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Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna

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Summary

Five bacterial strains with phosphate-solubilizing ability and other plant growth promoting traits increased the plant biomass (20–40%) by paper towel method. Glasshouse and field experiments were conducted using two efficient strains *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35. Increase in plant biomass (dry weight) was 99% with EB 67 and 94% with CDB 35 under glasshouse conditions. Increase in plant biomass at 48 and 96 days after sowing was 66% and 50% with EB 67 and 51% and 18% with CDB 35 under field conditions. Seed treatment with EB 67 and CDB 35 increased the grain yield of field-grown maize by 85% and 64% compared to the uninoculated control. Population of EB 67 and CDB 35 were traced back from the rhizosphere of maize on buffered rock phosphate (RP) medium and both the strains survived up to 96 days after sowing.

Introduction

After nitrogen, phosphorus (P) is an essential mineral fertilizer for plant growth and development and is the world's second largest agricultural chemical. Soluble P is often the limiting mineral nutrient for biomass production in natural ecosystems as well. In Vertisols, the pH is above 7.0, and most of the mineral P is in the form of poorly soluble calcium mineral phosphates (CaP) due to their buffering capacity (Ae et al., 1991). Plants utilize fewer amounts of phosphatic fertilizers that are applied and the rest is rapidly converted into insoluble complexes in the soil (Vassilev and Vassileva, 2003). This leads to the need of frequent application of phosphate fertilizers, but its use on a regular basis has become a costly affair and also environmentally undesirable (Reddy et al., 2002).

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Natural phosphate rocks have been recognized as a valuable alternative for P fertilizers. In India, it is estimated that there are almost 40 million tons of phosphatic rock deposits and this material should provide a cheap source of phosphate fertilizer for crop production (Halder et al., 1990). Microorganisms that dissolve poorly soluble CaPs are termed as 'mineral phosphate solubilizers' (MPS) (Dobbelaere et al., 2003; Goldstein et al., 2003). Phosphate-solubilizing microorganisms (PSMs) convert these insoluble phosphates into soluble forms through the process of acidification, chelation, exchange reactions and production of gluconic acid (Rodriguez et al., 2004; Chung et al., 2005). This process not only compensates for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil (Rajan et al., 1996). An increase in P availability to plants through the inoculation of phosphate-solubilizing bacteria (PSB) has been reported in pot experiments and under field conditions (Pal, 1998; Zaida et al., 2003).

We report the establishment of PSB strains (*Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35), screened using rock phosphate (RP) buffered medium in the rhizosphere and their effect on plant growth and yield of maize in glasshouse and field conditions.

Materials and methods

Performance of PSB in paper towel method

Two hundred and seven bacteria were isolated from farm waste compost, rice straw compost, *Gliricidia* vermicompost and body surface and excreta of macrofauna (earthworms, centipedes, slugs, snails etc – naturally present in farm waste compost) and screened for different plant growth promoting traits (unpublished data). Five (EB 27, EB 67, EB 75, CDB 35 and BWB 21) of the 207 isolates with P-solubilizing ability and other plant growth traits were evaluated for growth of maize (Surabhi) using paper towel method according to International Seed Testing Association (ISTA, 1993). Maize seeds were surface sterilized with 1% sodium hypochlorite for 5 min and washed 5 times with sterilized distilled water. Seeds were coated with peat-based inoculum (10^8–10^9 colony forming units (CFU)) using 1% carboxymethylcellulose (CMC) as adhesive, dried in air and the cell count was 10^6–10^7 CFU per seed. After bacterization, 50 seeds were placed in each germination paper and were incubated in glasshouse. *Enterobacter asburiae* PSI3 with phosphate-solubilizing activity was used as reference strain (Gyaneshwar et al., 1999). Seeds treated only with peat served as control. Four replications for each treatment were maintained. After 10 days, seed vigor index (ISTA, 1993) and plant weight were recorded.

