



P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(2): 1315-1320

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Received: 21-01-2020

Accepted: 25-02-2020

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A review on physiological and biochemical properties of plant growth promoting rhizobacteria (PGPR) and their effect on growth and development of maize (*Zea mays* L.)

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i2t.8948>

Abstract

Plant growth promoting rhizobacteria (PGPR) are a group of beneficial bacteria which inhabit various cereal crops including maize, colonize plant root interiors and migrate to different parts of plant. Under this study, four different endophytic bacterial strains namely *Burkholderia cepacia* (RRE3), *Rhizobium leguminosarum* bv. *Phaseoli* (RRE6), *Pseudomonas aeruginosa* (BHU3), *Pseudomonas saponiphila* (BHU8) were characterized for different physiological characters like tolerance to temperature, pH, salt and antibiotic resistance and biochemical parameters such as production of IAA, siderophore, and solubilization of phosphorus, zinc, potash. The combination of BHU8 with RRE3 proved to be most beneficial and efficient with regards to many of the growth parameters like shoot and root length, number of leaves, shoot and root fresh weight, shoot, root dry weight and chlorophyll content. In this review we have tried to compile the previous research and findings in a precise way.

Keywords: PGPR, endophytic bacteria, physiological characters, biochemical parameters

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops in the world. The crop is being regarded as a "Queen of Cereals" because of its carbon pathway (C_4), higher multiplication ratio, wider adaptability, desirable architecture, superior transpiration efficiency, highly versatile use, etc. It is grown over an area of 8.69 million hectares with a total production of about 21.81 million tons per year. For its cultivation maize requires a large amount of nitrogen (120kg/ha), phosphorus (60kg/ha), potassium (60kg/ha) as chemical fertilizer. This amounts to be very large input for crops and farmers have to invest money heavily. Annually more than 199.4 million tons of chemical fertilizer is used worldwide to increase the yield of plants. Though they have high efficiency in promoting crop yield, they have proved to be hazardous for soil health as well as for wellbeing of animals and human population. Plant growth promoting rhizobacteria (PGPR), which can be used as a tool for sustainable agriculture; is a bacterial group of microorganisms which are beneficial to crops and that colonize roots or rhizosphere soil of crop plants. To achieve maximum growth promoting interaction between PGPR and plant it is important to discover how the bacteria exert their effects on plant and whether the effects are changed by various environmental factors, including the presence of other microorganisms. Keeping this in view, the present experiment was carried out to characterize selected endophytic bacteria and to study their interaction in alone and their co-inoculation on growth promotion of maize plant.

The use of microbes as biofertilizers and/or antagonists of phytopathogens is a tool of sustainable agriculture which provides a promising alternative to chemical fertilizers and pesticides. In recent years, the use of Plant growth promoting rhizobacteria (PGPR) for sustainable growth in agricultural productivity has increased tremendously in various parts of the world. Plant growth promoting rhizobacteria (PGPR) is a bacterial group of microorganisms which are beneficial to crops and that colonize roots or rhizosphere soil of crop plants. The term PGPR was coined by Joe Kloepper presently at Auburn University in the United States in the later part of the 1980s. These bacteria associate with root surface of host plant and in general produce the beneficial effect through established mechanisms on host plants.

Endophytic bacteria

In the present study, the selected bacterial endophytes showed a wide diversity in accordance with their temperature tolerance. However, the best growth of all the bacteria was observed at 25°C and by increasing (35°C and 45°C) or by decreasing (15°C) the temperature the cessation in the growth was observed. This finding was similar to the result of Manivannan *et al.*, (2012) who found a good growth of all the bacterial isolates within the temperature range of 20°C to 28°C while studying 10 plant growth promoting rhizobacteria (PGB1 to PGB10).

