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Plant growth promoting activities of P solubilizing bacteria and their impact on disease resistance in groundnut, *Arachis hypogaea* L. against soil borne fungal pathogens

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Plant growth promoting (PGP) activities of soil bacteria directly help plants in taking up the nutrients, attuning the growth hormones and indirectly safeguard by inhibiting diverse groups of fungal pathogens. In this study, we explored the native P solubilizing bacteria (PSB) isolated from the acid soils (pH < 5.5) of Odisha for selection of efficient PGPR with antifungal potential. Five PSB strainswere checked for their P solubilization efficiencies with $Ca_3(PO_4)_2$,$AlPO_4$,$FePO_4$ and $Fe_3(PO_4)_2$. The bioconversion of P by all the five strains in the broth medium followed the order Ca-P > Fe(III)-P > Fe(II)-P > Al-P. The strains interestingly showed potential plant growthpromoting properties including indole acetic acid (IAA) and siderophore production in *in vitro* tests. These five strains also exhibited antifungal activities against fungal pathogens (*Pythium aphanidermatum*, *Fusarium oxysporum*, *Pythium debaryanum*, *Thanatephorus cucumeris* and *Aspergillus niger*) of groundnut. A field study was carried out with two of the above PSB strains [identified as *Bacillus amyloliquefaciens* (KT633845) and *Burkholderia cepacia* (KT717633)] with groundnut. Both the stains significantly influenced the plant growth (plant height, nodule no. and nodule dry weight) and pod yield. However, these two strains inoculated along with doses of inorganic phosphate (SSP, single super phosphate) resulted in significantly higher pod yield as well as residual soil P. Additionally; the prevalence of both seedling mortality and plant mortality due to collar rot and stem rot were found to be reduced significantly in the inoculated plots. The findings substantiate the growth promoting ability of the two P solubilizing strains, and thus qualifies to be used as biofertilizers either alone or as components of INM practices.

Keywords: Acid soils, Antifungal activity, Belly rot, Collar rot, Biofertilizer, Black mould, Fusarium wilt, Indole acetic acid production, Panama disease of banana, PGPR, Phosphate solubilization, Pod yield, Siderophore production, Stem rot, Water mould

Phosphorous is the most essential macronutrient; second only to nitrogen. However, unlike N, P availability is highly dependent on the type of soil reaction (pH) and no big atmospheric source is there to supplement the P requirement of crops. Again, compared to N and K, total phosphorous level of soils is low and usually one tenth to one fourth of N and one twelfth of K[1]. Phosphorus fixation is a major drawback in agricultural soil, where it forms compounds with calcium, aluminium and iron, making it unavailable for crop uptake[2]. Hence, P needs to be solubilized from fixed P into its available form; mediated by variety of microorganisms found in soil[3].

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Soil microorganisms termed as P solubilizing bacteria (PSB) possess the ability of solubilizing inorganic P and making the phosphate available for plant use, particularly in phosphorous deficient soils[4]. Although several genera of PSB occur in soil and in plant rhizosphere, the amount of phosphorous released by these bacteria is generally not sufficient to fulfill the requirements of growing plants[5]. Use of P solubilizing bacteria as biofertilizers or rather to say more appropriately as plant growth promoting rhizobacteria (PGPR) to maintain soil quality and health is a basic component towards sustainable agriculture[6].

In this context, here, we carried out experiment with groundnut (*Arachis hypogaea* L.) as the test crop. *A. hypogaea* L. is a major oil seed crop of India. However, diseases caused by soil borne fungal

pathogens inflict major yield loss in groundnut[7]. Thus, it necessitates developing a strong economical disease management strategy within the reach of small and marginal farmers for boosting the productivity besides maintaining soil fertility. Hence, we screened native PSBs from acid soil zones (five districts) of Odisha, evaluated their plant growth promoting activities, and also studiedtheir biocontrol activity against soil born fungal pathogens of groundnut.

Materials and Methods

Evaluation of P solubilization efficiency of PSB

Soil samples collected from five districts of Odisha i.e., Balasore, Cuttack, Khordha, Keonjhar and Mayurbhanj (Table 1) were stored at 4ºC for microbial analysis. For isolation of PSB, serial soil dilutions were spread plated on National Botanical Research Institute's phosphate (NBRIP) growth medium[8] with insoluble tricalcium phosphate (TCP) and the colonies were counted (cfu g^{-1} dry wt. of soil). The PSBs were spotted on NBRIP Agar plates for determination of P solubilization efficiency. The plates were incubated at 30±2ºC for 48 h and the diameter of the colony as well as the halo zone was measured. Psolubilizing index (PSI) and P solubilization efficiency (PE %) were calculated as:

$$PSI = Z / C, PSI (\%) = (Z-C) / C \times 100$$

where Z, halo zone diameter and C, colony diameter.

Screened PSBs were further examined in NBRIP liquid medium with inorganic phosphates [$Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and $Fe_3(PO_4)_2$]. The flasks were incubated at 30±2°C for 48 h and the contents were centrifuged at 10000 rpm for 30 min. Soluble free phosphate in culture supernatant was estimated from the absorbance values obtained using the calibration curve with KH_2PO_4 at 660 nm for each strain[9,10]. The potent strains were incubated till 8th day for estimation of soluble P content and P solubilization efficiency by using the above mentioned method.

Study of plant growth promoting activities

Antifungal activity

Dual culture method was employed to screen the antagonistic effect of bacterial strains against fungal pathogens. Each bacterial strain was grown on Nutrient Broth. The 24 h bacterial cultures were transferred by a single streak on two rows at 2.0 cm apart in the Nutrient Agar plate and incubated at 30°C for 24 h[11]. Seven days old fungal pathogens collected using sterilized cork borer at peripheral colony to get mycelial agar plug was transferred to the space in between the bacterial streaks. Fungal isolates grown on NA plates without bacterial cultures were taken as control. Percentage inhibition of colony growth (PICG) was expressed as percent[12,13].

