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ISOLATION AND CHARACTERIZATION OF PHOSPHATE-SOLUBILIZING BACTERIA FROM RHIZOSPHERE SOIL

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ABSTRACT

Different strains of phosphate-solubilizing bacteria (PSB) were isolated from the rhizosphere of different plants of Lahore District, Pakistan. The objective of the study was to explore the capabilities of PSB and evaluate their efficiency to enhance growth of sugarcane plants under greenhouse condition. The purified isolates were identified as Proteous vulgaris, Klebsiella pneumoniae, Enterobacter aerogenes, Burkholederi acepacia, Citrobacter freundii, Acinetobacter lwoffii, and Pseudomonas fluorescens, and the identification was based on the characteristic morphological and biochemical behavior. The efficiency of different PSB isolates for phosphate solubilization was evaluated from the zones they formed on agar plates of Pickovaskaya growth medium (PVK) by solubilizing the tricalcium phosphate of the medium. The efficiency of purified PSB was evaluated from pot experiments of two different genetically modified sugarcane varieties (SCMV resistant CAMB-I and CAMB-II) under greenhouse condition for their positive role in plant growth promotion. All the six PSB enhanced the growth rate significantly over that in the non-inoculated control. A significant increase in plant height, number of leaves, root length and dry matter contents was recorded and E. aerogenes and C. freundii were found significantly superior over the rest of the isolates for all the tested parameters. The efficiency gradient of different isolates for CAMB-I and CAMB-II varieties was recorded as C. freundii> K. pneumoniae> E. aerogenes> B. cepacia> A. lwoffii> P. vulgaris and E. aerogenes> C. freundii> A. lwoffii> B. cepacia> P. vulgaris, respectively. The results of this greenhouse evaluation are encouraging and need to be confirmed under field condition in combination with organic and chemical fertilizers.

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Keywords: phosphate-solubilizing bacteria, sugarcane, isolation and purification of bacteria

Introduction

The world of agriculture in the last few decades has been profoundly dependent on chemical fertilizers as source plant nutrients to meet the increasing demand for food. Conversely, in recent years environmentalist and agricultural scientists have realized that continued and persistent use of chemical fertilizers depletes the soil, and causes environmental pollution and unevenness in the soil microbial activity. Hence, increasing awareness is being built on the use of organics together with bio-fertilizers to sustain the soil richness and plant productivity.

Phosphorus is the major growth-limiting macronutrient of plants, only second to nitrogen. It is an integral part of plants and is usually deficient in the soil due to its rapid fixation. It has been estimated that in some soils almost 75 % of the applied phosphate fertilizer re-precipitates and becomes unavailable to the plants (35). Unlike nitrogen, phosphorus (P) is not abundant in the atmosphere in a form that can be made biologically available to plants (17). Phosphate-solubilizing bacteria (PSB) can convert insoluble phosphates into soluble forms (24, 28, 31, 39). PSB, therefore, find application in crop improvement as enhancers of solubilization of re-precipitated rhizospheric P (33, 44). Naturally occurring rhizospheric phosphorus-solubilizing microorganisms (PSM) have first been reported in 1903 (17).

Phosphorous is a key nutrient required for the higher sustained productivity of sugar for the sugarcane plant. It influences sugarcane yield and juice quality and application of P fertilizers has become an essential part of sugarcane cultivation (35). Therefore, purified strains of PSB are applied to the rhizosphere of Sugarcane Mosaic Virus (SCMV) resistant sugarcane lines to assess their efficiency for phosphate solubilization and positive role in plant growth.

A lot of bacteria have been reported to have a positive role for phosphate solubilization, both fixed soil phosphate and applied re-precipitated phosphate, ensuring higher crop yields when applied near plant roots. Many PSB, including *Pseudomonas*, *Bacillus*, *Rhizobium*, *Micrococcus*, *Flavobacterium*, *Burkholderia*, *Achromobacter*, *Erwinia*, and *Agrobacterium,*are used as biofertilizers or control agents for agriculture improvement (30, 37). Some of the most efficient PSB are *Pseudomonas* species. Owing to their multiple biofertilizing potential, they could serve as key bio-inoculants for improving soil nutrient status, secretion of plant growth regulators, and suppression of soil-borne pathogens (4, 40). Another very effective PSB is *Bacillus*, which is also anauxin-producing bacterium (6, 32).

The mechanism of mineral phosphate solubilization by PSB strains is generally considered to be associated with the release of low molecular weight organic acids. Their hydroxyl and carboxyl groups chelate the cations bound to phosphate and thus convert it into soluble forms. P-solubilization, however, is a complex phenomenon and depends on various nutritional and physiological factors, cultivation conditions, etc. (8, 21, 29).

PSB bacteria can grow in media containing calcium phosphate as the sole P source, and can solubilize, assimilate and release P in higher amounts. This reaction, manifested as a halo or a clear zone on the plate, is used for assessing the P-solubilizing activity of these bacteria. However, the reliability of this technique is questionable when it is the only one used in the detection of potential PSB (11, 30).

The aim of the present study was to optimize the conditions for isolation and purification of PSB from the rhizosphere of different plants; and to identify the PSB, using physiological and biochemical tests of bacterial isolates. Pot experiments were performed to estimate the efficiency of PSB to positively affect the plant growth parameters in green-house conditions.

