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Plant Growth Promoting Efficiency of Phosphate Solubilizing *Chryseobacterium* sp. PSR 10 with Different Doses of N and P Fertilizers on Lentil (*Lens culinaris* var. PL-5) Growth and Yield

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A B S T R A C T

Lentil is one of the important legume crop widely grown in India. The availability of low phosphorous in soil is one the major concern for the production of legume crop in the country. Thus, the present study was aimed to evaluate the plant growth promoting efficiency of phosphate solubilizing *Chryseobacterium sp.* PSR 10 with different doses of inorganic fertilizers (N and P) on lentil growth and yield under field conditions. The phosphate solubilizing potential of this bacterial strain through *In vitro* tricalcium phosphate solubilization in NBRI-BPB broth medium was 210.43µgml^{-1}. The field experiments were conducted for two "*Rabi*" crop seasons. To evaluate the potential of *Chryseobacterium sp.* PSR 10, lentil seeds were inoculated with the bacterium and applied with 30, 50 and 100% of recommended doses of nitrogen (N) and phosphorous (P) fertilizers along with unfertilized (without N and P) uninoculated control and fertilized (with N and P) but uninoculated control. Seed inoculation with 50% of recommended dose of nitrogen (N) and phosphorus (P) increased plant growth (agronomical parameters, chlorophyll content, nitrate reductase activity, phosphorus content and crop yield) significantly over control. Therefore, the study concluded that phosphate solubilizing plant growth-promoting bacterium *Chryseobacterium* sp. PSR10 broadens the spectrum of phosphate solubilizers available for field applications and might be used together with 50% dose of nitrogen and phosphorous.

Introduction

The every crop needs an effective fertilizer recommendation for good seed quality, yield and soil health. The continuous use of inorganic fertilizers may adversely effect to the crop yields, in order to sustain the efficiency of soil and crop yield and reduce the dependency of chemical fertilizers, the combined use of organic manures, biofertilizers and fertilizers is very much essential (Kumar *et al*., 2003). In order to

reduce the dependency of chemical fertilizers the need of the hour is to use alternative strategy to retain sustainable agriculture. The best alternative strategy is to utilize bio resources of microorganisms as biofertilizers. Among the group of microorganisms some of the bacteria associated with the roots of crop plants can exert beneficial effect on their roots and enhanced crop growth and seed yield (Singh *et al*., 2010, Yadav *et al*., 2016), collectively known as plant growth promotory rhizobacteria (PGPR). PGPR promote plant growth and health by providing fixed nitrogen, synthesizing siderophore, producing phytohormones, solubilizing phosphorous and out competing pathogenic soil microorganisms (Kloepper *et al*., 1989).

In this view the present study was planned to get the plant growth promotion effect of phosphate solubilizing *Chryseobacterium* sp. PSR 10 on lentil (*Lens culinaris* var. PL-5). Phosphorous (P) is an important nutrient required for normal growth and metabolic process occurring in plants (Singh and Satyanarayana, 2011). P influences many plant processes like seed germination, seed maturity, and plant growth rate, which includes root development of stalk and stem of the plants, flower and seed formation, N_2-fixation, energy metabolism, synthesis of nucleic acid, photosynthesis, respiration, crop quality and resistance against various biotic and abiotic stresses (Khan *et al*., 2009, Singh *et al*., 2013, Wang *et al*., 2013, Singh and Prasad, 2014). Most soil P is usually as insoluble metal chelates (Vassilev *et al*., 2006); moreover, substantial amount of applied chemical phosphate fertilizers are also rapidly converted into insoluble phosphate. The role of microorganisms in solubilizing inorganic phosphates in soil and making them available to plants is well known (Bhattacharya and Jain, 2000). These microorganisms are called phosphate solubilizers and they convert insoluble phosphates to soluble phosphates by acidification, chelation, an ion-exchange reaction and production of low molecular mass organic acids (Barroso *et al*., 2006).