During the study of selected bacterial tolerance to salt, the best growth was observed at 0.1% NaCl concentration and with the increase in salt concentration the growth was retarded. Whereas, maximum growth was observed at pH 7 and with an increase in acidity or alkalinity the growth of bacteria ceases. Shetta *et al.*, (2011) studied the ability of *Rhizobium* strains to thrive in extreme pH (4.5 to 10) and salinity condition. All the bacteria grew at 2% w/v NaCl concentration. Most of the isolates were found at 3% w/v NaCl concentration. At 4% w/v concentration also two strains ASR and LLR were found to grow. This result was antiparallel to the observations recorded here. Kloepper *et al.* (1980) [16] presented an evidence that plant growth promoting rhizobacteria (PGPR) exert their plant growth-promoting activity by depriving native microflora of iron. PGPR produces extracellular siderophores (microbial iron transport agents) which efficiently from the complex with environmental iron, making it less available to certain native microflora. Lalande *et al.* (1989) [17] sampled and studied the rhizobacteria (including organisms from the ectorrhizosphere, the rhizoplane and endorhizosphere) of 20 different maize hybrids from different locations. *Pseudomonas* spp. was the prominent strain found in the rhizoplane and in the ectorrhizosphere. *Bacillus* spp. and *Serratia* spp. were also detected but in smaller numbers. In the endorhizosphere, *Bacillus* spp. and *Pseudomonas* spp. were detected in order of importance. Screening for plant growth-promoting ability by these rhizobacteria was carried out in three soils with different physical and chemical characteristics. The results depended on the soil used, but two isolates (*Serratia liquefaciens* and *Pseudomonas* sp.) consistently caused a promotion of plant growth. Freitas and Germida (1990) [12] studied the association of winter wheat (*Triticum aestivum* L. cv. Norstar) with root-colonizing rhizobacteria in pot experiments in a growth chamber. Thirty-six known bacteria and 75 isolates obtained from the rhizosphere. These were tested for their effects on plant growth and development in two different soils. They showed plant growth promoting the effect of *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, and *Pseudomonas putida*. They demonstrated the potential use of plant growth promoting rhizobacteria as inoculants for winter wheat. Fisher *et al.* (1992) [11] studied on the distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). Endophytic bacteria and fungi were isolated from healthy maize plants collected in a field in Devon. The average bacterial counts in the stem core tissues showed that the plant parts closer to the soil were more heavily colonized by bacteria than those near the top of the plants and that the lower and middle part of the stems hosted the most frequently isolated bacterial species. Chen *et al.* (1995) [8] reported that the endophytic bacteria are efficient biological control agents of vascular pathogens. They isolated 170 bacterial strains from internal tissues of cotton. The bacterial strains (49 strains with known biological control activity

