Bacillus cereus and *B. circulans* – novel inoculants for crops

Millions of microbes inhabit the root zone of plants. Some plants are capable of exploiting these microorganisms to meet their hormonal and nutritional needs. To meet the demand for increasing productivity of economically important crop plants, several new plant–microbe associations have been reported[1–4]. It has been our endeavour to isolate new rhizobacteria, which may enhance the yield of crop plants. Among the variety of bacteria isolated from the rhizosphere of maize (*Zea mays*), isolates identified as *Bacillus cereus* and *B. circulans* merit attention, since they are being reported as plant growth promoting rhizobacteria (PGPRs).

A variety of nutrient media were used to isolate rhizobacteria, after subjecting the dilutions of the rhizosphere soil from maize plants to 100°C for 25–30 min. Isolates JKR and Bc were obtained from nutrient agar supplemented with glucose[5]. These strains were subjected to biochemical tests for identification. *Azotobacter chroococcum*, *Azospirillum brasilense* and *Pseudomonas fluorescens* strains were obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi and were maintained in Jensen medium, Okon medium and Kings medium respectively[6]. Establishment of *Bacillus cereus* and *B. circulans* in rhizotic zones was assessed at 60 days of crop growth by plating the serial dilutions in modified glucose–yeast extract–salts medium[7] containing kanamycin (25 µg/ml) and tetracycline (5 µg/ml) and incubated for 72 h at 30°C (± 1°C).

The strains were Gram-positive, spore-forming bacteria, able to grow at 45°C and in the presence of 7% NaCl. They grew well between pH 5.7 and 9.0, were able to produce acid but not gas from D-glucose and D-fructose, and utilized citrate and propionate as sole sources of carbon. Both the stains were catalase and oxidase-positive, efficiently hydrolysed starch, gelatin, casein, Tween 80 and esculin. On the basis of biochemical characteristics, Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ), Braunschweig, Germany designated the strain JKR as *Bacillus cereus* (DSM ID 04-715). The physiological characteristics of this strain point to *Bacillus thuringiensis*, but parasporal crystals were not detectable. Further, the profile of fatty acids of the strain DSM ID 04-715 is typical for the *B. cereus* group and the partial sequencing of the 16Sr DNA showed similarities with *B. cereus* strain (Tilak, unpublished). The strain Bc was identified as *B. circulans*.

In carrier(lignite)-based cultures, organisms were able to retain high million cells (10^8) population per gram of inoculum up to one year of storage at ambient temperature (30 ± 1°C). Effect of seed bacterization with carrier-based (1% w/w inoculum/seed) inocula of different PGPRs, namely *B. cereus* (DSM ID 04-715), *B. circulans* (Bc), *A. chroococcum* (Ac) and *A. brasilense* (SpCd) and *P. fluorescens* (S1-6) were field-tested on yield of maize (*Z. mays*) cv. GS 2, pigeonpea (*Cajanus cajan*) cv. P 921, wheat (*Triticum aestivum*) cv. HD 2285. For inoculation with PGPR strains, the seeds (100–120 g of seed per plot of 16 m^2) were first sterilized with 2% (w/v) chloramine-T for 30 min, followed by several washings in sterile distilled water. The sterilized seeds were then treated with the inoculum of the respective strains and sown immediately. The seeds received around 10^4 cells per gram of inoculum. The field soil was of sandy-loam type with pH 7.2 and poor in nitrogen, phosphorus and organic carbon content. Nitrogen, phosphorus and potassium (NPK) were applied at the rate of 80, 40 and 40 kg/ha as urea, single superphosphate and muriate of potash, respectively at the time of sowing. No fertilizer was applied when seed was inoculated with the bacterial cultures. At full maturity, the crops were harvested and grain yields were recorded. The experiments were laid out in a randomized block design with four replications. In all there were 24 plots with each crop and plot size was 4 m × 4 m (16 m^2). Maize and pigeonpea were grown during *kharif* (rainy) season and wheat was grown in *rabi* (winter) season for two successive years during 2002–04. Data were analysed statistically[8] and the level of significance was taken as 1%.

