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CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM SEASONAL FLOWER BEDS

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Key words: PGPR, IAA, Phosphate solubilization

Abstract

The present investigations were carried out to screen the PGPR isolates from five separate seasonal flower beds (*Catharanthus roseus, Portulaca grandiflora, Celosia argentea, Gomphrena globosa* and *Impatiens balsamina*) of Curzon Hall, University of Dhaka. The bacterial load of the collected soil samples ranged in between 7.60 ± 6.01 × 10^6 and 9.49 ± 5.44× 10^8 cfu/g, 5.34 ± 4.06×10^6 and 13.4 ± 4.8×10^6 cfu/g and 1.01 ± 0.59×10^6 and 1.19 ± 0.95×10^8 cfu/g on Nutrient Agar (NA), Luria-Bertani (LB) and Yeast Extract Mannitol Agar (YEMA), respectively. All the selected eight isolates were Gram positive and rod shaped. All isolates were found to be IAA producer and 7 showed varying levels of phosphate solubilizing activity. IAA production and phosphate (P) solubilization of the selected isolates ranged in between 9.76 ± 5.98 to 17.99 ± 1.865 U/ml, and 1.17 ± 0.59 to 1.84 ± 0.499 mg/l. Maximum production of both IAA and phosphate solubilization was achieved in 24h of incubation period at 37°C and pH 7.0. Germination test against Mung Bean (*Vigna radiata*) revealed positive plant growth promoting activities and could be used as PGPR inoculant.

Introduction

Plant growth promoting rhizobacteria (PGPR) are the bacteria that can enhance plant growth by a wide variety of mechanisms. Based on their activities PGPR can be grouped as biofertilizers, phytostimulators, rhizoremediators, and biopesticides(1). The plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility(2). In recent years considerable attention has been paid to PGPR to replace agrochemicals for the plant growth promotion by a variety of mechanisms that involve soil structure formation, decomposition of organic matter, recycling of essential elements, solubilization of mineral nutrients, producing numerous plant growth regulators, degrading organic pollutants, stimulation of root growth, crucial for soil fertility, biocontrol of soil and seed borne plant pathogens and in promoting changes in vegetation(3). The bacterial genera such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas, Allorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium* belongs to PGPR. Rhizospheric soil bacteria have the potentiality of producing different

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types of phytohormones like auxin, gibberellins, cytokinins, ethylene and abscisic acid and so on. Inole-3-acetic acid is produced by various PGPR involved in the plant growth processes and development. Effects of IAA on plants are significant and some of them are apical dominance, phototropism, geotropism, prevention of leaf and fruit abscission and induction of adventitious roots. Therefore, IAA has profound influence on crops. The rhizospheric bacteria utilize the root exudates to produce IAA as part of their secondary metabolism[4]. IAA-production by root associated bacteria is a major mechanism of plant growth promotion and it has great impacts on the development of the root system under the influence of rhizosphere microbes. Among PGPR which can solubilize phosphate called phosphate solubilizing bacteria (PSB) solubilizes the insoluble complex structured phosphates and make it easy to absorb by plants. Phosphorus is one of the essential macronutrients, which are required for maximum yield of agricultural crops. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants[5]. Farmers are thus suggested to apply phosphorus fertilizers in several-fold excess in order to overcome this problem. Plant root-associated PSB have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield[6]. Considering all possible importance the present study was aimed to isolate and characterize the plant growth promoting Rhizobacteria from seasonal flower beds.

Materials and Methods

Soil samples (winter season) were collected in sterile plastic bags from rhizospheric region of five separate seasonal flowering plant beds from Botanical Garden, Curzon Hall, University of Dhaka *viz*. *Catharanthus roseus, Portulaca grandiflora*, *Celosia argentea, Gomphrena globosa* and *Impatiens balsamina*. The pH of the soil samples were measured by a portable pH meter (ToA-Dkk, HM-31P, Japan) immediately after the samples were brought into the laboratory. Nutrient agar (NA), Luria-Bertani (LB) and Yeast Extract Mannitol Agar (YEMA) media were used for the enumeration and isolation of bacteria associated with the collected rhizospheric soil samples. The pH of the medium was adjusted to 7.0 ± 0.2 prior to the addition of agar and sterilization.