against *Rhizoctonia solani* in cotton, and 25 strains were known to induce resistance to *Colletotrichum orbiculare* in cucumber) were screened for biological control potential against vascular wilt of cotton caused by *Fusarium oxysporum* f. sp. *Vasinfestum* and introduced as endophytes in the Rowden cultivar of Cotton. Six strains reduced disease severity in two separate experiments. Six strains namely INR-B, JM-1128, JM-1137, CC-186, 89B-61, and JM-869, which reduced the disease severity. These were identified as *Aureobacterium saperdae*, *Bacillus pumilus*, *Phyllobacterium rubiacearum*, *Pseudomonas putida*, *P. putida*, and *Burkholderia solanacearum* respectively. Benhamou *et al.* (1996) [5] studied the endophytic bacterium *Bacillus pumilus* strain SE34 in plant defence reactions using an in-vitro system in which root-inducing T-DNA pea (*Pisum sativum* L.) roots were infected with the pea root-rotting fungus *Fusarium oxysporum* fsp. pisi. In non-bacterized roots, the pathogen multiplied abundantly, whereas, in prebacterized roots, pathogen growth was restricted to the epidermis and the outer cortex. The results revealed that endophytic bacteria may induce plant disease resistance. Roberts *et al.* (1997) [23] treated the seeds of cucumber with combinations of *Escherichia coli* S17R1 and *Burkholderia cepacia* Bc-B to test their biocontrol activity in cucumber rhizosphere. The results provided significantly greater ($P \leq 0.05$) suppression of cucumber seedling pathogens like *Pythium* and *Fusarium* spp in a field than seeds treated individually with strains Bc-B, S17R1, or *Enterobacter cloacae* 501R3. Although strain S17R1 had no effect on disease severity when applied alone and did not colonize cucumber rhizosphere, it encouraged the biocontrol activity of strain Bc-B. Sturz *et al.* (1997) [25] conducted an experiment to assess the effects of the endophytic nodule bacteria, alone and in combination with *Rhizobium* spp., on the growth and development of red clover seedlings. Thirty-one bacterial species from 14 different genera were recovered from within the foliage, roots and nodules of red clover plants cv. AC Charlie. Recovery of *Rhizobium* species was not restricted to the nodules, and species of this genus were systemic throughout the plant. Clover root nodules were host to 12 bacterial species other than rhizobia, of which 8 were specific to this tissue. Using non-selective media, *R. leguminosarum* bv *trifolii* constituted only 8.8% of all the root nodule bacteria recovered. In root bacterization experiments, species of nodule bacteria promoted the growth of red clover more often when applied in combination with *R. leguminosarum* bv *trifolii* than when applied singly. However, *Bacillus megaterium*, *Bordetella avium* and *Curtobacterium luteum* consistently promoted growth either individually or in combination with *R. leguminosarum* bv *trifolii*. Nodulation was promoted when *R. leguminosarum* bv *trifolii* was co-inoculated with *Bacillus insolitus*, *B. brevis* or *A. rhizogenes*.

Andrade *et al.* (1998) [1] studied the impacts of the antibiotic-producing *Pseudomonas fluorescens* strain F113 and its non-antibiotic subordinate *P. fluorescens* F113G22 and indigenous *Rhizobium* on nodulation using undisturbed sandy loam soil cores heavily infested with mycorrhizae. Furthermore, the effects of the different microbial inocula on the colonization of the pea roots by mycorrhizae were studied. It was found that *P. fluorescens* F113 enhanced nodulation by *Rhizobium* fourfold, while the nodules produced were much larger and strongly pigmented (pink) compared with those in other treatments. The extent of roots colonized by arbuscular mycorrhizae was not essentially influenced by the different treatments. Bevivino (1998) [4] studied *Burkholderia*