The first sign of the adaptability of these PGPR strains to the root system of maize, pigeonpea and wheat was the proliferation and clumping of bacteria around the root tips followed by entry into cortical cells of the root. In the case of maize, the PGPRs, particularly *Azospirillum* and both the species of *Bacillus* were able to enter into vascular tissues of the root system. Thus these PGPRs are endophytic in nature (Figure 1).

Beneficial effects of inoculation with different PGPRs on growth of the three crops were noticed. Seed inoculation enhanced grain yield (Table 1) compared to uninoculated control treatment. Biological activities are markedly enhanced by microbial interactions in the rhizosphere of crop plants. Such syntrophic associations are of ecological importance with implied agricultural significance. The PGPRs can influence plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of biocontrol agents against soil-borne phytopathogens[9]. Among the three crops tested, maize recorded maximum response to inoculation with *B. circulans* and *B. cereus*. The increase in grain yield of maize cv. GS 2 due to seed bacterization with *B. cereus* and *B. circulans* was 43.8 and 50.8% respectively over uninoculated control. That the organisms could establish in rhizotic zones of maize and presence of organism in the

Table 1. Response of maize, pigeonpea and wheat to inoculation with *Bacillus cereus* and *Bacillus circulans* (mean of two-year field trails)

	Grain yield (q/ha)		
Treatment	Maize (cv. GS 2)	Pigeonpea (cv. P 921)	Wheat (cv. HD 2285)
Uninoculated control	24.2	15.0	42
Azotobacter chroococcum	28.5 (17.8)	16.5 (10.0)	44 (4.8)
Azospirillum brasilense	27.9 (12.8)	17.2 (14.7)	48 (14.3)
Pseudomonas fluorescens	28.9 (19.4)	18.5 (23.3)	49 (16.7)
Bacillus cereus	34.8 (43.8)	20.8 (38.6)	58 (38.1)
Bacillus circulans	36.5 (50.8)	21.2 (41.3)	60 (42.9)
CD (P = 0.01)	4.5	3.8	6.3

Figures in parentheses denote percentage increase over uninoculated control.