Production of indole acetic acid

PSBs were assayed for their ability to produce indole acetic acid (IAA) by the colorimetric method using Salkowski's reagent (Ferric chloride–Perchloric acid)[14]. The strains were inoculated in the minimal medium (g L^{-1}): KH_2PO_4- 1.36; Na_2HPO_4- 2.13; $MgSO_4.7H_2O$- 0.2, glucose- 10; L-tryptophan- 1.0; Yeast extract- 0.1)[15] at 30ºC on a rotary shaker (140 rev min^{-1}) and incubated for 48 h. Culture supernatant was analyzed for IAA production using Salkowski's reagent wherein development of pink colour was assayed with a spectrophotometer (Visible Spectrophotometer- CL 320) at 530 nm.

Production of siderophore

Siderophore production was detected by observing orange halos around bacterial growth on CAS agar plates[16] after 72 h. Nutrient Agar medium was prepared and to it chromazurol, ferric chloride, cetyl trimethyl ammonium bromide were added in appropriate proportion. Orange halo zones were measured after 72 h of incubation.

Germinating seed bioassay

For seedling bioassay, the five bacterial strains were grown in Nutrient Agar at 30ºC for 48 h. The inoculants for treating seeds were prepared by suspending cells from agar plates in a Nutrient Broth (NB) at 28ºC and 120 rpm for 72 h. The cultures were grown to achieve optical density of 0.9 (10^8 to 10^9 CFU mL^{-1}) at 620 nm wavelength. The broths were then centrifuged at 12000 rpm, the pellets obtained were washed thrice with 0.1 M phosphate buffer (pH 7.0) and then dissolved in phosphate buffer (cell count 3.0×10^8 CFU mL^{-1}). Groundnut (*A. hypogaea* L cv. Tag 24) seeds were brought from Department of Seed Science and Technology, College of Agriculture, OUAT, Bhubaneswar. Seeds were surface sterilized with 1 % NaOCl for 6 min and then repeatedly (6 times) rinsed with sterile distilled water for 15-20 min. Sterilized seeds were then placed in glass Petridish and soaked in phosphate buffer for 2 h[17]. For each seed, 5 mL of phosphate buffer was used. The inoculated and uninoculated seeds were sown separately in sterile sand. The length of roots and seedlings were measured after 5 days and expressed in cm and compared with the uninoculated.

Field experimentation

The field trial was conducted with groundnut (*A. hypogaea* L cv. Tag 24) in the Agronomy Research Field, OUAT, Bhubaneswar located at 25º15'N latitude and 80º52'E longitude and altitude of 30 m above mean sea level during rabi season, 2017-18. The experimental site experienced a high temperature in summer and mild temperature during winter season and a medium rainfall.

Application of fertilizers and biofertilizers

Fertilizer application

The fertilizer sources N (20 kg ha^{-1}) as urea and K_2O (40 kg ha^{-1}) as muriate of potash (MOP) were given to all the treatments while P_2O_5 @40 kg $ha^{-1}$was applied as single super phosphate (SSP) following the treatment schedule.

Bioinoculation

P solubilizing bacteria (*Bacillus amyloliquefaciens* strain CTC12 and *Burkholderia cepacia* strain KHD08) as bioinoculants were applied as carrier based biofertilizer and seed inoculation.

Formulation of carrier based biofertilizer

The two strains were grown in nutrient broth at 28ºC and 120 rpm for 72 h. The cultures were grown to achieve optical density of 0.9 (10^8 to 10^9 CFU mL^{-1}) at 620 nm wavelength. The broths were mixed with sterilized lignite, $CaCO_3$ and gum acacia for formulation of biofertilizers. The solid carrier based biofertilizer formulation (1 kg) includes lignite 640 g, $CaCO_3$ 160 g, gum acacia 20 g and cultured broth 200 mL. Biofertilizers were applied at the time of sowing as per the treatment requirement.

Seed inoculation

The two selected strains were grown in nutrient broth at 28ºC and 120 rpm for 72 h. The cultures were grown to achieve optical density of 0.9 (10^8 to 10^9 CFU mL^{-1}) at 620 nm wavelength. The broths were centrifuged at 12000 rpm, the pellets obtained were washed thrice with 0.1 M phosphate buffer (pH 7.0) and then dissolved in phosphate buffer (cell count 3.0×10^8 CFU mL^{-1}). *A. hypogaea* L cv. Tag 24 seeds were surface sterilized with 1% NaOCl for 6 min and then repeatedly (6 times) rinsed with sterile distilled water for 1520 min. Sterilized seeds were soaked in phosphate buffer for 2 h[17]. The coated seeds were sown in field as per the treatment schedule.

Experimental details and treatment description

The field trial was conducted with *A. hypogaea* L cv. Tag 24 at the experimental plots (net plot area 5×2.2 m) randomized (RBD) comprising nine (9) treatments (T_1, control; T_2, *Bacillus amyloliquefaciens* CTC12; T_3*Burkholderia cepacia* KHD08; T_4, 100% P as SSP; T_5, 75% P as SSP; T_6, 75% P as SSP+CTC12; T_7, 75% P as SSP+KHD08; T_8, 100% P as SSP+CTC12; and T_9, 100% P as SSP+KHD08) andthree (3) replications.