Materials and Methods

Rhizosphere soil sampling

The soils adhering to roots and allied area of 12 different crop plants were collected from the Lahore District in March 2012. They were taken from depth of approximately 12 cm. The soil samples were collected in disposable autoclavable bags and were transported to the research laboratory of the Centre for Applied Molecular Biology Lahore and stored at 4 °C ± 1 °C until further use. Soils were analyzed for pH and organic carbon by the method of Walkely and Black (41). Available and total 'P' was analyzed according to Olsen et al. (23).

The isolation of PSB was made from *Pisumsativum* (pea), family Fabaceae; *Brassica campestris* (mustard), family Brassicaceae; *Solanum tuberosum* (potato), family Solanaceae; *Saccharum officinarum* (sugarcane), family Poaceae; *Gossypium hirsutum* (cotton), family Malvaceae; *Gladiolus communis* (gladulous), family Iridaceae; *Coriandrum sativum* (coriander), family Apiaceae; and *Oryza sativa* (rice), family Poaceae.

Isolation, purification, and solubilization index of PSB

Bacterial strains were isolated using 10-fold serial dilutions (15). Serially diluted soil samples (up to 10^{-5}) were spread on Pikovskaya agar in disposable plates (25) and incubated at 28°C for 48 h. Pikovskaya mediumcontained: glucose (ICN Biomedicals, Inc, CAT 152527), 10 g/L; $Ca_3(PO_4)_2$ (LAB CHEM,CAT A2140-M), 5 g/L; $(NH_4)_2SO_4$ (Biobascic, CAT PCRB55), 0.5 g/L; NaCl (Merck, CAT 7782-63-0), 0.2 g/L; $MgSO_4 \cdot 7H_2O$ (SIGMA-ALDRICH, CAS 7487-88-9), 0.1 g/L; KCl (Fisher Scientific, CAT P 330-500), 0.2 g/L; yeast extract (Biochemika, CAT 70161-500G), 0.5 g/L; $MnSO_4 \cdot H_2O$ (SAI CHEMICALS, CAT 7786-87-7), 0.002 g/L; $FeSO_4 \cdot 7H_2O$ (Fisher Scientific, CAT 7782-63-0), 0.002 g/L; and gar (Biotechnology Grade, CAT 40100044-1), 18 g/L, and was sterilized at 121 °C and 105 kPa for 15 min. Single PSB colonieswere streaked onfresh plates of Pikovskaya medium and incubated at 28 °C for 48 h. Then, the appearance of halo zone was used for confirmation of the presence of PSB. Single colonies with clear zones around them were picked up and streaked on new plates of the same medium to obtain pure cultures. The solubilization index was calculated from the measurements recorded after 7 days of growth of pin point inoculation on PVK at 28 °C. The solubilization index (SI) was calculated using the following formula (9): SI = (colony diameter + halo zone diameter)/colony diameter.

Colony and cell morphology

The PSB colony was streaked on plate count agar (Sigma, CAS 9002-18-0) and incubated at 28 °C for 48 h to study the morphological aspects of the colonies (5, 18, 24).

Gram-staining and catalase test

Gram-staining of purified PSB strains was performed according by the Vincent method (38) and was observed under a microscope (Van Guard 1400 series). For biochemical identification of PSB, catalase test was performed and a drop of 30 % hydrogen peroxide was dropped on a glass slide. A 24-hour-old pure bacterial colony of each isolate was taken from the PVK plate and gently mixed with a drop of hydrogen peroxide. The breakdown of hydrogen peroxide into water and oxygen indicates the presence of catalase in the bacteria (15, 19).

QTS 24-Miniaturized Identification System

Following the method of Yasmin and Bano (42), physiological and biochemical tests of bacterial isolates were performed to identify the bacterial isolates. QTS-24 miniaturized identification system (DESTO Laboratories, Karachi, Pakistan) was used for this purpose. Twenty-four-hours-old bacterial cultures grown on MacConkey agar (Fluka, P1400) were used. The results were scored after 18 h of incubation at 30 °C.

Pot experiments

Pot experimentswere carried out in the green house of the Centre for Applied Molecular Biology Lahore in 2012. Pots were filled with 2.5 kg of sterilized soil. The soil used in the pot culture experiment had a pH of 7.5, 0.89 % of organic carbon , 186 kg/ha of available nitrogen and 493.85 kg/ha of available K.

The growth-promoting effects of selected PSB isolates were studied on two-week-old sugarcane plants of CAMB I and CAMB II (genetically modified SCMV resistant) varieties and 24-hour-old selected bacterial cultures (5 mL/plant) were treated with sugarcane and sown in pots (18 cm^2 × 19 cm^2) containing sterilized soil. The pots were placed in a green house with a temperature range of 20 °C to 30 °C, 70 % humidity and 10 h to 15 h natural light. A total of 28 pots were sown, four plants per pot (7 treatments, 4 replicates) and were placed at the same depth (approximately 7.0 cm in each pot) and were arranged in randomized complete block design. Urea 0.32/200 mL

was applied to each pot after 3 to 4 days of sowing for better growth. The pots were watered slowly over the top soil every 1 or 2 days and plant protection measures were taken to protect the plants against insect pest damage.