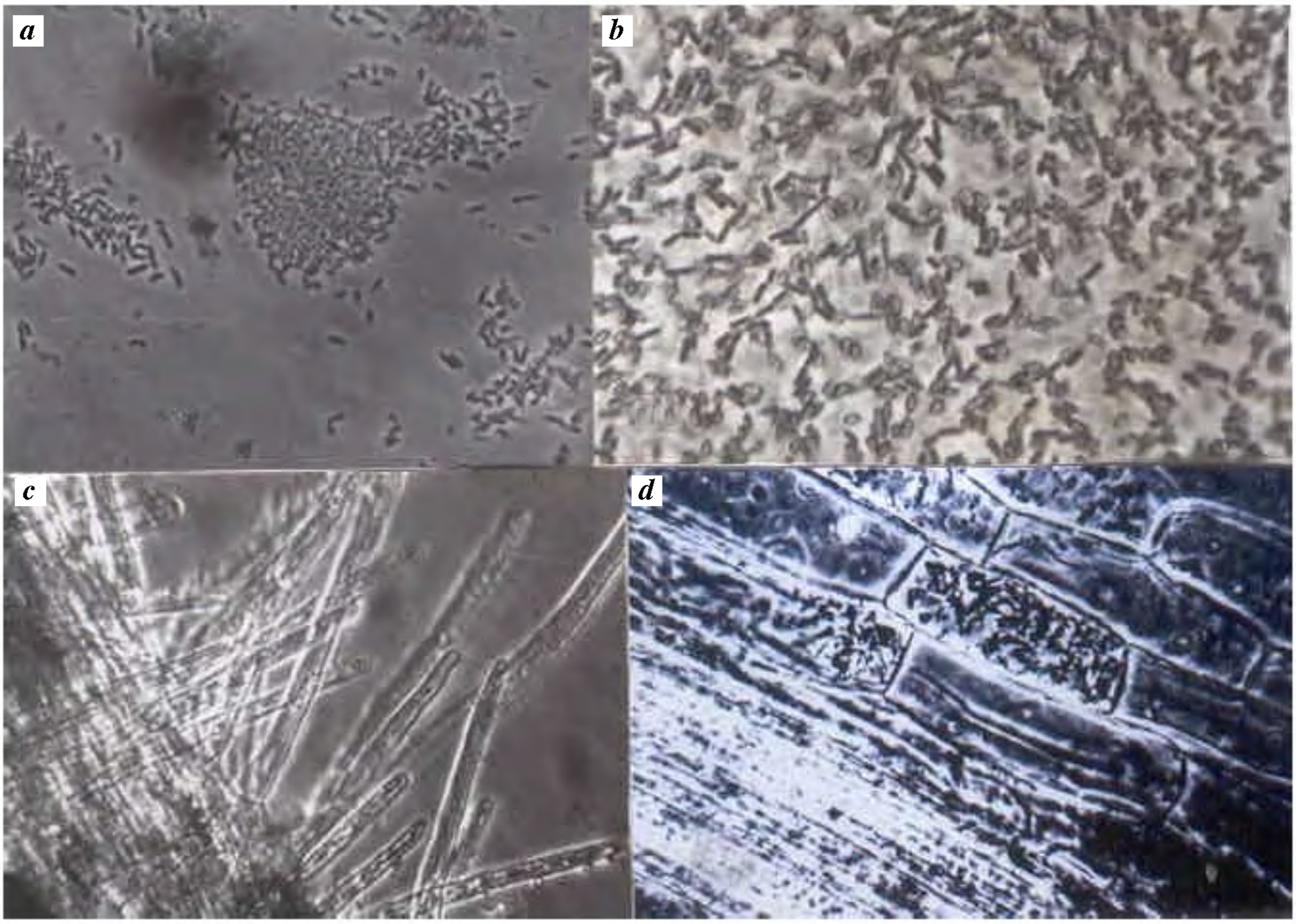

Figure 1. ***a***, Vegetative cells of *Bacillus circulans* (× 1000). ***b***, With profuse endospore formation (× 1000). ***c***, Cluster of root hairs of maize showing bacteria inside root hairs (× 600). ***d***, Presence of bacteria in cortical cells of maize (× 600).

Table 2. Population dynamics of *Bacillus* in rhizotic zones of maize at 60 days of plant growth

	Log number of cells/g fresh weight root		
Treatment	Rhizosphere	Rhizoplane	Endorhizosphere
Uninoculated control	1.25	0.80	0.51
B. cereus	5.02	5.85	4.53
B. circulans	5.61	6.24	5.66
CD ($P = 0.01$)	0.75	0.92	1.05

Each value is the mean count of 10 plates containing glucose–yeast extract salts agar medium with kanamycin (25 µg/ml) and tetracycline (5 µg/ml) as antibiotic markers.
The base 10 log transformation was applied to individual estimation of root colonization prior to statistical analysis.

rhizosphere, rhizoplane and endorhizosphere was noticed (Table 2). Further, these strains were good phosphate solubilizers which might have contributed towards increased yields in maize. Enhanced N_2-fixation in maize cultivars due to N_2-fixing *Azospirillum* has been reported by Bulow *et al.*[10] from Brazil. Similarly, pigeonpea and wheat also recorded significant increases in grain yield due to seed inoculation with these rhizobacteria. The yield of pigeonpea due to bacterization with PGPRs was low, possibly due to the differential response of PGPRs with *Rhizobium* population in soil. However, strains *Bacillus cereus* and *B. circulans* resulted in 38.6 and 41.3% increase in grain yield over uninoculated control. Tilak *et al.*[6] reported that dual inoculation of PGPR and *Rhizobium* sp. enhanced nodulation and nitrogen fixation in pigeonpea grown in semi-arid soils.

The results are of agronomic significance in selecting suitable PGPRs for enhancing the productivity of maize, wheat and pigeonpea. Such beneficial effects of introducing novel microorganisms are in concurrence with current views on the role of plant growth-promoting and soil-supporting bacteria in enhancing plant yields[6,11].

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ACKNOWLEDGEMENTS. We thank Ms Krishna, Agro-Bio Products, Hyderabad for permitting us to use the facilities for conducting field trials. We are grateful to Drs A. K. Saxena and K. K. Pal for supplying the cultures.

Received 15 November 2005; revised accepted 21 November 2005

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