The two strains [*B. amyloliquefaciens* CTC12 (KT633845) and *B. cepacia* KHD08 (KT717633)] were compared sole and in combination with 100% P as SSP. The field soil was loamy [sand 79.5%, silt 14.75% and clay 5.75%] having pH 4.18, organic carbon 0.38 %, available N 59.77 mg kg^{-1}, available P 5.32 mg kg^{-1} and available K_2O 42.05 mg kg^{-1}. Inorganic P fractions of the soil include Ca-P (40.25 mg kg^{-1}) and nonoccluded Al-P and Fe-P (400.69 mg kg^{-1}), respectively.

Soil analysis

Soil pH was determined in 1: 2.5 soil: water ratio using pH meter (Systronics Digital pH meter 335)[18]. The organic carbon content of soil samples were determined by Walkley and Black's rapid titration method[19]. Available phosphorous in the soil at harvest was determined by Bray's 1 method[19,20].

Disease incidence

Under field condition, the incidence of soil borne fungal diseases often caused by species of fungi *viz*., *Pythium*, *Thanatephorus* and *Fusarium*; collar rot by *Aspergillus niger* and stem rot by *Sclerotium rolfsii* was monitored. No artificial inoculation was done in the experimental plots. Seedling disease in groundnut is favoured by cool and wet soils which slow down the seed germination and seedling growth. Collar rot usually appears between 20-30 days of crop growth. Seedling mortality was monitored at 30 DAS. Seedlings infected with *A. niger* were identified by observing the black spores of *A. niger* and softening of the tissues at the collar region. The infected seedlings dried up after a couple of days. The number of seedlings dying was counted and mortality per cent was calculated in each plot. Similarly, incidence of stem rot usually starts from 45 DAS and it is typically identified by the presence of white mycelial growth of the organism at the stem region and it also attacks the developing pods. The number of plants infected with *S. rolfsii* was counted in each plot at 60 DAS and the mortality of the plants was determined[17].

Yield attributes of groundnut

Due care and maintenance were followed till 110 days for growth of plants in the treated plots till

maturity and then harvested. The plants from different treatments were collected at the time of harvest of the crop, by moistening the rhizosphere, uprooting the plants without disturbing the roots with the help of spade. The entire root and adhered soils wereloosened in a bucket of water, saving the roots, which were further washed thoroughly and dried. Average height (ground level to tip of the leaf in cm) of 10 plants measured from each plot was recorded as Plant height. Similarly, nodule no. and nodule dry weight were averaged over ten (10) groundnut plants uprooted from each plot at 45 DAS. At harvest, the plants were measured for total no. of pods (per plant) and pod yield expressed as t ha^{-1}.

Statistical analysis

Statistical analysis was performed by the software R version 3.2.2 and were tested with Duncan's new multiple range test at 5% critical range using the package "agricolae". The values are the means of three replicates.

Results

Five PSB strains isolated from acidic soils of five districts *viz*., Balasore, Cuttack, Khordha, Keonjhar, and Mayurbhanj of Odisha, were tested for their PSB solubilization efficiencies and plant growth promoting traits. Five isolates BLS18, CTC12, KHD08, KJR03, K1 were identified respectively as *Bacillus cereus* (KT582541), *Bacillus amyloliquefaciens* (KT633845), *Burkholderia cepacia* (KT717633), *Burkholderia cepacia* (KT717634), *Burkholderia cepacia* (KM030037) through with molecular (16S ribosomal RNA (rRNA) analysis (data not shown).

P solubilization efficiencies of the five PSB strains on NBRIP agar plates

These P solubilizing bacteria were screened for their P solubilizing efficiency, biocontrol and crop growth promotion effects. Among five PSBs isolated, BLS18 and CTC12 were Gram-positive rods and the rest three were Gram negative rods. BLS18 and CTC12 showed maximum clearing zone (31 mm) and KJR03 showed the minimum (20 mm) (Table 1).

Evaluation of P solubilization efficiency of the five PSB strains

Phosphorous solubilization efficiency (PE) of the five PSB strains were characterized and presented in Fig. (A) and (B). All the strains solubilized Ca-P efficiently followed by Fe(III)-P till 192 h of incubation. At 48 h incubation strain CTC12 recorded

Table 1 — P solubilizing bacterial (PSB) strains from five districts of Odisha, India with P solubilizing efficiencies

Districts	Blocks	Village	Latitude (North)	Longitude (East)	PSB strains	Diameter of clearing zone (mm)'A'	Diameter of bacterial colony growth (mm) 'B'	P solubilization Index (SI)	P solubilization Efficiency PE (%)
Balasore	Nilagiri	Sarupala	$N21^{0}30.666'$	$E86^{0}47.575'$	BLS18	31	6	5.17	416.67
Cuttack	Athagarh	Echhapur	$N20^{0}31.105'$	$E85^{0}33.624'$	CTC12	31	6	5.17	416.67
Khordha	Begunia	Balarampur	$N20^{0}11.881'$	$E85^{0}27.827'$	KHD08	21	6	3.50	250.00
Keonjhar	Bansapal	Rangadihi	$N21^{0}36.787'$	$E85^{0}27.219'$	KJR03	20	6	3.33	233.33
Mayurbhanj	Muruda	Chitrada	$21^{0}50.487'$	$86^{0}56.986'$	K1	26	6	4.33	333.33