Preparation of peat-based formulation

Based on plant growth promotion by paper towel method, two bacterial strains, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35, were selected for plant growth studies and were prepared as peat-based inoculants. Neutralized peat (Biocare Technology Pvt. Ltd., Australia) (30 g) was packed in HDPE bags and sterilized by autoclaving at 121 °C for 20 min. Thirty milliliters of bacterial cells (10^8–10^9) harvested from mid-log phase culture grown in LB broth were injected aseptically into an individual pack and covered with a label at the injecting point. Inoculated packets were thoroughly kneaded to ensure uniform adsorption of the bacterial cells into the carrier material and incubated at 30 ± 1 °C for a period of 10 days. Later on, peat packets were preserved at room temperature (26 °C) or cold room (4 °C), based on further use.

Survival of *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in peat

Viability of both the PSB in the peat formulation was determined at 30-day intervals up to 180 days after inoculation (DAI). Ten grams of the peat-based inoculum was diluted in 90 mL water blanks and serially diluted. Appropriate dilutions were plated on RP buffered medium (Gyaneshwar et al., 1998). The plates were incubated at 30 ± 1 °C and the number of colonies after 96 h in case of RP media was expressed as log CFU g^{-1}. The experiment was repeated thrice with two replications in each treatment.

Intrinsic antibiotic resistance of EB 67 and CDB 35

Antibiotic resistant markers were developed for both the bacterial strains EB 67 and CDB 35 by testing their tolerance to intrinsic levels of different antibiotics (erythromycin, vancomycin, trimethoprim, nalidixic acid, gentamycin, streptomycin and rifamycin) on buffered RP medium. A marker was developed with combination of resistant levels of different antibiotics to trace back the bacteria from soil. Both the strains were subcul-

tured 10 times on buffered RP antibiotic plates to confirm their stability of antibiotic resistance.

Evaluation of PSB EB 67 and CDB 35 in glasshouse and field conditions

Maize cultivar (Surabhi) was used as host plant to evaluate the performance of PSB under glasshouse and field conditions. Nitrogen was applied in the form of urea at 80 kg N ha^{-1} in two-split dose (initially during sowing and during flowering stage). P was applied as 20 kg P ha^{-1} either as single superphosphate (SSP) or RP based on the treatments. Peat-based formulation of the bacteria at 150 g ha^{-1} was applied as seed coat with 1% CMC as adhesive. Viable cell count as determined by dilution plating was 10^6–10^7 CFU per seed. *E. asburiae* PSI3 (Gyaneshwar et al., 1999) with P-solubilizing ability in buffered conditions and *Bacillus coagulans* that did not perform well in buffered conditions (Gyaneshwar et al., 1998) were used as reference strains. The experiment had the following treatments: (i) uninoculated (control), (ii) N_{80}+SSP_{20}, (iii) N_{80}+RP_{20}, (iv) *E. asburiae* PSI3+N_{80}+RP_{20}, (v) *B. coagulans*+N_{80}+RP_{20}, (vi) *S. marcescens* EB 67+N_{80}+RP_{20} and (vii) *Pseudomonas* sp. CDB 35+N_{80}+RP_{20}.

Pot trials

The experiments were conducted in glasshouse in 21 cm diameter plastic pots using unsterile soil from BP2C field at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, that had pH 7.8, OC% 1.1, total N 1200 ppm, total P 484 ppm and available P 5 ppm. The treatments were arranged in a completely randomized block design with four replications and repeated twice. Harvesting of the plants was done after complete growth period of plants and plant growth measurements were recorded.

Field trials

The experiment was conducted in field BP2C at ICRISAT, during January–April 2004. Each treatment was raised in eight rows, each of 9 m length $\times$ 6 m width, with an intra- and inter-row spacing 10 and 60 cm. The treatments were arranged in a completely randomized block design with five replications. Subsamples of 10 plants from four replications were sacrificed and plant growth measurements were taken. Growth parameters were studied at 24, 48, 72 and 96 days after sowing (DAS). After harvesting, the dry matter yield of grain and stover was calculated. Shoot and grain of maize was analyzed for N and P (Okalebo et al., 1993).

Rhizosphere population dynamics of EB 67 and CDB 35

The rhizosphere soil from three treatments, uninoculated (control), *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 inoculated pots/plots, was collected at 24, 48, 72 and 96 DAS from glasshouse and field treatments. Population dynamics of introduced bacteria was studied using the intrinsic antibiotic marker. Roots were washed to remove adhering soil particles and appropriate dilutions were plated onto RP buffered antibiotic medium and incubated at 30 ± 1 °C and observed for number of CFU after 96 h.