The plants harvest was made 90 days following inoculation and the root and shoot length were measured. Plants were dried at 70 °C (air oven) for 72 h to a constant weight. After that, the dry weight of shoots and roots was recorded (40).

Statistical analysis

The data were analyzed statistically by analysis of variance (one-way ANOVA) and POST HOC test, using SPSS software version 20.

Results and Discussion

Isolation, purification and solubilization index of PSB

Bacterial isolates were identified as PSB based on their ability to solubilize tricalcium phosphate $Ca_3(PO4)_2$ by the formation of visible dissolution halos on Pickovskaya agar (**Fig. 1**). Similar results on the occurrence and isolation of PSB were found by El-Komy (10) and Neelam and Meenu (22).

The solubilization index based on colony diameter and halo zone for each PSM isolate is presented in **Fig. 2**. The results showed that, among the 12 isolates, the BR2 isolate showed a maximum solubilization index of 2.23, followed by all the other bacterial strains. The bacterial isolates produced the largest halos of approximately 1.52 cm to 2.23 cm within 7 days of incubation. Similar results were also reported by Alam et al. (2) and Yasmin et al. (43).

According to De Freitas et al. (8), good phosphate-solubilizers produce halos around their colonies with diameters larger than 15 mm (1.5 cm). It has been reported that some strains of PSB lose their phosphate-solubilizing ability after several cycles of inoculation. We corroborated the persistence of this trait in all PSB isolates by successive subcultures, which is in accordance with Alam et al. (2)

Morphology of bacterial isolates

All PSB isolated from rhizosphere soil of different plants were odorless, had round raised colonies with a smooth and shiny surface. Most of them had entire margins, except PR2, BR1, BR2, GR1 and OR1, which had wavy margins. The color of PR1, PR2, SR2, PTR1, and CR1 was skin; of FR1, yellowish; and of PR4, BR1, BR2, SR1, GR1, and OR1, off-white. The cells of all PSB isolates were rod-shaped. Among them, some were paired and some were scattered in arrangement. All the 12 PSB isolates were Gram-negative (**Fig. 3**).

Catalase and oxidase test

All the isolates from rhizosphere soil of different plants were tested for catalase activity. PR2, PR4, BR1, BR2, GR1, PTR1, and FR1 bacterial isolates were positive in the catalase test, while the other bacterial isolates, viz. PR1, SR1, CR1, OR1, and SR2, were negative.

Oxidase test was performed to determine the presence of oxidase enzyme in all bacterial isolates. BR1, BR2, GR1, and PTR1 were oxidase-positive, while the other bacterial isolates, viz. PR1, PR2, PR4, SR1, SR2, FR1, CR1, and OR1 were negative for oxidase.