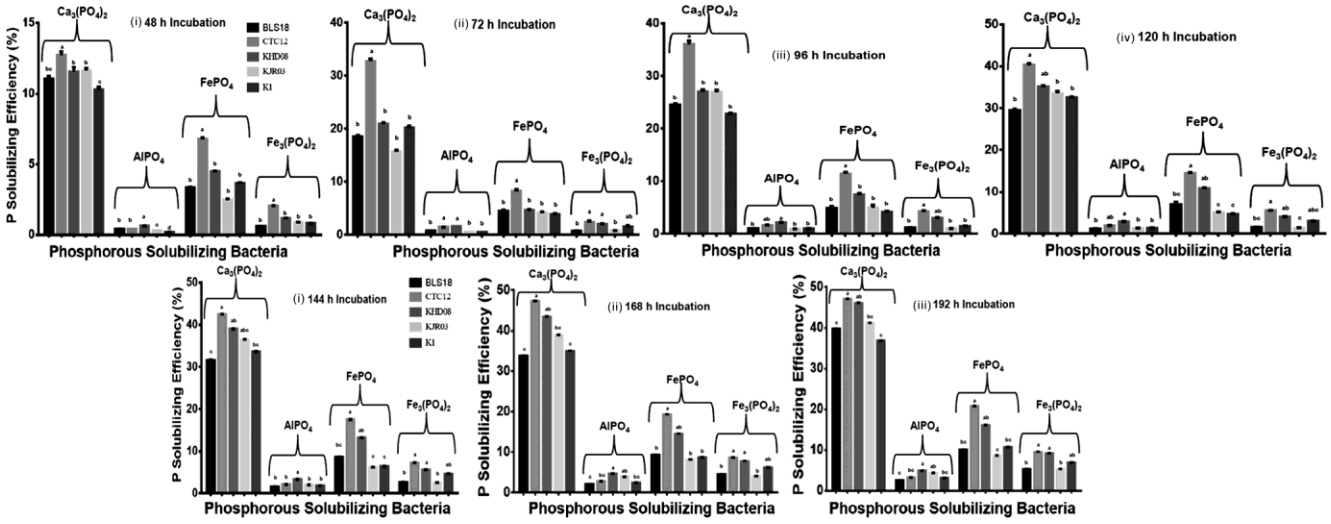

Fig. 1 — P solubilizing efficiency of five (BLS18, CTC12, KHD08, KJR03 and K1) P solubilizing bacterial (PSB) strains in NBRIP medium with inorganic P sources [$Ca_3(PO_4)_2$,$AlPO_4$,$FePO_4$ and $Fe_3(PO_4)_2$] at (A) (i) to (iv) 48, 72, 96 and 120 h; and (B) (i) to (iii) 144, 168 and 192 h of incubation, respectively

maximum PE in the medium supplemented respectively with $Ca_3(PO_4)_2$ (12.73%), $FePO_4$ (6.79%) and $Fe_3(PO_4)_2$ (2.05%) but strain KHD08 recorded maximum PE with $AlPO_4$ (0.64%).

At 48 h of incubation strain K1 measured lowest PE in mediums supplemented with Ca-P (10.28%) and Al-P (0.19%) and KJR03 in Fe(III)-P (2.51%) and BLS18 in medium supplemented with Fe(II)-P (0.61%). The PE of all the strains increased with increase in the incubation duration and continued till 192 h (8^{th} day). Results further revealed that, after 8^{th} day of incubation, strain K1 recorded least P solubilization efficiency in Ca-P (36.78%) while KJR03 in Fe(III)-P (8.55%) and BLS18 in Al-P (2.50%) and Fe (II)-P(5.20%).

Study of plant growth promoting activities

The strains were further tested for their efficacy as biocontrol agent against soil borne fungal pathogens and production of indole acetic acid as well as siderophore.

Antifungal activity

Antifungal activity of the five strains incubated along with soil borne fungal pathogens viz; *Pythium aphanidermatum*, *Fusarium oxysporum*, *Pythium debaryanum*, *Thanatephorus cucumeris*and *Aspergillus niger*were recorded [Table 2 and Fig. 2 (A-C)]. The growth of *P.aphanidermatum* inhibited by 64.29% with strains CTC12, KHD08 but the strain K1 could inhibit up to 78.57%. It was further observed that KHD08 and K1 could inhibit the colony growth of *F. oxysporum* up to 64.29% while 78.57% inhibition was due to the effect of BLS18, CTC12 and KJR03. Similarly, when *P. debaryanum* was inoculated with these PSB strains, it showed 80% growth inhibition with KHD08, KJR03 and K1 and only 60% with BLS18 and CTC12. The colony growth of fungal pathogen *T. cucumeris* was inhibited 80% by CTC12 whereas it was only 30% with rest of the strains. Similarly, CTC12 inhibited 76% colony growth of *A. niger* which was only 60% with KHD08, KJR03 and K1.

Production of indole acetic acid and siderophore

All the strains were tested for production of indole acetic acid (IAA) (Table 3). Data revealed that CTC12, KHD08, KJR03 and K1 could produce 8.55,

Table 2 — Antifungal effect of the five PSB strains

PSB strains	Colony diameter (mm) of fungal pathogens				
	PA	FO	PD	TC	AN
BLS18	13^a (53.57)	6^b(78.57)	10^a (66.67)	21^a (30.00)	11^a (56.00)
CTC12	10^b (64.29)	6^b(78.57)	10^a (66.67)	6^b (80.00)	6^b (76.00)
KHD08	10^b (64.29)	10^a (64.29)	6^b (80.00)	21^a (30.00)	10^a (60.00)
KJR03	6^c (78.57)	6^b (78.57)	6^b (80.00)	21^a(30.00)	10^a (60.00)
K1	10^b (64.29)	10^a (64.29)	6^b (80.00)	21^a (30.00)	10^a (60.00)

Tested by Duncan's Multiple Range Test with 5% critical range. Values with similar letters are not significantly different. [*Colony diameter (mm) of control plates (absence of the PSB): (PA) *Pythium aphanidermatum* 28 mm; (FO) *Fusarium oxysporum* 28 mm; (PD) *Pythium debaryanum* 30 mm; (TC) *Thanatephorus cucumeris* (30 mm; and (AN) *Aspergillus niger* 25 mm. Figures in parenthesis indicate the % growth inhibition of fungi as influenced by PSBs]