Statistical analysis

Glasshouse and field experiments were arranged in completely randomized block design. The data were subjected to analysis of variance (ANOVA) using Genstat 6.1 statistical package (Lawes Agricultural Trust, Rothamsted, UK).

Results

Selection of efficient PSB

Five of the 207 isolates produced gluconic acid and solubilized RP (data not shown). When tested for growth of maize by paper towel method, all the strains enhanced root length (6–7%) and shoot length (4–5%). Plant biomass (wet weight) ranged between 15% and 43% more when compared to control (Table 1). Based on plant growth by paper towel method and RP-solubilizing ability, two PSB, S. *marcescens* EB 67 and *Pseudomonas* sp. CDB 35, were selected for plant growth studies as peat formulations. Population of EB 67 and CDB 35 in peat up to 180 DAI was log 6.2 and 7.3 CFU g^{-1} on buffered RP agar medium (Fig. 1).

Intrinsic antibiotic markers of EB 67 and CDB 35

Antibiotic resistance pattern of both the strains was determined and the marker developed was used to study the population densities in the rhizosphere. Both the strains showed tolerance of 50 μg mL^{-1} of erythromycin, vancomycin, trimethoprim and

Table 1. Growth of maize seedlings inoculated with phosphate-solubilizing bacteria (PSB) by paper towel method

Treatments	Root length (cm)	Plumule length (cm)	Plant weight (mg)	Germination (%)	Seed vigor index
Control (uninoculated)	21	17	1510	81	3080
E. asburaie PSI3	33*	22*	1957 (30)*	90*	5021*
E. cloacae EB 27	30*	18	1738 (15)*	89*	4235*
S. marcescens EB 67	34*	27*	2168 (43)*	94*	5787*
Serratia sp. EB 75	33*	22	1852 (23)*	89*	4892*
Pseudomonas sp. CDB 35	31*	25*	1940 (28)*	89*	4985*
Pseudomonas sp. BWB 21	32*	23	1796 (19)*	87*	4826*
Mean	30	22	1851	88	4689
LSD (5%)	2.8	5.1	56.1	3	676.7
CV (%)	5	13	2	2	8

Values in parentheses are percent increase over control.
LSD: least significant difference; CV: coefficient of variance.
*Means are significantly different from control at $P = 0.05$ when compared by LSD.

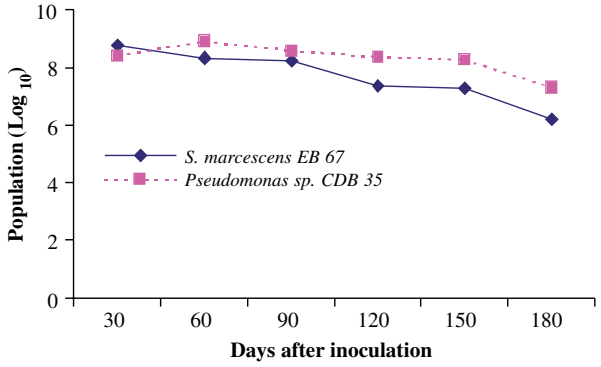

Figure 1. Survival of PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in peat-based formulation on buffered RP agar medium.

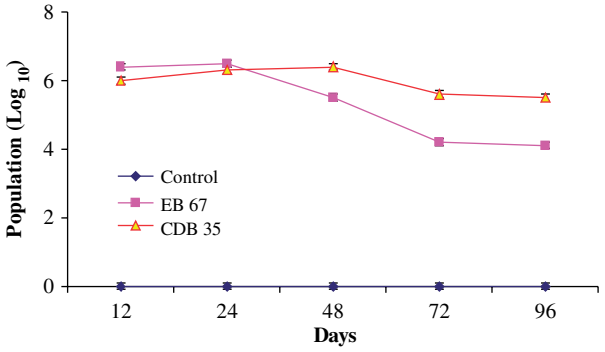

Figure 2. Survival of inoculated PSB, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35, in rhizosphere of maize under glasshouse conditions.