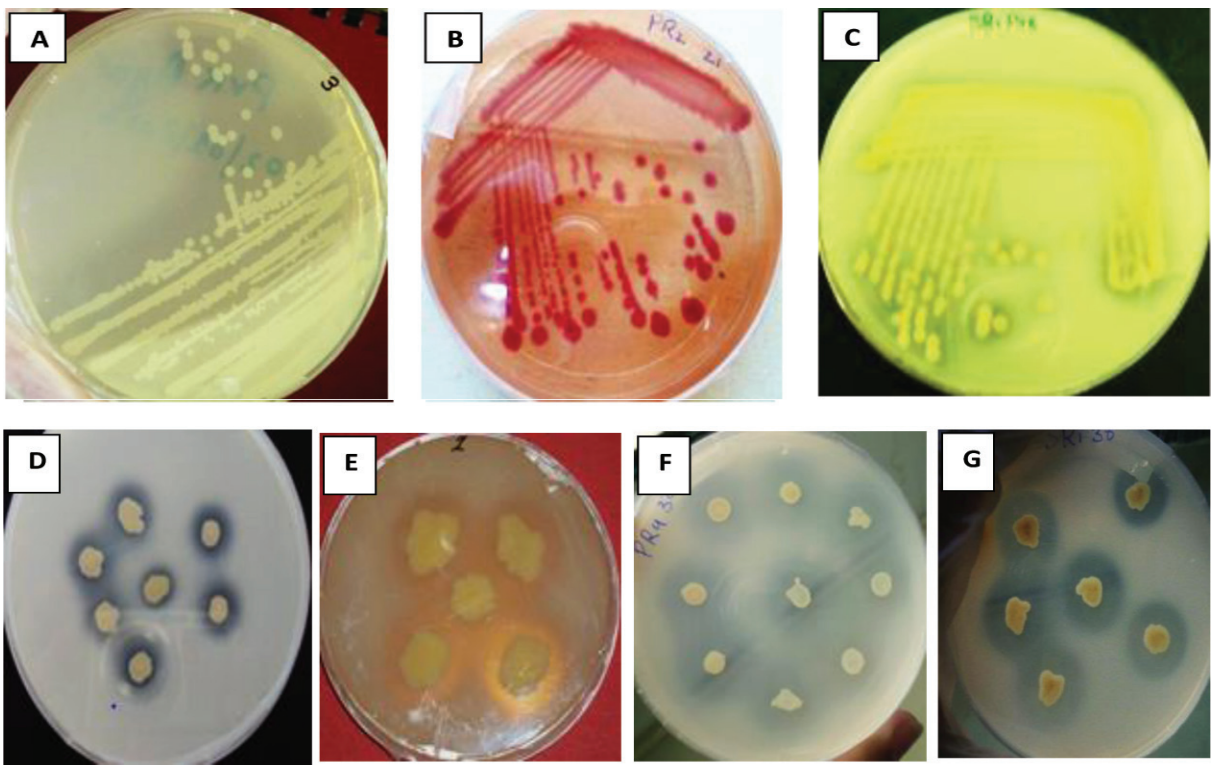

Fig. 1. Purified colonies of phosphate-solubilizing bacteria. Colonies of BR2 on PVK agar (**A**), PR2 on MacConkey agar (**B**), SR1 on PVK agar (**C**); halo formation around the colony due to solubiliziation of phosphorous by BR1 (**D**), BR2 (**E**), PR4 (**F**), and SR1 (**G**) on PVK agar.

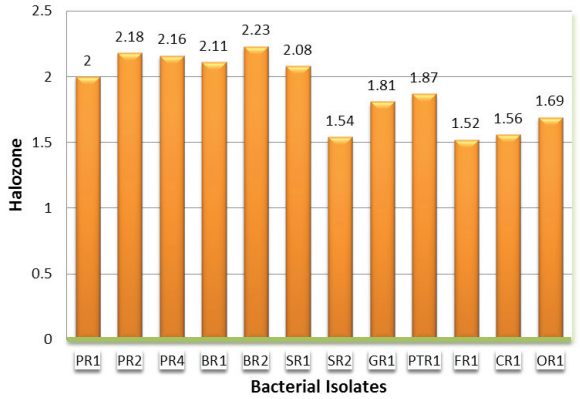

Fig. 2. Phosphate solubilization index of different purified strains of PSB.

Identification of PSB isolates by QTS-24 Miniaturized Quantification System

The microbial identification kit QTS- 24 used is based on carbon/nitrogen utilization. The dominant representatives identified are listed in **Table 1**: *Proteous vulgaris* (PR1), *Klebsiella pneumoniae* (PR2), *Enterobacter aerogenes* (PR4), *Burkholederia cepacia* (BR1), *Citrobacter freundii* (BR2), *Acinetobacter lwoffii* (SR1), *Proteous vulgaris* (SR2), *Pseudomonas fluorescens* (GR1), *Burkholederia cepacia* (PTR1), *Klebsiella pneumoniae* (OR1), *Citrobacter freundii* (FR1), *Acinetobacter lwoffii* (CR1). Similar results related to isolation, screening and identification have been found by different scientists (7, 13, 21, 27, 36). Similarly, Haque and Dave (14) isolated *A. lwoffii* and studied the rhizosphere soil of different regions and examined the promotion of plant growth.