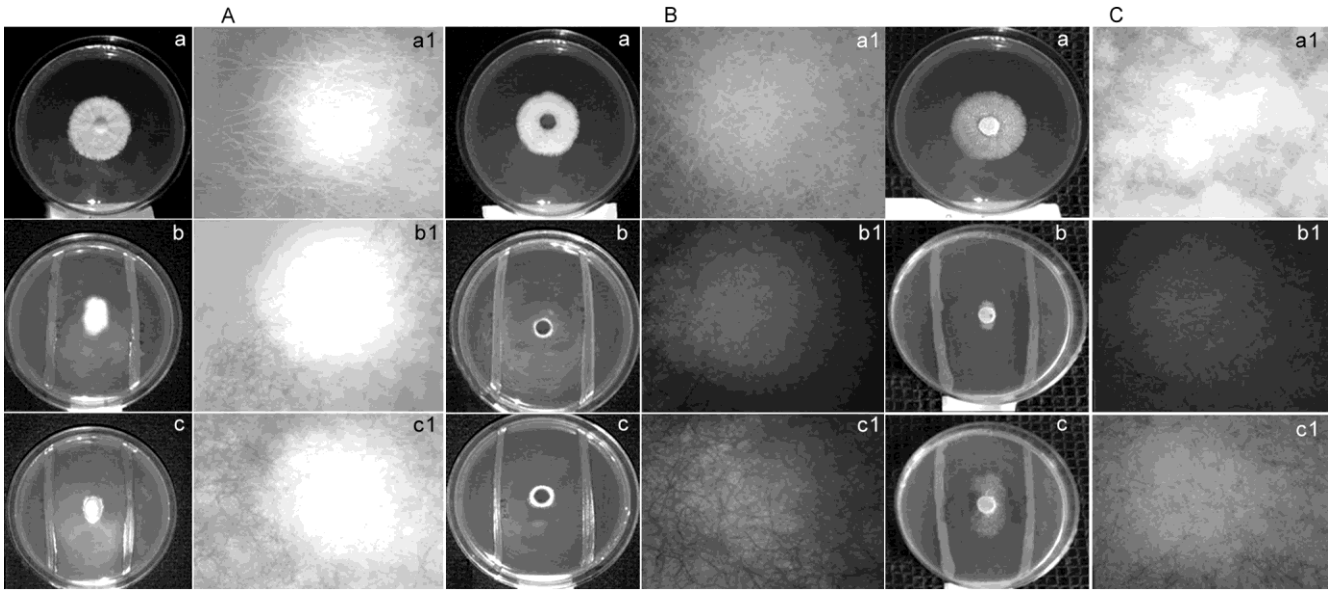

Fig. 2 — Antifungal activity of CTC12 and KHD08 strains against phytopathogen (A) *Pythium debaryanum*; (B) *Fusarium oxysporum*; and (C) *Thanatephorus cucumeris.* [(a) Fungus growth (Control), (a1) Stereo microscopic structure under 5X (Control); (b & c) Coinoculation of respective fungal pathogen with CTC12 and KHD08 strains, respectively; and (b1 & c1) Stereo microscopic structure of coinoculated respective pathogenwith CTC12 and KHD08 strains under 5X]

Table 3 — Production of indole acetic acid and germinating seed bioassay

PSB strains	IAA (µg mL^{-1})	Siderophore (mm)	Root length of seedling $plant^{-1}$ (cm)	Seedling length $plant^{-1}$ (cm)
Control	-	-	4.9	10.6
BLS18	-	-	12.3	17.8
CTC12	8.55	20.0	12.9	20.9
KHD08	8.33	14.0	12.9	20.4
KJR03	8.40	14.0	13.0	19.1
K1	7.32	11.0	11.6	17.6

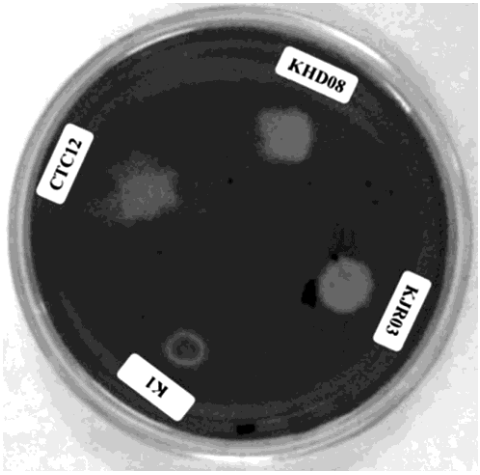

Fig. 3— Production of siderophore by four (CTC12, KHD08, KJR03 and K1) PSB strains

8.33, 8.40, 7.32 µg mL^{-1} IAA, respectively in broth cultures supplemented with tryptophan. However, strain BLS18 failed to produce IAA in the same broth. Four (CTC12, KHD08, KJR03 and K1) of the PSB strains showed siderophore production (Table 3 and Fig. 3). CTC12 recorded highest zone diameter (20 mm) followed by KHD08 and KJR03.

Germinating seed bioassay

Five PSB strains (BLS18, CTC12, KHD08, KJR03 and K1) were studied for germinating seed bioassay (Table 3 and Fig. 4) for enhancement of root growth and seedling length in groundnut *in vitro*. Maximum seedling length (20.9 cm) and root length (12.9 cm) recorded when strain CTC12 inoculated with groundnut seeds followed by KHD08. The uninoculated control recorded the minimum seedling length (10.6 cm) and root length (4.9 cm).

