Developing novel bacterial based bioformulation having PGPR properties for enhanced production of agricultural crops

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Plant growth promoting rhizobacteria (PGPR) are beneficial rhizobacteria which enhance plant growth as well as the productivity by a variety of mechanisms. PGPR were isolated from the rhizosphere region of som plants (*Machilus bombycina* King) maintained at the Central Muga Eri Research and Training Institute, Lahdoigarh, Jorhat. A bacterial based bioformulation was prepared and sprayed over the experimental crops including tomato (*Solanum lycopersicum*), cauliflower (*Brassica oleracea var botrytis*), chili (*Capsicum annuum*) and brinjal (*Solanum melongena*). Biochemical analysis was done on these PGPR treated crops as well as the untreated crops. The bioformulations prepared from *Bacillus cereus* (MTCC 8297), *Pseudomonas rhodesiae* (MTCC 8299) and *Pseudomonas rhodesiae* (MTCC 8300) was found to be the most effective in increasing the shoot height, number of leaves, early fruiting and total biomass content of the plants after treatment.

**Keywords:** *Bacillus cereus*, *Brassica oleracea*, Brinjal, Cauliflower, *Capsicum annuum*, Chilli, *Machilus bombycina*, Plant growth promoting rhizobacteria (PGPR), *Pseudomonas rhodesiae*, *Solanum lycopersicum*, *Solanum melongena*, som plants, Tomato

The term “Plant Growth Promoting Rhizobacteria” (PGPR) was first used by Kloeper and Schroth (1978) to describe soil bacteria that colonize the roots of many plant species. PGPR have been applied to a wide range of crops and agricultural conditions for the purpose of enhancing plant growth and health, hence improving crop yields1-4. Plant growth promotion by rhizobacteria can occur directly or indirectly, e.g. (i) by solubilising phosphate, potassium, oxidizing sulphur, fixing nitrogen, chelating iron and copper; (ii) suppression of soil borne pathogens by the production of hydrogen cyanide, siderophores, antibiotics; (iii) improving plant stress tolerance to drought, salinity, metal toxicity; and (iv) productions of phytohormones such as IAA, etc5. Different soil bacteria that have been reported as PGPR belong to the genera which exert a beneficial effect to the plant growth includes *Pseudomonas, Bacillus, Azospirilium, Agrobacterium, Azotobacter, Arthrobacter, Alcaligenes, Serratia, Rhizobium, Enterobacter, Burkholderia, Beijerinckia, Klebsiella, Clostridium, Vario-voxax, Xanthomonas* and *Phyllobacterium*6-13. Over the years, the PGPR have gained worldwide importance and acceptance for agricultural crops, which lead to enhanced growth and biomass content14-16. PGPR from *Pseudomonas fluorescense* have shown a potential activity in increasing the height of some graminaceous plants17. PGPR have also been reported to enhance nutrient accumulation, growth of oil palm seedlings18 and production of high yielding quality banana19. PGPR have also shown its positive effects in germination rate and drought tolerance under adverse conditions. It has shown antibacterial and antifungal properties against many plant diseases20. In the present study, we prepared a bioformulation from the bacterial strains having potential PGPR properties and explored its effect on plant growth and productivity of some selected agricultural crops.

**Materials and Methods**

Isolation and identification of strains having PGPR properties—A total of 45 soil samples were collected from the rhizosphere region of the Som plant (*Machilus bombycina* King), from CMERTI,
Lahdoigarh, Jorhat. Each soil sample was examined for pH and its urease activity. Isolation and identification of microorganisms were done according to Cappucino and Sherman and Bergey’s manual on five different media, viz. nutrient agar, potato dextrose agar, King agar B, yeast malt agar and actinomycetes agar.

Selection and preparation of bioformulation of PGPR strains—Pure culture of isolated bacterial strain was maintained in nutrient agar slants. For inoculums preparation, 3.25 g of nutrient broth was suspended in 250 mL distilled water. A loopful of consortium from RB-1, RB-4 and RB-5 strain were inoculated into a single broth and was kept in a shaker at 200 rpm at 28 °C for around 48 h. This bioformulation was collectively known as consortium.

Mode of treatment—For the field experiments, a few selected agricultural crops having specificity of early fruiting such as tomato (Solanum lycopersicum), chili (Capsicum annuum), cauliflower (Brassica oleracea var botrytis), and brinjal (Solanum melongena) were considered. Bacterial bioformulation (5 mL) was given near the root region of individual treated plants and a 20% dilution of the bacterial bioformulation was sprayed on leaves, stems near the collar region of each plant. Spraying was done at an interval of 5 days till the onset of flowering. The measurements related to height and numbers of leaves were taken before the start of spraying as well as at an interval of 10 days after spraying till the fruiting stage. Finally the total biomass content of both the treated and control plants were measured.

Biochemical analysis—For biochemical analysis, the fruits of all the vegetables were considered. Here, four variables (carbohydrate, protein, lipid and total amino acid concentrations) of both the treated and control plants were analysed. The carbohydrate content was determined by Anthrone method (Hedge et al.) and protein concentrations were measured by Folin-Ciocalteau’s method as described by Lowry et al. The lipid content was determined by extracting the sample with chloroform and methanol (2:1) following the method of Folch et al. Free amino acids were estimated by the method described by Lee et al.