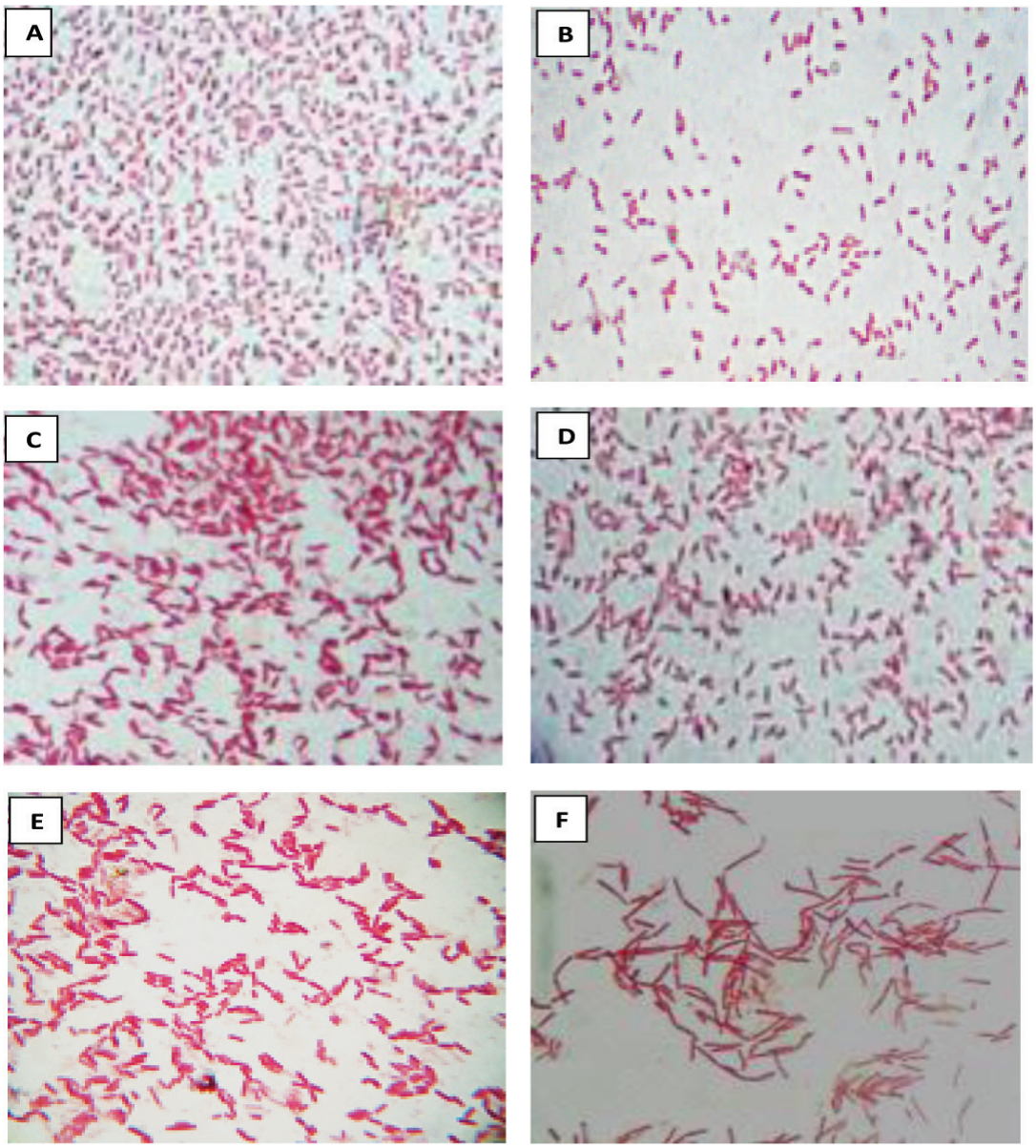

Fig. 3. Microscopic examination of phosphate-solubilizing bacteria. Rod-shaped PR1 (**A**), rod-shaped PR2 (**B**), rod-shaped BR2 (**C**), rod-shaped PR4 (**D**), rod-shaped BR1 (**E**), long chain SR2 (**F**).

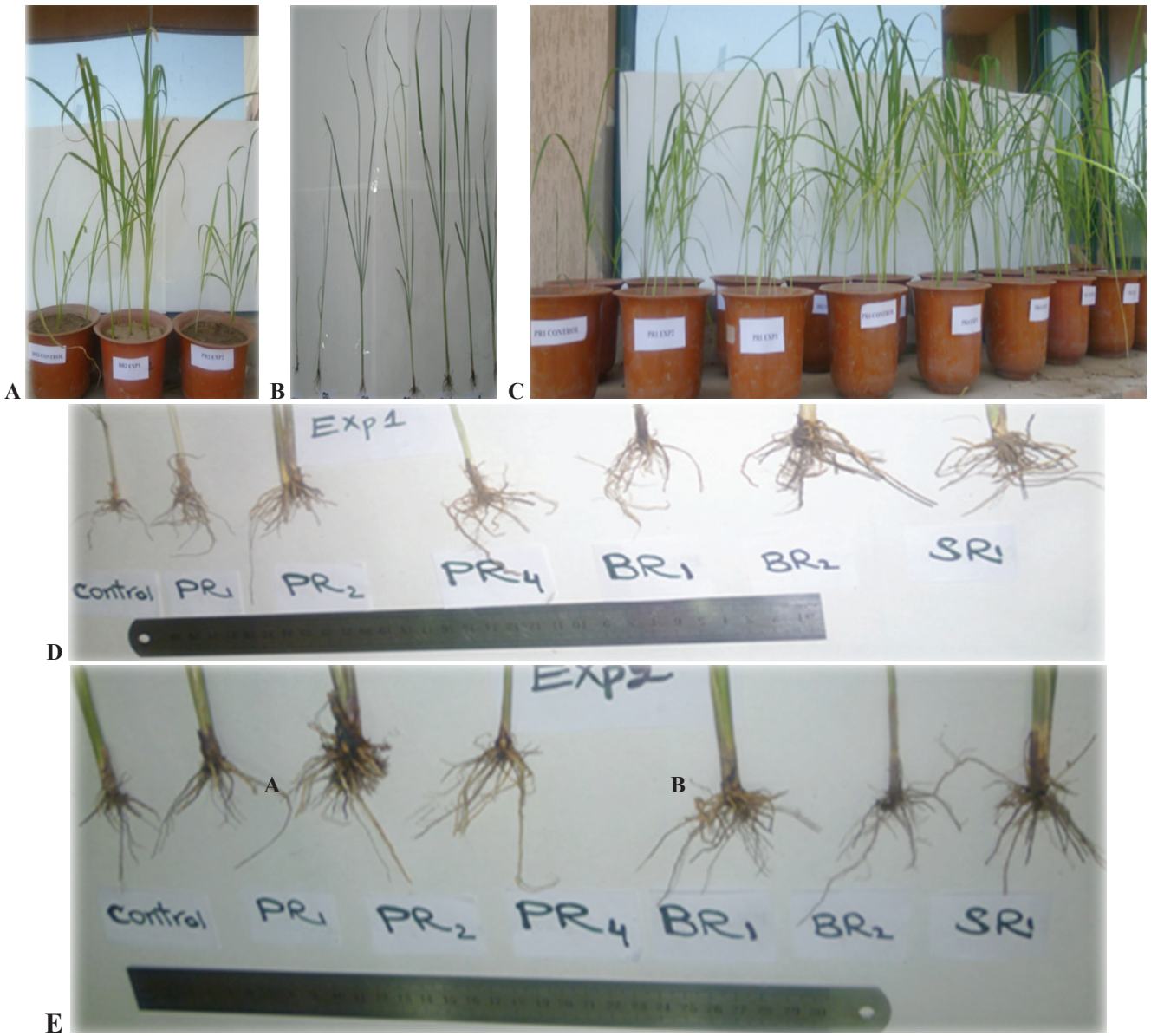

Fig. 4. Growth promotion of sugar cane plants by PSB isolates (**A**). Replicates of sugar cane plants (**B**). PSB-treated sugarcane plants in greenhouse (**C**). Roots of sugarcane plants of the CAMB I (**D**) and CAMB II (**E**) variety after 90 days of treatment with PSB isolates.