cepacia in the rhizosphere of maize for their biocontrol activity and plant growth promoting (PGP) activity by metabolic and molecular profiling. The results revealed that *B. cepacia* strains formed a tight phenetic cluster which includes *B. cepacia* LMG 11351 in the rhizosphere. They also demonstrated the biocontrol activity of *B. cepacia* strains like antibiosis against phytopathogenic fungi. Chiarini *et al.* (1998) ^[9] conducted an experiment on *Burkholderia cepacia* strain PHP7 to test its growth promoting ability in *Sorghum bicolor* alone or in combination with *Enterobacter* sp. Strain BB23T4d or *Pseudomonas fluorescens* strain A23T3c. These all were found to colonize the root of sorghum but only *B. cepacia* and *P. fluorescens* promoted plant growth in single-strain inoculation tests. They concluded that dual strain inocula were no more effective than single ones. Barazani and Friedman (1999) ^[2] estimated the phytotoxic or promoting impact of bacterial secretions on root development of lettuce seedlings (*Lactuca sativa*) under axenic conditions. It was expected that the inhibitory or advancing impacts of either pernicious rhizobacteria (DRB) or of plant growth promoting rhizobacteria (PGPR) were mediated by auxin. Auxin discharge rate, assessed by thin-layer chromatography (TLC), demonstrated that all bacteria analyzed, discharged indole-3-acetic acid (IAA). Large amounts of IAA (76.6 μ M) were discharged by four DRB (*Micrococcus luteus*, *Streptovorticillium* sp., *Pseudomonas putida*, and *Gluconobacter* sp.) during 84 hr of incubation. Nejad & Johnson, (2000) ^[20] conducted an experiment to determine plant growth-promotion potential of bacteria isolated from plant tissue of tomato and oilseed rape. They also tested these bacteria for their biological control activity against soilborne diseases. Seeds and young plants of oilseed rape (*Brassica napus* L. cv. Casino) and tomato (*Lycopersicon esculentum* L. cv. Dansk export) were inoculated with individual bacterial isolates or mixtures of bacteria that originated from symptomless oilseed rape, wild and cultivated. They were isolated after surface sterilization of living roots and they found isolates that not only significantly improved seed germination, seedling length, and plant growth of oilseed rape and tomato but also, when used for seed treatment, significantly reduced disease symptoms caused by their vascular wilt pathogens *Verticillium dahliae* and *Fusarium oxysporum* fsp. *Lycopersici* (Sacc.), respectively. Balandreau *et al.* (2001) ^[3] conducted a study on polyphasic taxonomy including DNA-DNA hybridization, protein electrophoresis and 16S rDNA analysis. They isolated a group of *Burkholderia cepacia*-like organisms from the rhizosphere or tissues of maize, wheat, and lupine belong to *B. cepacia* genomovar III, a genomic species associated with "cepacia syndrome" in cystic fibrosis patients. Strobel *et al.*, (2004) ^[26] reported that the bacterial endophytes prevent disease development through the endophyte-mediated *de-novo* synthesis of novel compounds and antifungal metabolites. Investigation of the biodiversity of endophytic strains for novel metabolites may identify new drugs for effective treatment of diseases in humans, plants and animals. Mercado-Blanco & Prieto (2013) designed a bioassay to score the colonization process of olive roots cv. Arbequina by strain *P. fluorescens* PICF7 under nongnotobiotic experimental conditions. Fluorescently tagged bacteria quickly colonized the root surface and were mostly found in the differentiation zone. Thereafter, PICF7 tagged populations gradually disappeared from the surface and increasingly colonized inner root tissues. However, endophytic colonization by the introduced bacteria was highly localized and limited over

time. Bacterial microcolonies were visualized in the intercellular spaces of the root cortex and colonization of the root xylem vessels was never detected. The same strategy was also employed to confirm simultaneous endophytic colonization of strain PICF7 and another biocontrol strain (*Pseudomonas putida* PICP2) but using *in-vitro* propagated olive plants (cv. Manzanilla) and a gnotobiotic study-test system. Interestingly, root hairs were demonstrated to play an important role in the endophytic colonization of inner root tissues by both biocontrol strains. Singh *et al.* (2015) ^[24] screened ten plant growth promoting bacterial isolates from the rhizosphere of maize growing at the Indo-Gangetic plain of eastern Uttar Pradesh, India. Based on 16S rDNA gene sequence strains BHU1, BHU2, BHU3, BHU8, BHU10 showed 99.6%, 99.8%, 99.8%, 99.7% and 99.9% sequence alignment respectively with *Pseudomonas brassicacearum*, *Pseudomonas taiwanensis*, *Pseudomonas aeruginosa*, *Pseudomonas saponiphila* and *Pseudomonas oryzae* respectively. Maximum production of phytohormone auxin (IAA) in the tryptophan supplemented medium was found in strain BHU8. Highest Phosphate solubilization activity was observed in isolate BHU8 followed by BHU3, BHU9 and BHU10.