$2.5\,\mu g\,mL^{-1}$ of streptomycin and rifamycin when used in combination.

Rhizosphere colonization of EB 67 and CDB 35 in glasshouse and field conditions

Seed treatment with peat-based formulation of EB 67 and CDB 35 increased the seedling emergence, shoot length and dry biomass and grain yield of maize. Rhizosphere soil from the control (uninoculated) plots was also used to compare and study for any PSB present that could grow on buffered RP antibiotic medium. In glasshouse conditions, there was no growth of any PSB from control on buffered RP antibiotic plates. Population of EB 67 ranged from $6.4\,\log_{10} g^{-1}$ soil at 12 DAS to $4.1\,\log_{10} g^{-1}$ of soil at 96 DAS. Population of CDB 35 ranged from $6.0\,\log_{10} g^{-1}$ soil at 12 DAS to $5.5\,\log_{10} g^{-1}$ at 96 DAS (Fig. 2). Similar pattern was observed in field conditions, as there was no count of any PSB from control plots till 24 days. But 48 DAS from control plot, there was population of $3.8\,\log_{10} g^{-1}$ soil and it was $3.2\,\log_{10} g^{-1}$ at 72 and remained same upto 96 DAS. In inoculated plots, the population of EB 67 was $6.0\,\log_{10} g^{-1}$ soil 12 DAS to $4.7\,\log_{10} g^{-1}$ soil at 96 days and CDB 35 was from $6.9\,\log_{10} g^{-1}$ soil 12 DAS to $5.1\,\log_{10} g^{-1}$ soil at 96 days (Fig. 3).

Growth parameters of maize under glasshouse conditions in unsterilized soil

PSB, EB 67 and CDB 35, which performed better in vitro, were evaluated for growth of maize in glasshouse conditions. In addition to this, two reference strains *E. asburiae* PSI3 and *B. coagulans* (Gyaneshwar et al., 1998, 1999) were also used in the study (Table 2). Control treatments included uninoculated, SSP and RP applied separately. Under

glasshouse conditions, significant improvement in shoot length, leaf area and dry weight (except where SSP was applied) were observed because of bacterial inoculation. Maximum increase in dry weight was by EB 67 (99%) followed by CDB 35 (94%) and was similar to the reference strain *E. asburiae* (96%) used in the study. Both strains, EB 67 and CDB 35, and the reference strain *E. asburiae* showed 42–47% increase over *B. coagulans* (which showed P solubilization in unbuffered conditions) (Table 3).

Growth parameters of maize in field conditions

Seed treatment with peat-based formulation of two PSB, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 under field conditions improved the growth parameters and yield of maize. At vegetative stage (48 DAS), except the treatments where SSP was applied, all the other treatments showed significant difference in plant dry weight than the uninoculated control. Maximum increase in plant dry weight at 48 DAS was with *S. marcescens* EB 67 (66%) followed by *B. coagulans* (57%) and *Pseudomonas* sp. CDB 35 (51%) (Table 3). At flowering stage (96 DAS), increase in dry weight (50%) was significant and maximum with *S. marcescens* EB 67 and was similar to *E. asburiae* used in the study (Table 3). Throughout the study, *S. marcescens* EB 67 was better than both the reference strains, *B. coagulans* and *E. asburiae*, in most of the growth parameters observed. *Pseudomonas* sp. CDB 35 was better than *B. coagulans* but was similar or less than *E. asburiae* used in the study.

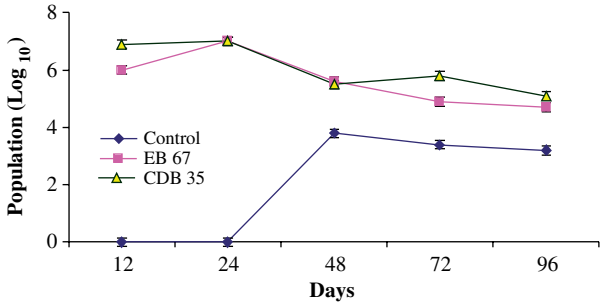

Figure 3. Survival of inoculated PSB, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35, in rhizosphere of maize under field conditions.