Statistical analysis—The mean values as well as the standard deviation for each parameter were calculated from 6 replicates to find out the effect of the consortium on number of leaves, early fruiting and total biomass after treatment as compared to control.

Results
Altogether 120 morphologically different bacterial colonies were isolated from 45 rhizosphere soil samples. The strains were identified at Gene Bank (MTCC), CSIR-IMTECH, Chandigarh. The results of identification by CSIR-IMTECH, Chandigarh, India are as follows: RB1 (MTCC 8297) Bacillus cereus, RB4 (MTCC 8299) Pseudomonas rhodesiae, RB5 (MTCC 8300) Pseudomonas rhodesiae, RB3 (MTCC 8298) Streptomyces luteireticulii and RB8 (MTCC 8071) Chromobacterium violaceum. Although many consortia prepared from different bacterial strains were attempted to check their effect on agricultural crops, the consortium prepared from RB1, RB4 and RB5 were found to be more effective with respect to the growth and productivity.

Field experiment with bioformulation prepared from Consortium (RB-1, RB-4 and RB-5)—Effect of bioformulation on crop plants such as tomato (Solanum lycopersicum), chilli (Capsicum annuum), cauliflower (Brassica oleracea var botrytis), brinjal (Solanum melongena) was observed in terms of increase in the shoot height, number of leaves and total biomass after treatment. The field experiments that were laid to see the potency of three bacterial strains as “PGPR” are presented in Figs. 1 and 2. The results showed increased height, early fruiting, and number of leaves as well as the biomass content of the treated plants as compared to the control crops.

![Fig. 1](image_url)—Comparative value of Biomass (g/100g) in treated and control crops.
Table 1—Comparative account of carbohydrate, lipid content of treated and control crops

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Carbohydrate content (g/100 g)</th>
<th>Lipid content (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Tomato</td>
<td>3.4± 3.4</td>
<td>11.3± 3.4</td>
</tr>
<tr>
<td>Chili</td>
<td>1.7 ±0.01</td>
<td>7.5 ± 3.6</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>1.4 ± 1.5</td>
<td>4.6 ± 1.4</td>
</tr>
<tr>
<td>Brinjal</td>
<td>1.6 ± 2.2</td>
<td>7.8 ± 0.70</td>
</tr>
</tbody>
</table>

Table 2—Comparative account of protein, total amino acid content of treated and control crops

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Protein content (g/100 g)</th>
<th>Total amino acid content (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Tomato</td>
<td>0.10 ± 0.07</td>
<td>0.20 ± 1.4</td>
</tr>
<tr>
<td>Chili</td>
<td>0.087 ± 0.05</td>
<td>0.14 ± 0.9</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>0.11± 0.65</td>
<td>0.32 ±0.39</td>
</tr>
<tr>
<td>Brinjal</td>
<td>0.07.± 0.83</td>
<td>0.18 ±0.42</td>
</tr>
</tbody>
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Fig. 2—Comparative account of height and number of leaves in selected crops after treatment. [BC, Brinjal control; BT, Brinjal treated; TC, tomato control; TT, tomato treated; CC, chili control; CT, chilli treated; CaC, cauliflower control; CaT, cauliflower treated.]

Discussion

Plant growth promoting rhizobacteria (PGPR) is a heterogeneous group of bacteria which are found in the rhizosphere region at root surfaces and in association with roots, which can improve the extent of quality of plant growth directly and/or indirectly. A huge amount of artificial fertilizers is used to replenish soil N and P, resulting in high costs and increased environmental pollution. However, free-living PGPR also can be used as bio fertilizers. PGPR shows its contribution towards the agro-ecosystems by N2-fixation, P-solubilisation, production of antibiotics and other plant growth promoting substances such as indole-3 acetic acid (IAA), gibberellic acid (GA3), zeatin, ethylene and abscisic acid (ABA), etc. Although there are various hypothesis such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization, and promotion of mineral nutrient uptake which are believed to be involved in PGPR mechanism regarding the stimulation of plant growth, but the actual mechanism is not clearly established. In our experiment, altogether 120 morphologically different bacterial colonies were isolated from 45 rhizosphere soil samples. Out of these, on the basis of morphological and biochemical studies, a bioformulation (consortium) prepared from the RB1 (MTCC 8297) Bacillus cereus, RB4 (MTCC 8299) Pseudomonas rhodesiae and RB5 (MTCC8300) Pseudomonas rhodesiae was found to possess the best PGPR...
properties among others. Statistical analysis through mean and standard deviation shows that they are effective in the growth progression as well as the enhancement of productivity of the said crops as compared to the control crops. Similarly, biochemical estimation of plants treated with consortium showed high nutrient content such as carbohydrate, protein, lipid and total amino acid in treated crops.

Thus, we can conclude that the bioformulation prepared from RB1-*Bacillus cereus*, RB4-*Pseudomonas rhodesiae* and RB5-*Pseudomonas rhodesiae* are effective as a growth enhancer as well as nutrient content enhancer for selected agricultural crops.

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References