Serial dilution technique[7] was used for the isolation of bacteria using nutrient agar NA, LB and YEMA media for enumeration of aerobic heterotrophic bacteria. The pH of the media was adjusted to 7.0 ± 0.2. Inoculated plates were inverted and incubated at 37°C for 24 h in an incubator (Memmert GmbH + Co Kg 8540 Schwabach, Germany). After 24 h, plates having well discrete colonies were counted. All the suspected colonies were screened for phosphate solubilization on Pikovskayas medium[8]. Isolates showing phosphate solubilizing ability were spot inoculated at the Pikovskayas agar plate and incubated at 37 ^{0}C Diameter of clearance zone was measured successively after 24 hours upto 7 days. The ability of the bacteria to solubilize insoluble phosphate was described

by the solubilization index (PSI) [= the ratio of the total diameter (colony + halo zone) to the colony diameter[9].

Phosphate solubilizing broth medium was used for the estimation of phosphate solubilization. The medium consisted of (% w/v) : 1 g glucose, 0.5 g $MgCl_2.6H_2O$, 0.025 g $MgSO_4.7H_2O$, 0.02g KCl, 0.01g $(NH_4)_2SO_4,7H_2O$, 2.0 g NaCl as well as 0.5 g tri-calcium phosphate amended as in-soluble P source[10]. Bacterial isolates from their respective slants were inoculated in 100 ml of LB broth under aseptic condition. Flasks were placed in a shaker incubator at 37°C, 120 rpm for 24 h. After 1 day of incubation, growth (turbidity) was appeared in inoculated broth and this preparation was directly used as a source of inoculum. Optical Density (OD) of each inoculum was fixed to 1.00 at 600 nm using UV-spectrophotometer (UV-1800 Shimadzu, Japan). From LB broth culture, 1 ml of inoculum was transferred in 100 ml of production medium. All experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml liquid medium. The flasks were autoclaved at 121°C under 15-atmosphere pressure for 15 min prior to adding tri-calcium phosphate. Culture conditions for the isolates were initial pH 7.0 and temperature 30°C with rotary shaking at 100 rpm for seven days. Phosphate solubilization activity was assayed as pure isolates. Growth rate and P solubilization were measured at daily intervals.

Two milliliters of media was centrifuged at 10,000 rpm for 30 min, and then the supernatant was taken to measure the P concentration using ascorbic acid method[10]. Bacterial isolates from their respective slants were inoculated in 100 ml of Luria broth. Flasks were placed in a shaker incubator at 37°C, 120 rpm for 24 h. After 1 day of incubation, growth (turbidity) was appeared in inoculated broth and this preparation was directly used as a source of inoculum. Optical density (OD) of each inoculum was fixed to 1.00 at 600 nm using UV-spectrophotometer (UV-1800 Shimadzu, Japan).

For the estimation of IAA production, 1 ml of inoculum from LB broth culture was transferred in 25ml of LB broth amended with 50 µ/ml tryptophan. All experiments were carried out in 100 ml Erlenmeyer flasks. The flasks were autoclaved. They were incubated for 24 h at 28°C on rotary shaker. Cultures were centrifuged at 10,000 rpm for 15 min. 2 ml of supernatant was taken and 2 to 3 drops of orthophosphoric acid was added. Four ml of Salkowski reagent was added and incubated for 25 min. at room temperature and development of pink colour indicates the IAA production. Absorbance was read at 530 nm. Auxin production was determined by using a standard graph.

The efficiency of phosphate solubilization and IAA production of the selected isolates were studied at incubation temperature 25°C, 30°C, 37°C and 40°C and media was adjusted at different pH values 5, 6, 7, 8 and 9 with incubation period of 24h, 48h, 72h and 96h. Gram positive bacteria were provisionally identified following Bergey's Manual of Systematic Bacteriology Vol. II[11]. Twelve Mung Bean (*Vigna radiata*) seeds were used to test the effect of hormone on germination. The seeds were placed on

circular Whattman's filter paper in a Petri plate and 1 ml of supernatant was added accordingly for 8 days. Seed germination effect was observed every day interval.

Results and Discussion

A good number of aerobic heterotrophic bacteria were found to be associated with the collected soil samples. Aerobic heterotrophic bacterial load of the samples ranged from 7.60 ± 6.01 × 106 to 9.49 ± 5.44× 10^8, 5.34 ± 4.06×10^6 to 13.4 ± 4.8×10^6 cfu/g and 1.01 ± 0.59×10^6 cfu/g on NA, LB and YEMA media, respectively (Table 1). The highest mean bacterial counts (10.91 ± 3.41 × 10^6 cfu/g on NA, 13.4 ± 4.8 × 10^6 cfu/g on LB and 18.06 ± 12.2×10^6 cfu/g on YEMA) were observed.