Seed treatment with *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the grain yield of field-grown maize by 85% and 64% compared to uninoculated control (Table 4). *S. marcescens* EB 67 showed 19% increase over *E. asburiae*, 33% over *B. coagulans*, 35% over urea+rockphosphate (N_{80}+RP_{20}) treatment and 43% over urea+SSP (N_{80}+SSP_{20}). *Pseudomonas* sp. CDB 35 showed 12% increase over *B. coagulans*, 14% over N_{80}+RP_{20}, and 19% over N_{80}+SSP_{20}. Increase in P uptake of shoot and grain of maize was 13 and 27 kg ha^{-1} with EB 67, 9 and 22 kg ha^{-1} with CDB 35 and 5 and 11 kg ha^{-1} with control (Table 5). N uptake in maize shoot was 54 kg ha^{-1} with EB 67 and 48 kg ha^{-1} with CDB 35, and 30 kg ha^{-1} with control.

Discussion

Bacteria belonging to genera *Bacillus*, *Pseudomonas*, *Serratia*, *Enterobacter*, etc., are reported to solubilize the insoluble phosphatic compounds and aid in plant growth (Rodriguez and Fraga, 1999). In the present investigation, *S. marcescens* EB 67 (isolated from slug body surface) and

Table 2. Plant growth of maize inoculated with phosphate-solubilizing bacteria (PSB) under glasshouse conditions

Treatments	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)
Control	130	578	44
N_{80}+RP_{20}	189 (45)*	788 (36)*	73 (65)*
N_{80}+SSP_{20}	192 (48)*	720 (25)	61 (38)*
E. asburiae PSI3+N_{80}+RP_{20}	204 (57)*	1092 (89)*	87 (96)*
B. coagulans+N_{80}+RP_{20}	186 (43)*	1082 (87)*	67 (52)*
S. marcescens EB 67+N_{80}+RP_{20}	221 (70)*	1148 (99)*	88 (99)*
Pseudomonas sp. CDB 35+N_{80}+RP_{20}	192 (48)*	1105 (91)*	86 (94)*
Mean	188	930	72
LSD (5%)	16.5	202.3	9.6
CV %	7	16	10

Values are means of four replications from two independent experiments and data calculated per plant. N = urea at 80 kg ha^{-1}; SSP = single superphosphate and rock phosphate (RP) at 20 kg ha^{-1}. Values in parentheses are percent increase over control.
LSD: least significant difference; CV: coefficient of variance.
*Means are significantly different from control at $P = 0.05$ when compared by LSD.

Table 3. Effect of PSB on growth parameters of maize under field conditions, 48 and 96 days after sowing (DAS)

Treatments	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)
At vegetative stage (48 DAS)			
Control	122	2927	35
N_{80}+RP_{20}	140 (15)	3959 (35)	53 (51)[a]
N_{80}+SSP_{20}	117	3142 (7)	37
E. asburiae PSI3+N_{80}+RP_{20}	142 (16)	3782 (29)	51 (46)*
B. coagulans+N_{80}+RP_{20}	149 (22)	3951 (35)	55 (57)*
S. marcescens EB 67+N_{80}+RP_{20}	143 (17)	4097 (40)*	58 (66)*
Pseudomonas sp. CDB 35+N_{80}+RP_{20}	143 (17)	3790 (29)	53 (51)*
Mean	137	3664	49
LSD (5%)	22.6	992.7	14.0
CV%	11	19	20
At flowering stage (96 DAS)			
Control	1604	157	127
N_{80}+ RP_{20}	1727	166 (6)	135 (5)
N_{80}+ SSP_{20}	1817 (7)	161 (3)	147 (14)
E. asburiae PSI3+N_{80}+RP_{20}	2073 (29)	181 (15)*	191 (50)*
B. coagulans+N_{80}+RP_{20}	1331	174 (11)	168 (31)
S. marcescens EB 67+N_{80}+RP_{20}	1910 (13)	174 (11)	192 (50)*
Pseudomonas sp. CDB 35+N_{80}+RP_{20}	2004 (24)	183 (17)*	152 (18)
Mean	1781	171	127
LSD (5%)	768.4	20.4	47.7
CV%	29	8	21

Values are means of four replications and data calculated per plant. Single superphosphate (SSP) and rock phosphate (RP) at 20 kg ha^{-1}.
Values in parentheses are percent increase over the control.
LSD: least significant difference; CV: coefficient of variance.
*Means are significantly different from control (uninoculated) at $P = 0.05$ when compared by LSD.