Phosphate deficiency in soil can severely limit plant growth productivity of legumes, where both the plants and their symbiotic bacteria are affected and this may have a deleterious effect on nodule formation, development and function (Alikhani *et al*., 2006). However, phosphate solubilization is a complex phenomenon that depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes *et al*., 1999). Interest has focused on the inoculation of PSB into soil to increase the availability of native fixed phosphate and to reduce the use of chemical fertilizers. Many PSB viz., *Pseudomonas*, *Bacillus*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Micrococcus*, *Aerobacter*, *Enterobacter*, *Flavobacterium* and *Erwinia* genera have been isolated from soils (Rodriguez and Fraga, 1999; Gulati *et al*., 2007; Singh *et al*., 2011) and are being used for plant growth promotion. These PSB facilitate mobilization of insoluble phosphates in the soil and increase plant growth under conditions of poor phosphorus availability.

The introduction of many species, either crop, forest, ornamental vegetation with several plant growth-promoting bacteria (PGPB) has frequently resulted in healthier and greener plants (Swedrzynska and Sawicka, 2000; Singh and Prasad, 2014; Prasad *et al*., 2016), suggesting enhanced photosynthesis (Alam *et al*., 2001). Nitrate reductase is the first and most important enzyme in overall nitrogen metabolism of the plants (Solomonson and Barber, 1990). The input of reduced nitrogen to a plant is determined by the activity of nitrate reductase, which catalyses the first step and determines the rate of this assimilating process. Therefore, we evaluated the influence of PSB on total chlorophyll content and nitrate

reductase activity of lentil plants. In this context, the utilization of phosphate solubilizing microorganisms are considered an important bioinoculant to convert soil insoluble phosphate to soluble phosphate in natural and agriculture ecosystem, which also helps to impart in soil, plant health and ultimately leads to better plant growth and crop yield. In the present study, effect of phosphate solubilizing *Chryseobacterium* sp. PSR 10 was investigated on lentil and their combined effect with nitrogen (N) and phosphate (P) fertilizers on plant growth promotion under field conditions with aim to reduce fertilizer supply using selected PSB in the cultivation of lentil (*Lens culinaris* var. PL-5).

Materials and Methods

Bacterial isolates and formulation

The phosphate solubilizing bacterial (PSB) strain *Chryseobacterium* sp. PSR10 was originally isolated from soybean rhizosphere and collected from Department of Microbiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, India. The phosphate solubilizing potential of bacterial (PSB) strain *Chryseobacterium* sp. PSR10 was previously confirmed by Singh *et al*. (2013). The bacterial strain was maintained on nutrient agar slants at 4°C and in glycerol stock at -20°C. Seed bacterization was placed through talc based formulation and prepared according to Commare *et al*. (2002) and at the time of application, the population of PSB in the formulation was 1.9×10^{8} cfug^{-1}. Before bacterization, seeds of lentil were disinfected for 3 minutes with 0.1% mercuric chloride solution, afterwards disinfected again with 70% ethanol for 3 minutes. Subsequently, seeds were washed ten times with sterilized distilled water. Afterwards, seeds to be treated were weighed and moistened with sterilized distilled water for surface inoculation with talc based formulation and shade dried for two hours as described by Lokesha and Benagi (2007) and Singh and Goel (2015).

Plant growth promotion

Plant growth promotion was studied under field conditions on lentil (*Lens culinaris* PL-5). Field experiments were conducted at Crop Research Centre, G. B. pant University of Agriculture and Technology, Pantnagar, India and the experimental site lies at 29°N latitude and 79.3° E longitude with elevation of 243 m above sea level. The field experiments were conducted for two cropping season in the year 2005-06 and 2008-09. The experiments were laid out in randomized block design with three replications per treatment. There were four rows in each plot of 1.2 m width and 2 m length. The experiment was designed with five treatments designated with T_1 to T_5. Treatment T_1 was an uninoculated control (without nitrogen and phosphorus supply), whereas treatment T_2 was uninoculated but fertilized with 100% of the recommended dose of nitrogen and phosphorus. The recommended dose of nitrogen and phosphorus for lentil was 30 and 60 kg ha^{-1}, respectively. Treatments T_3, T_4 and T_5 comprised PSB *Chryseobacterium* sp. PSR10 with combinations of 30 and 60 kg ha^{-1} (100%), 15 and 30 kg ha^{-1} (50%), and 9 and 18 kg ha^{-1} (30%) of the recommended dose of nitrogen and phosphorus, respectively. Subsequently, agronomical as well as physiological growth parameters (root length, shoot length, fresh and dry weight), chlorophyll content, nitrate reductase activity, plant P content and crop yield were determined. The agronomical growth parameters were recorded for each replication of every treatment on the eve of the pod setting stage. However, chlorophyll content, nitrate reductase activity and plant P content were determined at the flowering stage.