Inoculation in maize

Prikryl *et al.* (1985) ^[22] isolated bacteria of the species *Pseudomonas putida* and *Pseudomonas fluorescens* from the rhizosphere of maize and bean plants. They reported that these rhizobacteria produce indol-3-acetic acid and some other auxins when grown in liquid cultures. The amounts found varied between 1.6 and 3.3 μ g IAA per ml of media which corresponded to 100–200 μ g per gram of growth promoting bacterial dry mass. Hebbar *et al.* (1992) ^[15] isolated different species of bacteria associated with rhizosphere and roots of maize exhibited antagonism towards *Fusarium moniliforme*. *Pseudomonas fluorescens*, *P. putida*, *P. cepacia*, *Flavobacterium*/CDC group II, *Enterobacter cloacae*, *E. agglomerans*, *Acinetobacter calcoaceticus*. *Bacillus* spp and various actinomycetes were the antagonistic bacteria, found in maize rhizosphere. Gram-negative bacteria isolated were inhibitory to *F. moniliforme* than the other antagonists. *Pseudomonas* group of bacteria (*P. fluorescens*, *P. putida* and *P. cepacia*) showed 88% antagonism. They also revealed that Antibiotic production seems to be responsible for the antifungal activity of these endophytic bacteria.

Chabot *et al.* (1996) ^[7] conducted an experiment on *Rhizobium leguminosarum* bv. *Phaseoli* strains P₃₁ and R₁, *Serratia* sp. Strain 24 and *Rhizopus* sp. Starin 68 for their plant growth promoting potential on lettuce and forage maize. The results revealed that rhizobia especially chose for solubilisation function as plant growth promoting rhizobacteria with maize and lettuce. The P solubilization effect was found to be the most important mechanism of plant growth promotion in moderately fertile and very fertile soils when P uptake was increased with rhizobia and other P Solubilizing Micro-organisms.

Cavaglieri *et al.*, (2009) ^[6] studied the influence of plant growth stages on the population size of culturable bacteria and fungi associated with rhizosphere and endo-rhizosphere of maize grown in the field and to establish the community structure of total culturable bacteria and fungi. Density, diversity and community structure of culturable rhizosphere and endo-rhizosphere populations at different maize plant growth stages were estimated. Plant development did not have an influence on total culturable microflora density but it

selectively influenced some bacterial and fungal groups present in the rhizosphere.

Montañez *et al.* (2012) ^[19] isolated twenty-two putative endophytic bacteria from maize plants were identified and characterized by the presence of *nifH*, IAA production, siderophores and phosphate solubilizing capacity. In addition, inoculation experiments to evaluate plant growth promotion were conducted under laboratory conditions. High diversity of diazotrophic bacteria associated with maize plants was found, including the genera *Rhanella*, *Pantoea*, *Rhizobium*, *Pseudomonas*, *Herbaspirillum*, *Enterobacter*, *Brevundimonas* and *Burkholderia*. All strains produce IAA *in vitro* but only *P. fluorescens* (EMA68) produces siderophores. Phosphate solubilization capability was detected in eighteen strains and *Rhanella* spp. (EMA83) showed the highest potential.

Co-inoculation

Lalande *et al.* (1989) ^[17] identified rhizobacteria from maize and determined their plant-growth promoting potential. They conducted this study in 20 different maize cultivars. In the endorhizosphere, *Bacillus* spp. and *Pseudomonas* spp. were detected in order of importance. *Pseudomonas* spp. was found prominently in the ectorhizosphere. *Bacillus* spp. and *Serratia* spp. were also detected but in smaller numbers. Screening for plant growth-promotion was carried out in three soils with different physical and chemical characteristics. The results depended on the soil used, but two isolates (*Serratia liquefaciens* and *Pseudomonas* sp.) consistently caused a plant growth promotion.

Roberts *et al.* (1997) ^[23] observed that the seed treatments containing combinations of *Escherichia coli* S17R1 and *Burkholderia cepacia* Bc-B provided significantly greater ($P \leq 0.05$) suppression of cucumber seedling pathogens in a field soil naturally infested with *Pythium* and *Fusarium* spp. than seeds treated individually with strains Bc-B, S17R1, or *Enterobacter cloacae* 501R3. Although strain S17R1 had no effect on disease severity when applied alone and did not colonize cucumber rhizosphere, it enhanced the biocontrol effectiveness of strain Bc-B.