Table 4. Effect of PSB on growth and yield of maize under field conditions

Treatments	Grain weight (t ha^{-1})	Total dry matter (t ha^{-1})	Stover yield (t ha^{-1})
Control	2.8	7.8	5.0
N_{80}+RP_{20}	4.2 (50)	10.6 (35)	6.4 (14)
N_{80}+SSP_{20}	4.0 (42)	9.5 (22)	5.5 (11)
E. asburiae PSI3+N_{80}+RP_{20}	4.8 (71)*	11.6 (48)*	6.7 (21)
B. coagulans+N_{80}+RP_{20}	4.2 (52)	10.6 (36)	6.4 (14)
S. marcescens EB 67+N_{80}+RP_{20}	5.2 (85)*	12.2 (57)*	7.0 (27)*
Pseudomonas sp. CDB 35+N_{80}+RP_{20}	4.6 (64)*	11.4 (46)*	6.8 (23)*
Mean	4.3	10.5	6.3
LSD (5%)	1.4	2.88	1.81
CV%	26	21	22

Values are means of four replications and data calculated per plant.
Single superphosphate (SSP) and rock phosphate (RP) at 20 kg ha^{-1}.
Values in parentheses are percent increase over control.
*Means are significantly different from control (uninoculated) at $P = 0.05$ when compared by LSD. t = tons. LSD: least significant difference; CV: coefficient of variance.

Pseudomonas sp. CDB 35 (isolated from rice straw compost) solubilized P in buffered RP medium (data not shown). Both the strains enhanced root and shoot length, plant biomass and seed vigor index of maize by paper towel method (Table 1). Previous studies have shown that *Pseudomonas fluorescens* and *Bacillus* spp. enhanced germination and seedling vigor of different crop plants (Amruthesh et al., 2003). In addition to P solubilizing ability, both the strains, *S. marcescens* EB 67 and *Pseudomonas*

Table 5. Effect of PSB on nutrient uptake by maize shoot and grain in field experiment

Treatments	P uptake[a] (kg ha^{-1})	P uptake[b] (kg ha^{-1})	N uptake[a] (kg ha^{-1})
Control	5	11	30
N_{80}+ RP_{20}	7	19*	42
N_{80}+ SSP_{20}	6	18	35
E. asburiae PSI3+N_{80}+RP_{20}	9*	23*	50*
B. coagulans+N_{80}+RP_{20}	7	19*	42
S. marcescens EB 67+N_{80}+RP_{20}	13*	27*	54*
Pseudomonas sp. CDB 35+N_{80}+RP_{20}	9*	22*	48
Mean	8	20	43
LSD	3.9	6.8	18.7
CV (%)	37	26	33

LSD: least significant difference; CV: coefficient of variance.
[a]N and P uptake in shoot.
[b]P uptake in the grain tissue.
*Means are significantly different from control (uninoculated) at $P = 0.05$ when compared by LSD.

sp. CDB 35, showed various plant growth promoting traits (Hameeda et al., 2006). PSB are also reported to produce metabolites such as phytohormones, antibiotics and siderophores and aid in plant growth (Kloepper et al., 1989). *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 inoculated in peat had good shelf life up to 180 DAI. Population of CDB 35 was comparatively higher than EB 67 (Fig. 1). Their viability in peat up to 180 days further indicates that these strains can be tested and used with other low-cost carriers as biofertilizers. It further substantiates the work of Vidhyasekaran and Muthamilan (1995) of the possibility of developing microbial formulations with long shelf life without decreasing its effectiveness.