Evaluation of efficient PSB isolates for plant growth under greenhouse condition

The performance of six efficient PSB isolates, PRI, PR2, PR4, BR1, BR2, and SR1, with sugarcane varieties CAMB I and CAMB II (genetically modified SCMV resistant) shoots and roots were evaluated in a green-house experiment (**Fig. 4**). The control sugarcane plants both for the CAMB I and the CAMB II variety, for evaluating the efficiency of PSB isolates, were of similar apparent characteristics. Therecorded observations revealed that theresponse of the CAMB II variety to different PSB treatments was more positive as compared to that of CAMB I.

Ninety days following inoculation, the treatment variant BR2 showed the maximum plant height (78.2 cm), which was a 1103 % increase over the initial height, followed by PR2 with 71.8cm, which was a 1070 % increase over the initial height (**Fig. 5**, **Fig. 4 A, B**). Both of the isolates gave a significant increase in plant height over the non-inoculated isolates. All the other isolates showed significant results over the non-inoculated controls (**Table 2**, **Fig. 4 A, B, C**). The order of PSB isolates for elongation of root and shoot length and number of leaves was as follows: BR2> PR2> PR4> BR1> SR1> PR1 for CAMB I, and PR2> PR4> BR2> SR1> BR1>PR1 for the CAMB II sugarcane variety.

The growth of shoots and proliferation of roots is accredited to the improvement of cell elongation and exponentiation due to P application or this may be due to the assembly of plant-growth-supporting substances (22). PSB solubilize the immovable soil P and useful phosphates, causing greater crops production (12, 28, 36). An increase in plant height

and biological yield has been reported due to soil inoculation withPSM and these results are in close conformity with the finding of Mehravarz et al. (20).

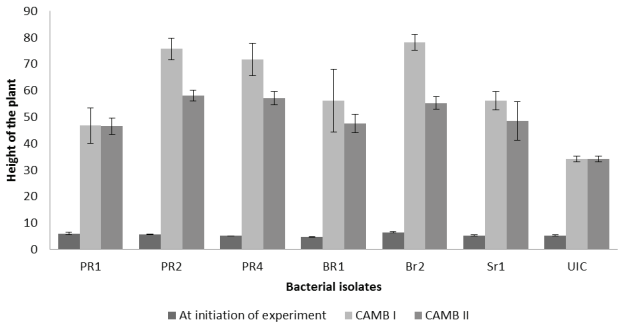

Fig. 5. Comparison of plant height [cm] of the CAMB I and CAMB II variety. UIC: uninoculated control.

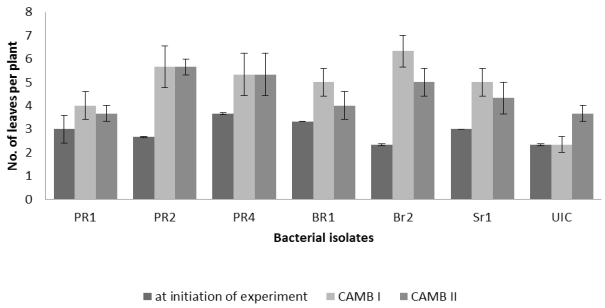

Fig. 6. Comparison of leaves of the CAMB I and CAMB II variety. UIC: uninoculated control.

Response of plant growth parameters

Number of leaves per plant. Among the inoculated variants, the highest number of leaves were recorded with isolates BR2 and PR2 (6.33, which was a 171 % increase; and 5.66, which was a 45 % increase over the initial stage, respectively) in the case of the CAMB I variety, while isolates PR4, BR1, SR1, and PR1 also showed a significant higher number of leaves per plant over the control (**Fig. 6**). For the CAMB II variety, among the inoculated variants, the highest numbers of leaves were recorded with isolates PR2 and PR4 (5.33, which was a 54 % increase; and 5.33, which was a 60 % increase over the initial stage, respectively). Both were significantly superior over many other isolates (**Table 2**). Other PSB isolates BR1, BR2, SR1, and PR1 also showed a significantly higher number of leaves per plant over the controls. The increase in leaf count is due to the ample fertilizer nutrition, as a result of enhanced root enlargement and increased translocation of carbohydrates from the source to developing points in well fertilized plots (34).

Shoot and root length. The shoot and root length of sugarcane plants inoculated with BR2 ranked the highest increase by 78.2 cm and 10.8 cm, respectively, as compared to the control, followed by PR2, PR4, BR1, SR1, and PR1 in the case of the CAMB I variety. PR2 ranked the highest increase by 58.2 cm and 7.9 cm, respectively, as compared to the control, followed by PR4, BR2, SR1, BR1, and PR1 in the case of the CAMB II variety (**Table 2**, **Fig. 7**).

