&2):213-216.

[19] Ramamoorthy V, Viswanathan R., Raguchander T, Prakasan V, Samiyappan R. Introduction of systemic resistance by Plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection. 2011; 20: 1-11.

[20] Scher FM, Baker R. Effect of Pseudomonas putida and synthetic iron chelator on induction of soil suppressiveness to Fusarium wilts pathogens. Phytopathology. 1982; 72:1567-1573.

[21] Schroth, MN, Hancock JG. Disease-suppressive soil and root-colonizing bacteria. Science. 1982; 216:1376-81.

[22] Schwyn B, Neilands JB. Universal Chemical Assay for the detection and determination of siderophores. Anal. Biochem. 1986;140: 47-56.

[23] Vincent JM. Distortionn of fungal hyphae in presence of certation inhibitors. Nature. 1927; 59, pp. 850.