In this study, tracing back of inoculated strains, EB 67 and CDB 35, from maize rhizosphere on buffered RP antibiotic medium has shown a similar pattern of inocula survival under glasshouse and field conditions (Figs. 2 and 3). Determining the dynamics of root colonization by the introduced bacteria is essential for their effective use as it is critical in plant growth promotion and biological control (Kloepper and Beauchamp, 1992). There was no growth of any PSB from control (uninoculated) pots in this study. It may be possible that bacteria present in uninoculated (control) rhizosphere are unable to solubilize RP in buffered conditions. Tracking back the introduced *Rhizobium* strains in field trials using serological and antibiotic resistant markers has been reported (Schwinghamer and Dudman, 1973). *P. fluorescens* isolated from *Lupinus hispanicus* colonized pepper roots (Lucas García et al., 2003) and *Pseudomonas aeruginosa* isolated from phylloplane colonized the groundnut ecto- and endorhizosphere (Kishore et al., 2005). The relationship between source of isolation (compost and macrofauna) and colonization of rhizosphere environment need to be investigated further to know the factors involved in root colonization by both the strains of this study.

Plant biomass (dry matter) with *S. marcescens* EB 67 was better than the chemical control (SSP) and was on par with the reference strain *E. asburiae* used in this study (Table 3) (Gyaneshwar et al., 1999). EB 67 showed significant difference in grain and stover yield of maize when compared to control (uninoculated) and also where urea and RP were applied (Table 4). P uptake by shoot and grain was also more with the application of EB67 in our study (Table 5). Several studies have shown increase in plant growth and P uptake due to the addition of PSB. Kundu and Gaur (1984) reported increased P uptake and plant growth in various crops inoculated with PSMs. *Penicillium bilaii* effectively colonized roots of canola and increased the P uptake and crop in field conditions (Kucey and Leggett, 1989). Phosphate-solubilizing and phytohormone-producing *Azotobacter chroococcum* showed increase in grain and straw yield of wheat (Kumar et al., 2001). Stimulation in growth and yield of maize by inoculation with *Rhizobium leguminosarum* or *Penicillium rugulosum* in glasshouse or field conditions is reported (Chabot et al., 1996; Reyes et al., 2002). Phosphate-solubilizing *P. fluorescens* is reported to enhance growth, increase in yield, N and P contents in shoot and kernels of peanut (Dey et al., 2004).

Phosphorus, one of the most critical elements for the growth of plants, is not a renewable resource and its future use in agriculture will be impacted by declining availability and increased cost. In India, about 46% of soil is classified as P-deficient soil where available P concentration is not higher than 10 μM (Fried and Shapiro, 1961). P gets precipitated

with calcium, iron and aluminum and becomes unavailable to plants. Theoretical estimates have suggested that the accumulated P in soil is sufficient to sustain crop yields worldwide for about 100 years (Goldstein et al., 1993). Phosphatic biofertilizers in the form of microorganisms can help in increasing the availability of accumulated phosphates for plant growth by solubilization or by applying low-grade RP along with PSMs. In addition, the microorganisms involved in P solubilization as well as better scavenging the soluble P (P biofertilizers) can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of plant growth promoting substances and other trace elements. Previous studies involving plants inoculated with PSMs showed growth enhancement and increased P contents but large variations were found (Kucey et al., 1989). It depends on the survival and colonization of the inoculated PSMs in the rhizosphere or their inability to solubilize soil phosphates. Similarly, no soluble P was liberated in alkaline vertisols even when supplemented with nutrients (Gyaneshwar et al., 2002). This clearly suggests that reasons leading to the failure of screened PSMs in field trials should be elucidated and further development of PSMs should be carried out in buffered conditions taking into account the high buffering capacity of alkaline vertisols (Gyaneshwar et al., 1998). In this study, screening of bacteria using RP buffered medium helped in selecting novel bacteria that survived and solubilized P in maize rhizosphere and were efficient in enhancing plant growth and grain yield under glasshouse and field conditions. Use of these bacteria *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 either as seed inoculants or in compost preparation would increase plant productivity and replace use of chemical fertilizer P. To establish the effectiveness of a potential organism as a commercial inoculant, which has been shown to colonize the rhizosphere and solubilize P in the laboratory and glasshouse, must be tested under the full range conditions that will be experienced by the farmers. This research must be proactive and the field trials must be established across a broad range of soil and environmental conditions and must be conducted within the context of current and/or future farming practices.

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