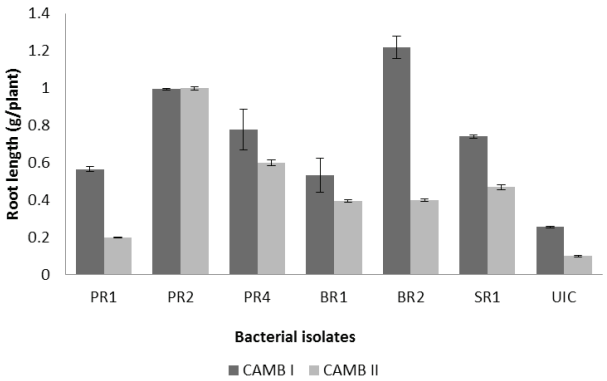

Fig. 7. Comparison of root length of the CAMB I and CAMB II variety. UIC: uninoculated control.

Almost all lateral roots of the inoculated variants were densely covered by root hairs, whereas very few or none developed on non-inoculated control plants (**Fig. 4 D, E**). Hence, dry matter accretion increased significantly with the use of PSB and was significantly higher as compared to non-inoculated controls. Such results have also been reported by Khan and Joergensen (16).

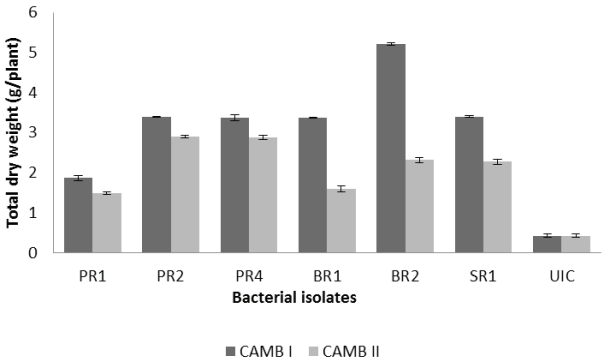

Fig.8. Comparison of total dry weight of the CAMB I and CAMB II variety. UIC: uninoculated control.

Shoot and root dry weight. A significant increase in shoot and root dry weight was shown in all inoculants as compared to non-inoculated controls. The variants that exhibited the maximum increase as compared to the control were CAMB I/BR2 (by 5.209 g/plant root and shoot dry weight) and CAMB II/PR2 (by 2.899 g) (**Table 2**, **Fig. 8**). These results also are in accordance with several other reports (1, 3) that showed an increase in plant growth, dry matter content and nutrient uptake of diverse crop plants owing to inoculation of PSB.

Statistical analysis

Significant differences ($P < 0.005$) were observed between the values obtained for growth promotion, number of leaves, root length and total dry weight of sugarcane plants 90 days following PSB inoculation as compared to non-inoculated control plants (**Table 2**).

Based on all these aspects, the use of biofertilizers containing PSB could be considered an important and safe way to help plant growth and development through the enhancement of P availability. As it was previously pointed

TABLE 1

Biochemical test for the identification of PSB isolates by the QTS-24 Miniaturized Quantification System

No.	Biochemical tests	PR1	PR2	PR4	BRI	BR2	SRI	SR2	GR1	PTR1	OR1	FR1	CR1
Gram test		-	-	-	-	-	-	-	-	-	-	-	-
Catalase Test		-	+	+	+	+	-	-	+	+	+	-	-
1	ONPG	-	+	+	-	+	-	-	-	-	+	+	-
2	CIT	-	+	-	+	+	-	-	-	+	+	+	+
3	MALO	-	+	+	-	+	-	-	-	-	+	+	-
4	LDC	-	+	+	+	+	-	-	-	+	+	-	-
5	ADH	-	-	-	-	-	-	-	+	-	-	-	-
6	ODC	-	-	+	-	+	-	-	-	-	-	+	-
7	H_2S	-	-	-	+	-	-	-	-	+	-	-	-
8	UREA	+	+	-	-	-	+	+	-	-	+	-	-
9	TDA	-	+	-	+	+	+	-	-	+	+	+	+
10	IND	+	-	-	-	-	+	+	-	-	-	-	-
11	VP	-	-	-	-	-	-	-	-	-	-	-	-
12	GEL	-	-	+	+	+	+	-	-	+	-	+	+
13	GLU	+	+	+	-	+	+	+	+	-	+	+	-
	NO_3/N_2	+	-	+	+	+	-	+	-	+	-	+	+
14	MALT	+	+	+	+	+	+	+	+	+	+	+	+
15	SUC	+	+	+	+	+	+	+	+	+	+	+	+
16	MANN	+	+	+	-	+	+	+	-	-	+	+	-
17	ARAB	+	+	+	+	+	+	+	+	+	+	+	+
18	RHAM	+	+	+	+	+	+	+	+	+	+	+	+
19	SORB	+	+	+	-	-	-	+	-	-	+	-	-
20	INOS	+	+	+	-	-	-	+	-	-	+	-	-
21	ADO	+	-	+	-	-	+	+	-	-	-	-	-
22	MEL	+	+	+	-	-	-	+	-	-	+	-	-
23	RAF	+	-	+	-	-	-	+	-	-	-	-	-
24	MOT	-	-	-	-	-	-	-	-	-	-	-	-
25	CO	-	-	-	+	+	-	-	+	+	-	-	-