Field study

The field study was conducted with two of the strains i.e. *Bacillus amyloliquefaciens* CTC12 (KT633845) and *Burkholderia cepacia* KHD08 (KT717633)], and were compared sole and in combination with single super phosphate (SSP).

Table 4 — Effect of PSB on incidence of seedling, collar rot and stem rot diseases in groundnut

Treatments	Seedling mortality (%) due to collar rot and other seedling diseases	Plant mortality (%) due to stem rot
Control	6.5±0.436[a]	8.0±0.200[a]
B amyloliquefaciens strain CTC12	3.2±0.173[b]	3.7±0.346[b]
B cepacia strain KHD08	3.5±0.100[b]	3.5±0.173[b]
100% P as SSP	6.8±0.346[a]	7.8±0.264[a]
75% P as SSP	7.1±0.300[a]	8.2±0.529[a]
75% P as SSP+CTC12	3.6±0.265[b]	3.8±0.200[b]
75% P as SSP+KHD08	3.6±0.360[b]	4.0±0.100[b]
100% P as SSP+CTC12	3.0±0.200[b]	3.7±0.200[b]
100% P as SSP+KHD08	3.4±0.173[b]	4.2±0.200[b]
CV (%)	34.276	36.601

[Tested by Duncan's Multiple Range Test with 5% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean(SEM). SSP, single super phosphate]

Fig. 4 — Germinating seed bioassay with groundnut cv. Tag 24

Incidence of seedling, collar rot and stem rot diseases

The incidence of both seedling diseases and collar rot in groundnut (*A. hypogaea* L cv. Tag 24) were monitored at 30 DAS while stem rot at 60 DAS under field conditions. In general, incidence of diseases *viz*., seedling diseases by species of fungi *viz*., *Pythium*, *Thanatephorus* and *Fusarium*; collar rot by *A. niger* and stem rot by *S. rolfsii* were below 10% in the uninoculated control (Table 4). However, inoculation of the PSBs, *viz*., CTC12 and KHD08 reduced the seedling mortality from 6.5% in the uninoculated control to 3.2 and 3.5%, respectively. Inoculation of CTC12 and KHD08 also reduced the incidence of stem rot severity and subsequently, the plant mortality from 8.0% in the uninoculated control to 3.7 and 3.5%, respectively.

Soil available P status

Residual soil available P was recorded at harvest of crops (Table 5). The strain *B. cepacia* KHD08 in

Table 5 — Effect of PSB on pod yield and soil P status

Treatments	Pod yield (t ha^{-1})	Total no. of pods	Soil available P (mg kg^{-1})
Control	1.689±0.301^{e}	17.4±0.721^{d}	6.04±0.056^{b}
B amyloliquefaciens strain CTC12	2.136±0.035^{cd}	20.8±0.624^{c}	6.50±0.251^{b}
B. cepacia strain KHD08	2.105±0.086^{d}	20.6±1.000^{c}	6.55±0.087^{b}
100% P as SSP	2.445±0.193^{abc}	24.8±0.872^{ab}	5.89±0.400^{b}
75% P as SSP	2.348±0.192^{bcd}	24.0±1.400^{b}	6.06±0.083^{b}
75% P as SSP+CTC12	2.638±0.219^{ab}	26.4±1.637^{ab}	8.68±0.223^{a}
75% P as SSP+KHD08	2.605±0.155^{ab}	26.0±1.732^{ab}	9.67±0.096^{a}
100% P as SSP+CTC12	2.745±0.126^{a}	27.0±2.307^{ab}	8.33±0.061^{a}
100% P as SSP+KHD08	2.739±0.203^{a}	27.4±2.163^{a}	8.46±0.031^{a}
CV (%)	7.553	7.352	12.158

[Tested by Duncan's Multiple Range Test with 5% critical range. Means represented by the same letter are not significantly different. Data given in above are average values of three replicates ± standard error of mean (SEM)]

combination with lower dose of SSP (75% P) recorded highest soil available phosphorous (9.67 mg kg^{-1}). However, no significant differences were measured among the four treated plots where two of the PSB were applied in integration with doses of SSP. Further, the plots treated with either PSB or SSP were also found at par with respect to soil available P. Un-inoculated and unfertilized i.e. control plot recorded lowest amount of soil available P (6.04 mg kg^{-1}) at harvest.

Total no. of pods and pod yield

The inoculation of PSB along with SSP significantly influenced pod yield and total no. of pods (Table 5). PSBs (KT633845 and KT717633) inoculation showed significantly higher pod yield than the uninoculated control plot. Maximum pod yield (2.745 t ha^{-1}) was recorded in plots treated with CTC12 along with SSP (100% P) followed by the treatment (100% P as SSP + KHD08). However, maximum no. of total pods (27.4) were recorded inplots inoculated with KHD08 in combination with SSP (100% P). However, no significant differences were observed in terms of pod yield and total no. of pods among the plots treated with integrated application of PSBs (KT633845 and KT717633) and SSP (75 and 100 %) compared to plots applied with SSP (100% P) only.