Table 1. Heterotrophic bacterial load of the collected soil samples.

Flower Bed	Bacterial load (cfu/g) on NA, LB and YEMA media		
	NA	LB	YEMA
Catharanthus roseus	9.49 ± 5.44 × 10^8	10.71 ± 5.36 × 10^6	18.06 ± 12.2 × 10^6
Portulaca grandiflora	7.60 ± 6.01 × 10^6	7.24 ± 6.16 × 10^6	11.9 ± 2.7 × 10^6
Celosia argentea	10.91 ± 3.41 × 10^6	13.4 ± 4.8 × 10^6	15.7 ± 2.08 × 10^6
Impatiens balsamina	7.85 ± 6.10 × 10^6	5.34 ± 4.06 × 10^6	1.01 ± 0..59 × 10^6
Gomphrena globosa	9.58 ± 6.78 × 10^6	5.66 ± 4.66 × 10^6	1.19 ± 0.95 × 10^8

A total of 149 bacteria were isolated from the collected rhizospheric soil. Among them 8 isolates were evaluated for their plant growth promoting traits. Five best potential bacterial strains showing PGP activities were selected for characterization. All the bacterial isolates were rod shaped Gram positive bacteria (Table 2). The major biochemical tests were shown in the Table 3. The isolated bacteria were identified as *Bacillus alcalophilus*, *Bacillus firmus* and *Bacillus lentus* (Table 4).

Table 2. Microscopic observations of the isolates.

Isolate No.	Gram reaction	Vegetative cell characteristics	Spore
Cr6/S1/L	+	Long rods, occur in chains	-
Ib132/S11/L	+	Rod shaped, occur in both as single cells and chains	+
Ib133/S11/L	+	Rod shaped, occur in chains	+
Ib134/S11/L	+	Rod shaped, occur in chains	+
Ib140/S13/L	+	Rod shaped, occur in chains	+

Table 3. Biochemical characteristics of the isolates.

Isolates No.	V.P test	M.R test	Deep glucose agar	Utilization of		Lecithinase production	Nitrate reduction	Lipase
				Citrate	Propionate			
Cr6/S1/L	+	+	A	-	-	-	-	+
Ib132/S11/L	+	+	FA	-	-	+	-	+
Ib133/S11/L	+	+	FA	-	+	-	-	+
Ib134/S11/L	+	+	FA	-	+	-	-	+
Ib140/S13/L	+	+	FA	-	-	+	-	+

Table 4. Provisional identification of the isolated rhizospheric bacterial isolates.

Isolate No.	Provisionally identified bacteria
Cr6/S1/L	*Bacillus alcalophilus*
Ib132/S11/L	*B. alcalophilus*
Ib133/S11/L	*B. alcalophilus*
Ib134/S11/L	*B. firmus*
Ib140/S13/L	*B. lentus*

The soil samples were collected from different rhizosphere soil showed presence of PGPR showing PGP activities *i.e.* phosphate solubilization and IAA production (Table 5). Among 5 potential IAA producers, 3 were chosen for better production (Fig.1). The result showed that *B. lentus* (Ib/S13/L) showed the maximum (17.99 µg/ml). In a study the highest accumulation of IAA was observed after 96 h by *B. subtilis* WR-W2[12]. In the present study, optimum pH for the IAA production was found to be pH 7.0. Very similar report was observed in case of Baggam *et al*. findings[13]. In the present study it was

Table 5. Estimation of IAA production and phosphate solubilization.