ONPG: Ortho nitrophenyl β-D-galactopyranoside; CIT: Sodium citratrate; MALO: Sodium malonate; LDC: Lysine decarboxylase; ADH: Arginine dihydrolase; ODC: Ornithine decarboxylase; H2S: H_2S production; URE: Urea hydrolysis; TDA: Tryptophan deaminase; IND: Indole; VP: (Voges proskauer): Acetion; GEL: Gelatin hydrolysis; GLU: Acid from glucose; MAL: Acid from maltose; SuC: Acid from sucrose; MAN: Acid from mannitol; ARA: acid from arabinose; RHA: Acid from rhamnose; SOR: Acid from sorbitol; INO: Acid from inositol; ADO: Acid from adonitol; MEL: Acid from melibiose; RAE: Acid from raffinose. Where, (-) stands for negative in the test and (+) stands for positive in the test.

TABLE 2

Effect of PSB on different plant growth parameters

No.	Bacterial isolates	Plant growth parameters										
		At the start of experiment		90 days following inoculation								
				CAMB I				CAMB II				
		Plant height (cm)	No. of leaves per plant	Plant height (cm)	No. of leaves per plant	Root length (cm)	Total dry weight (g)	Plant height (cm)	No. of leaves per plant	Root length (cm)	Total dry weight
1	PR1	6.0 ± 0.400	3.00 ± 0.0588	46.8 ± 6.709	4.00 ± 0.588	$5.3 \pm 0.155^{b,e}$	$1.86 \pm 0.058^{b,c,d,e,f,g}$	46.5 ± 3.070	3.66 ± 0.339	5.5 ± 0.250	$1.48 \pm 0.023^{b,c,e,f,g}$
2	PR2	5.6 ± 0.089	2.66 ± 0.033	75.5 ± 3.971	5.66 ± 0.898	8.3 ± 0.089^{g}	$3.39 \pm 0.006^{a,e,g}$	58.2 ± 2.037^{b}	5.66 ± 0.339	$7.9 \pm 0.296^{f,g}$	$2.89 \pm 0.035^{a,d,e,f,g}$
3	PR4	5.1 ± 0.033	3.66 ± 0.033	71.8 ± 6.056	5.33 ± 0.898	6.5 ± 0.179^{e}	$3.37 \pm 0.078^{a,e,g}$	57.06 ± 2.496	5.33 ± 0.898	6.9 ± 0.425	$2.87 \pm 0.044^{a,d,e,f,g}$
4	BR1	4.8 ± 0.089	3.33 ± 0	56.2 ± 11.918	5.00 ± 0.588	6.3 ± 0.391^{e}	$3.36 \pm 0.012^{a,e,g}$	47.5 ± 3.508	4.00 ± 0.588	5.9 ± 0.425	$1.59 \pm 0.067^{b,c,e,f,g}$
5	BR2	6.5 ± 0.179	2.33 ± 0.033	78.2 ± 3.000^{g}	6.33 ± 0.679	$10.8 \pm 0.962^{a,c,d,g}$	$5.20 \pm 0.038^{a,b,c,d,f,g}$	55.3 ± 2.364	5.00 ± 0.588	6.0 ± 0.378	$2.31 \pm 0.058^{a,b,c,d,g}$
6	SR1	5.3 ± 0.155	3.00 ± 0	56.2 ± 3.490^{g}	5.00 ± 0.588	6.2 ± 0.424^{g}	$3.39 \pm 0.023^{a,e,g}$	48.5 ± 7.246	4.33 ± 0.679	6.0 ± 0^{b}	$2.26 \pm 0.058^{a,b,c,d,g}$
7	UIN	5.3 ± 0.206	2.33 ± 0.033	34.1 ± 1.052^{f}	2.33 ± 0.339	$4.4 \pm 0.176^{b,e,f}$	$0.41 \pm 0.042^{a,b,c,d,e,f}$	34.1 ± 1.052^{b}	2.33 ± 0.339	$4.4 \pm 0.176^{b,e}$	$0.41 \pm 0.042^{a,b,c,d,e,f}$

Each value is an average of three replicates; ± denotes standard error means. The means followed by different letters within each column are significantly different at $P < 0.005$.

out in the context of experiments with cotton plants (26), PSB are environmentally friendly, economical, and have greater agronomic utility to compensate the expensive inorganic sources of P fertilizers (44).

Conclusions

The finding of the present investigation highlighted that PSB from local soil could be easily isolated and may be exploited for local use. The advantageous effect of PSB on plant growth varied in a widerange depending on the environmental conditions, type of bacterial strain, host plant (sugarcane variety) and the condition of the soil. The combined application of phosphate fertilizers and PSB isolates with an organic source showed a considerably superior effectas compared to the control due to solubilization of tricalcium phosphate. This influence may be due to a changed soil environment, causing a steady increase in nutrients and resulting in a higher yield and nutrient uptake. Additional research work is required to explore the performance of these competent PSB isolates either alone or in combination with other biofertilizers, such as potassium-solubilizing and nitrogen-fixing bacteria, to increase the growth of sugarcane plants under field conditions.

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