Plant height, nodule no. and dry weight

Application of SSP along with inoculation of PSB positively influenced plant height of groundnut at harvest (Fig. 5A). Plant height values were found at par among the plots treated with PSB (CTC12 or KHD08) and doses of SSP (75 or 100% P). Maximum height (65.50 cm) was observed in plants inoculated with CTC12 and SSP (100% P). Further, no

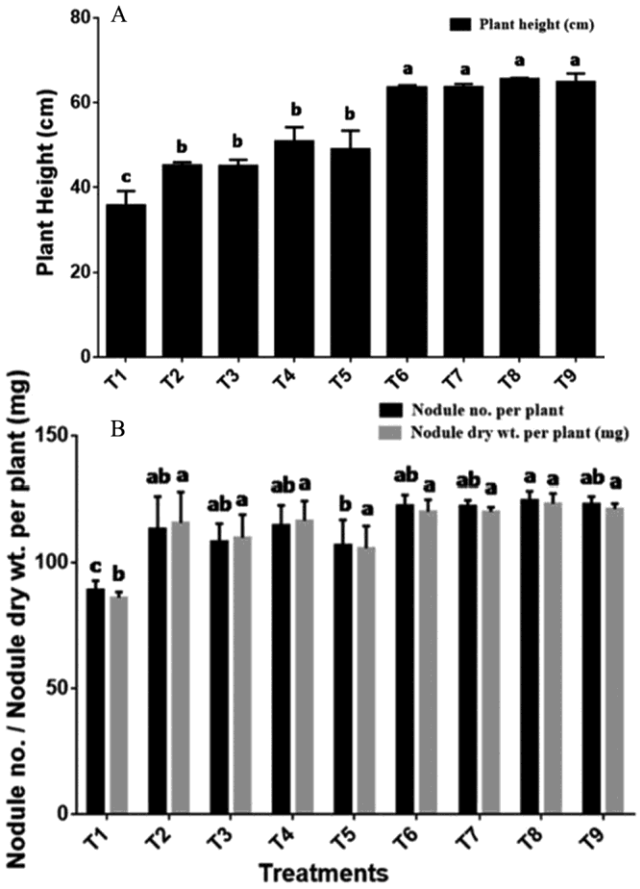

Fig. 5 — Effect of application of PSB strains (CTC12 and KHD08) on (A) Plant height; and(B) nodule dry wt. and nodule no. per plant in groundnut *cv*. Tag 24 [Treatments: T_1, control; T_2, *Bacillus amyloliquefaciens* CTC12; T_3, *Burkholderia cepacia* KHD08; T_4, 100% P as SSP; T_5, 75% P as SSP; T_6, 75% P as SSP+CTC12; T_7, 75% P as SSP+KHD08; T_8, 100% P as SSP+CTC12; and T_9, 100% P as SSP+KHD08]

significant differences with regard to height were found among the plants receiving either PSB or SSP. Plants grown in the unfertilized control plants recorded lowest height (35.80 cm). However, nodule parameters were found to be greatly influenced by availing phosphorous either through PSB or SSP or both (Fig. 5B). No significant differences were observed among all the treated plots with regard to nodule no. and dry weight collected at 45 days after sowing. Control plot recorded lowest nodule no. (89.10) and dry wt. (85.96 mg). Plants inoculated with CTC12 along with SSP (100% P) recorded highest nodule no. (124.70) and dry wt. (123.29 mg).

Discussion

Phosphorous, though one of the most indispensable elements of plant nutrition for effective growth, only

0.1% of total phosphorous present in the soil is available to plants for uptake[21]. The reason behind the low availability is phosphorous fixation (Al-P / Fe-P) in acid soils[22]. In India, about 30% of cultivated land falls under acidic soil category and in Odisha, acid soils occupy about 70 per cent of the total cultivated area (6.1 M ha)[23]. Poor nutrient status of acid soils is often compensated with erratic use of chemical fertilizers and by applying lime which deteriorates soil quality. Consequently, innovative efforts are being carried out by the researchers for manipulation of rhizosphere microflora to improve soil fertility and crop uptake.P solubilizing bacteria (PSBs) are capable of releasing phosphorus from the soil minerals; which often play a vital role in enhancing soil fertility[2] when P availability is low or P requirement is high.

In this context, we have isolated five P solubilizing bacteria from acid soils of Odisha and these were identified as *Bacillus cereus* BLS18 (KT582541), *Bacillus amyloliquefaciens* CTC12 (KT633845), *Burkholderia cepacia* KHD08 (KT717633), *Burkholderia cepacia* KJR03 (KT717634), *Burkholderia cepacia* K1 (KM030037). Out of these, *B. cereus* BLS18 and *B. amyloliquefaciens* CTC12 showed higher P solubilization zone on NBRIP agar plates with tricalcium phosphate. Since these organisms have been isolated from acid soils and in acidic agricultural soil, the most common insoluble P forms are variscite ($AlPO_4 \cdot 2H_2O$) and strengite ($FePO_4 \cdot 2H_2O$) both being stable minerals[24] and hard to dissolve. Hence, keeping this fact in view, we have tested the P solubilizing efficiencies in liquid mediums with $Ca_3(PO_4)_2$,$AlPO_4$,$FePO_4$ and $Fe_3(PO_4)_2$as insoluble P sources. The bioconversion of P by all the five strains in the broth medium followed the order Ca-P >Fe(III)-P > Fe(II)-P > Al-P. Most of the previous researchers had described PSB according to their solubilization index with tricalcium phosphate, which proved to be impractical in case of acidic soils[2,22]. *B. cepacia* KHD08 when tested with Ca-P in NBRIP agar plate recorded lower solubilizing zone, whereas the same organism recorded highest solubilizing efficiency when Ca-P was replaced with Al-P as P source. Moreover, *B. cepacia* KHD08 was found to be the second most efficient bacteria after *B. amyloliquefaciens* CTC12 in terms of P solubilization efficiency (with Ca-P, Fe(III)-P and Fe(II)-P.