Chiarini *et al.* (1998) ^[9] conducted an experiment and showed that the dual strain inoculum (*B. cepacia* and *P. fluorescens*) did not have any significant effect on plant growth in contrast to the separate inoculation of both strains. They concluded that Establishment of large populations of bacterial inoculants on roots did not appear to be essential for plant growth promotion. They also showed that the *B. cepacia* population was significantly reduced in the presence of *Enterobacter* sp. but not of *P. fluorescens* in dual strain tests.

Pandey *et al.* (1998) ^[21] conducted a field experiment using three strains of *Azotobacter chroococcum* and two of *Azospirillum brasilense* at two elevations on a local maize variety. The results revealed a significantly improved plant performance at the subtropical location where yield enhancement of 1.15-fold over control was recorded with one of the bacteria. The highest performance was seen by *Azotobacter chroococcum* W5 (originally a wheat isolate). This was due to the stimulation of already existing plant growth-promoting rhizobacteria in and around roots. Bacterial inoculation also resulted in significantly higher values for nitrogen and phosphorus content of plant components. Seed inoculation did not result in improvement of plant performance at the temperate location due to the inability of the introduced bacteria to establish or survive at lower temperatures.

Dobbelaere *et al.* (2002) ^[10] conducted a study on *Azospirillum* inoculation in spring wheat and grain maize. Wheat and maize plants were inoculated with different concentrations of the wild-type strains *A. brasilense* Sp245 and *A. irakense* KBC1, and grown in a substrate with varying concentrations of organic matter (OM) and N fertiliser. The effect of inoculation was most pronounced at low to intermediate N fertilisation levels, while the OM content of the substrate had no effect. Inoculation was found to affect early plant and root development, plant and root dry weight, grain yield and the N-uptake efficiency of plants. However, inoculation did not change the N concentration in plants or grains. In addition, a difference in the ability of both strains to stimulate plant growth and N uptake of wheat and maize was observed, with *A. brasilense* Sp245 having most effect on spring wheat and *A. irakense* KBC1 being more effective on grain maize.

Gamalero *et al.* (2004) ^[13] evaluated growth promoting ability of mixed inocula *Pseudomonas fluorescens* 92rk and P190r and/or the AMF *Glomus mosseae* BEG12 in tomato plants (*Lycopersicon esculentum* Mill. cv. *Guadalete*) by measuring shoot and root fresh weight and by analysing morphometric parameters of the root system. The effect of the microorganisms on phosphorus (P) acquisition was also assayed. The two bacterial strains and the AMF, alone or in combination, promoted plant growth. *P. fluorescens* 92rk and *G. mosseae* BEG12 when co-inoculated had a synergistic effect on root fresh weight. Moreover, co-inoculation of the three microorganisms synergistically increased plant growth as compared with singly inoculated plants. Both the fluorescent pseudomonads and the myco-symbiont, depending on the inoculum combination, strongly affected root architecture. *P. fluorescens* 92rk increased mycorrhizal colonization. The bacterial strains and the AMF, alone or in combination, improved plant mineral nutrition by increasing leaf P content. The results revealed the potential use of fluorescent pseudomonads and AMF as mixed inoculants for tomato.

Mishra *et al.* (2009) ^[18] conducted an experiment on co-inoculation of *Bacillus thuringiensis*-KR1 with *Rhizobium leguminosarum* in pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.). They showed that the beneficial effects of nodulation can be enhanced when rhizobial inoculation is combined with plant-growth-promoting bacteria (PGPB). The enhancement in nodulation due to co-inoculation was 84.6 and 73.3% in pea and lentil respectively compared to *R. leguminosarum*-PR1 treatment alone. The PGPB strain *Bacillus thuringiensis*-KR1 was found to promote plant growth of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) under Jensen's tube, growth pouch and non-sterile soil, respectively, when co-inoculated with *Rhizobium leguminosarum*-PR1.