Isolate No	Bacteria	IAA activity (U/ml)	Phosphate solubilization (mg/l)
Cr6/S1/L	*Bacillus alcalophilus*	15.90 ± 1.93	1.53 ± 0.42
Ib132/S11/L	*B. alcalophilus*	14.95 ± 4.08	1.17 ± 0.59
Ib133/S11/L	*B. alcalophilus*	9.76 ± 5.98	1.84 ± 0.49
Ib134/S11/L	*B. firmus*	9.93 ± 3.87	1.42 ± 0.41
Ib140/S13/L	*B. lentus*	17.99 ± 1.865	1.46 ± 0.35

Table 6. Effects of water and PGPR on the germination of Mung Bean (*Vigna radiata*) **seeds.**

Isolate No.	Bacteria	Average radicle length (mm) under water	Average radicle length (mm) under PGPR	Average plumule length (mm) under water	Average plumule length (mm) under PGPR
Cr6/S1/L	*Bacillus alcalophilus*	2.33	6.67	22.67	30
Ib132/S11/L	*B. alcalophilus*	4.33	1.63	4.00	3.00
Ib133/S11/L	*B. alcalophilus*	3.17	3.00	2.73	3.83
Ib134/S11/L	*B. firmus*	3.17	5.00	15.67	25.00
Ib140/S13/L	*B. lentus*	3.33	4.67	12.50	11.33

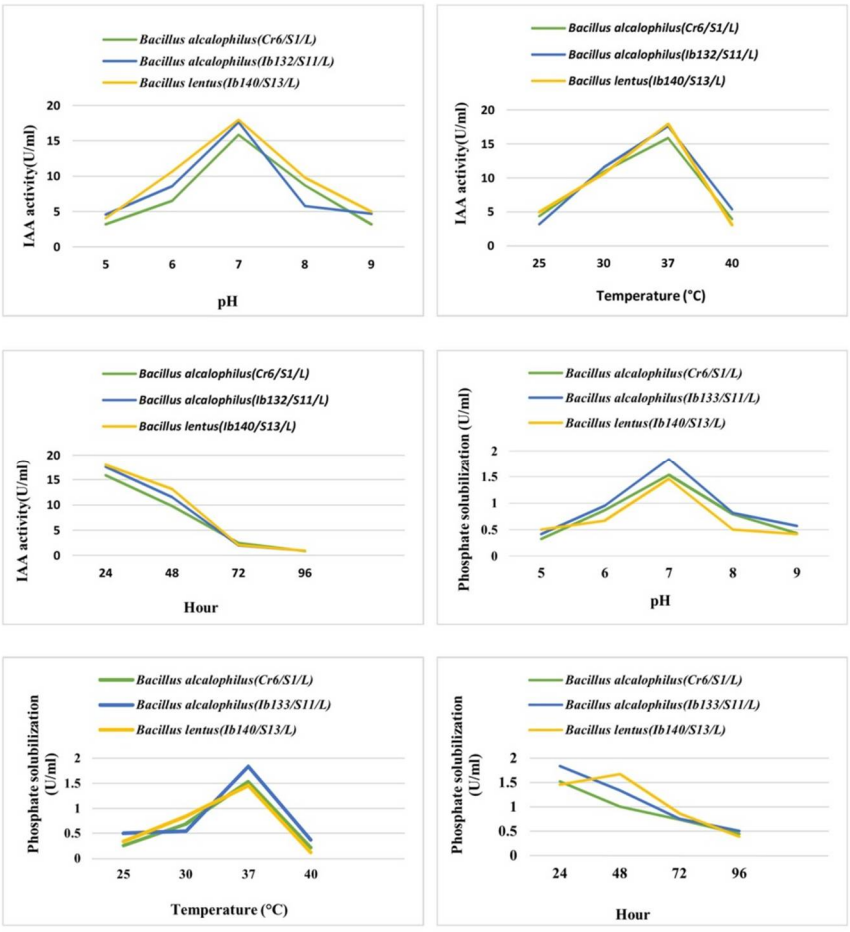

Fig. 1. Optimization of IAA production and phosphate solubilization by three best producer under varied levels of pH, temperature and incubation period.

observed that the optimum temperature was 37°C for IAA production which is also similar to the observation of Suliasih and Widawati[11]. Maximum phosphate solubilization activity 1.84 mg/L was showed by *B. alcalophilus* (Ib/S11/L) which was found to be better than that of Maheswar *et al.*[14]. Positive effects of PGPR were observed in seed germination in Mung Bean (Table 6). Best result was observed in case of *B. alcalophilus* (Cr6/S1/L) isolated from *Catharanthus roseus*. This is because due to better activities of IAA (15.90±1.93) of the *B. alcalophilus*. The highest average plumule length under PGPR was recorded as 30 mm by the isolated species *B. alcalophilus* (Cr6/S1/L). The isolated PGPR could be used in the better crop production.

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