All these organisms were further assessed for some of their plant growth promoting traits including biocontrol activities. For any bioinoculant besides P solubilization, antifungal effect and production of phytohormones are also useful traits. When these five PSB strains were tested for their antifungal activity against soil borne fungal pathogens (*Pythium aphanidermatum*, *Fusarium oxysporum*, *Pythium debaryanum*, *Thanatephorus cucumeris* and *Aspergillus niger*), they were found to successfully inhibit growth of fungi Antifungal activity in *Paenibacillus polymyxa*[25] and *Pseudomonas fluorescens*[26] have been reported. Out of the five strains, CTC12 showed highest antifungal activity against *Thanatephorus cucumeris*. Furthermore, all these strains except BLS18 could produce indole acetic acid and siderophore. Maximum IAA was produced by CTC12. Production of siderophore may have been involved in inhibiting pathogens in the rhizosphere and thus promoting plant growth[27]. Our observations corroborate the findings of previous researchers[28]. PGPR enhance the plant growth by several mechanisms, such as phosphate solubilization, nitrogen fixation, hormone production and by controlling phytopathogens[29,30]. In the present investigation, all the four (CTC12, KHD08, KJR03 and K1) strains except BLS18 when inoculated to groundnut showed similar effects *viz*., enhanced seedling length, root length, antifungal activity, release of IAA and siderophore.

Plant growth promotion is a collective term, often used to describe the activities of beneficial soil microflora. This beneficial soil microflora predominantly bacteria should be capable of colonizing the rhizosphere leading to the stimulation of plant growth, mobilization of nutrients and protection of plants from soil-borne phytopathogens[31,32]. However, for plants to acquire the beneficial effects from PGPR, as previously mentioned, it is crucial for PGPR to colonize the plant root system. Therefore, a field study was conducted with two strains *B. amyloliquefaciens* CTC12 and *B. cepacia* KHD08 and inorganic P fertilizer i.e. SSP to document the effects. The treatment variations were mainly done with P sources i.e. P fertilizer (SSP) and P solubilizing bacteria while N and K were common to all.

In the field study plants were found to be healthy due to fewer disease incidences. Under normal growth conditions mortality of the plants due to fungal disease was well below 10% in uninoculated control

plots. However, inoculation of *B. amyloliquefaciens* CTC12 and *B. cepacia* KHD08 further reduced the incidence of fungal diseases and thus seedling mortality. Inoculation of P solubilizing bacteria showed strong inhibition to *S. rolfsii* and reduced the incidence of stem rot severity. Earlier reports also suggest that strains of *Pseudomonas* produce some antifungal metabolites that can suppress soil-borne fungal pathogens[17,26,33,34].

Combined inoculation of PSB and SSP found to be effective in making higher bio-available P in the residual soil. The PSB treatment showed its effectiveness in solubilizing P from its insoluble forms. However, PSB inoculation with 75% P as SSP recorded maximum soil available P in comparison to plots inoculated with PSB and higher dose of SSP (100% P). *B. cepacia* KHD08 inoculation along with SSP (75% P) most effectively solubilized P in field condition. As part of acid soil characteristic soluble phosphorous forms are mainly fixed by aluminum and iron-free oxides and hydroxides[35] and these limits phosphorous availability in crops. Although a substantial reserve of phosphorous is present in soil, a large proportion is unavailable to plants and a considerable part of the fertilizer phosphates applied to soil is immobilized after application[12]. Plots treated with full doses of SSP (100% P), may have suffered higher immobilization with lower microbial activity limiting crop P uptake[2].

Seed bacterization of these two strains (CTC12 and KHD08) increased the plant height, nodulation, no. of pods and pod yield significantly over control. Nodulation is greatly influenced by application of phosphorous as P is necessary to maintain the quality of fruits, vegetables and grain, even biological N_2 fixation is heavily dependent on P, as it is vital for nodule formation[36]. The significant increase in plant height and pod yield in the PSB and SSP treated plots could be attributed to higher P uptake by the plants and also might be due to secretion of phytohormone by the two P solubilizing bacteria. Phosphate solubilization has also been reported to enhance crop yield in various field conditions[37,38].

Although use of chemical fertilizer has played a significant role in the green revolution, but its injudicious use has affected the soil biological health and crop quality gradually. Soil biological health, particularly pertains to the rhizosphere microflora, represent a wide variety of soil bacteria and actinomycetes. When they are grown in association with a host plant, it not only results in growth stimulation of their host but also enhance aerobic N fixation, organic matter decomposition and nutrient mineralization processes. Thereby it assists in releasing inorganic plant nutrients such as N, P and S to soil solution. This beneficial rhizosphere microflora, are more appropriately termed as Plant growth promoting rhizobacteria (PGPR). They either directly provide nutrients to the host plant, or indirectly by positively influencing root growth and morphology or by aiding other beneficial symbiotic relationships[39]. Further, many PGPR stimulate plant growth by aiding in control of pathogenic organisms and hence referred to as biocontrol agents[40].

Conclusion

The beneficial microflorae can be broadly categorized into groups according to their abilities either to mineralize nutrients or to inhibit soil borne pathogens or to release phytohormones, such as indole-3-acetic acid. These soil microorganisms are potential tools for sustainable agriculture and a new perspective for the future. Here, we screened native PSBs from acid soil zones of five districts of Odisha, evaluated their plant growth promoting activities, and also studied their biocontrol activity against soil born fungal pathogens of groundnut. The present investigation revealed two strains, *Bacillus amyloliquefaciens* CTC12 (KT633845) and *Burkholderia cepacia* KHD08 (KT717633) having potency to mobilize inorganic P fractions from sparingly soluble phosphate sources of acid soils for crop uptake. Inhibition of soil borne fungal pathogens, growth promoting effects on seedlings, release of indole acetic acid and siderophore could also serve as the indirect tools for improved growth and high yield in groundnut. In view of these beneficial properties, both the P solubilizing bacteria (PSBs) (KT633845 and KT717633) possess potential to be developed and formulated further as commercial biofertilizer and biocontrol agents for field application.

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