Montanez *et al.* (2012) ^[19] conducted an experiment on twenty-two cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars. They also studied their inoculation effects in-vitro. Rhizobacteria including the genera *Rhanella*, *Pantoea*, *Rhizobium*, *Pseudomonas*, *Herbaspirillum*, *Enterobacter*, *Brevundimonas* and *Burkholderia* were characterized by the presence of *nifH*, IAA production, siderophores and phosphate solubilizing capacity. Highest Phosphate solubilization capability was detected in *Rhanella* spp. (EMA83). All strains produce IAA *in vitro* but only *P. fluorescens* (EMA68) produces siderophores. Positive effects across maize cultivars were observed with 10 isolates from 22, whose inoculation resulted

in an increase on shoot biomass over uninoculated controls without *N. H. frisingense*, EMA117 was the only strain that showed a positive effect on both maize cultivars. A significant interaction between inoculation treatment \times maize cultivars in the shoot and root dry weight ($P < 0.05$) was observed. Only one strain, *Pseudomonas fluorescense*, increased significantly the growth of the radicle compared to that of the controls without inoculation.

Gholami *et al.* (2012) [14] conducted an experiment on the role of Plant growth promoting rhizobacteria (PGPR) in plant health and soil fertility. The experiment was conducted with two factors of *Azospirillum* and *Azotobacter*. The bacterial strains were *Azospirillum lipoferum* s-21, *A. brasilense* DSM 1690, *A. lipoferum* DSM 1691, *Azotobacter chroococcum* s-5, and *A. chroococcum* DSM 2286. The results revealed that leaf area index and crop growth index were significantly affected by bacterial treatments. It was also seen that growth promotion appeared from 45 days after inoculation (DAI), ear growth was observed after 75 DAI. Inoculation with PGPR also increased dry weights of leaf, stem, and grain and hence total biomass. The greatest grain weight was produced by *Azospirillum* s-21 inoculation. Dual inoculation with *Azotobacter* s-5 + *Azospirillum* s-21 increased total dry weight up to 115%.

Conclusion

Plant growth promoting rhizobacteria (PGPR), which can be used as a tool for sustainable agriculture; is a bacterial group of microorganisms which are beneficial to crops and that colonize roots or rhizosphere soil of crop plants. To achieve maximum growth promoting interaction between PGPR and plant it is important to discover how the bacteria exert their effects on plant and whether the effects are changed by various environmental factors, including the presence of other microorganisms. Keeping this in view, the present experiment was carried out to characterize selected endophytic bacteria and to study their interaction in alone and their co-inoculation on growth promotion of maize plant.

In our present study interaction effect of endophytic bacteria alone and their co-inoculation on growth and development of maize was recorded. The interaction of BHU8 with RRE3 proved to be highly beneficial and efficient with regards to most of the growth parameters. The PGPR enhances plant growth mainly through various direct and indirect effects. The direct effects mainly involve the production of IAA, siderophore, solubilization of phosphate, potash, zinc or in facilitating necessary nutrient uptake from soil. BHU8 (*Pseudomonas saponiphila*) the highest producer of siderophore, IAA and phosphate solubilizer while RRE3 (*Burkholderia cepacia*) being the maximum potash and zinc solubilizer. So, their co-inoculation was found to be most efficient plant growth promoter for the maize cultivar Malviya Hybrid Maize 2. Strain RRE3 was a representative of *Burkholderia cepacia* which belongs to beta subclass of proteobacteria while strain BHU8 was a representative of *Pseudomonas saponiphila* which belongs to gamma subclass of proteobacteria. The combination of these two isolates gave the best response on overall promotion of growth of maize plant and therefore it is recommended as a biofertilizer for their commercial exploitation in field trials.

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