Chlorophyll assay

The total chlorophyll content of plant flag leaves was measured according to Singh and Goel (2015). In brief, 0.05-g sample of leaf tissue was placed in a vial containing 10 mL dimethylsulfoxide (DMSO). Chlorophyll was extracted with fluid without grinding at 65°C by incubation for 3 h and was assayed immediately. A 3.0-mL sample of chlorophyll extract was transferred to a cuvette, the OD values at 645 and 663 nm were read by spectrophotometer against a DMSO blank and the chlorophyll content was calculated.

Nitrate reductase activity assay

The nitrate reductase activity of plant flag leaves was measured according to Hageman and Hicklesley (1971). In brief, 0.5-g sample of chopped leaves was placed in a beaker containing 25 mL of infiltration medium (0.1 M KNO3 and 0.15 M phosphate buffer, pH 7.5) and incubated at 30°C with gentle shaking. After incubation, aliquots of 0.2 mL were drawn twice after 10 and 40 min and added to separate test tubes containing 1.8 mL of distilled water. Two millilitres of a 1:1 (v/v) mixture of 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) and 1% sulfanilamide prepared in 1.5 M HCl were added to each test tube. The test tubes were kept in dark for ~15 min for colour development. Absorbance was read at 540 nm with the help of spectrophotometer against water blank and nitrate reductase activity was calculated.

Plant phosphorus content estimation

Phosphorus (P) determination in plant samples were employed as described by Singh *et al*. (2013). In brief, 10 plants were randomly selected for each replication of every treatment at the flowering stage of the crop. The experiments were laid out in a randomized block design with three replications and data were analysed statistically at the 5% level of significance. Plant samples were oven dried at 65°C for 3 days and ground before analysis. An ion chromatograph system DIONEX model DX-600 instrument was used for P analysis of plant samples. The mobile phase was 2.7 mM Na_2CO_3 + 0.3 mM $NaHCO_3$ at a flow rate of 1.4 mL min^{-1} and a pump pressure of 1400 psi. Oven-dried plant material (0.10 g) was placed in a crucible and mixed with 0.5 g $NaHCO_3$ and 0.02 g Ag_2O. A guard layer of 0.5 g $NaHCO_3$ was placed on the top of the ignition mixture. The crucible was placed in a muffle furnace and heated to 550°C for 3 h. The ignition residue was dissolved in 15 mL of 1 M acetic acid, heated to near boiling on a sand bath at 200°C and the final volume was made up to 100 mL with deionized water. The solution was cooled to room temperature and filtered through a 0.22-mm membrane filter to remove any particulates before analysis. To estimate phosphorus, a standard curve was developed using a standard solution of phosphate (20 mg L^{-1}, KH_2PO_4).

Results and Discussion

Phosphate solubilizing bacteria (PSB)

The potential of phosphate solubilizing bacterial strain *Chryseobacterium* sp. PSR10 was confirmed in the previous study of Singh *et al*. (2013) through *In vitro* tricalcium phosphate solubilization in NBRI-BPB broth medium with phosphate solubilization potential of 210.43µgml^{-1}. Singh *et al.*, (2013) identified this strain through 16S rDNA sequencing with accession number DQ-118018.

Field experiment

Phosphorous (P) is second most essential elements after nitrogen for the growth and development of plants. It's exist in soil as phosphate anions and extremely reactive and

are immobilized by soil cations and thus make it unavailable for plants. There are some 'P' solubilizing microorganisms (PSM) that are capable to solubilize unavailable form of phosphorous into available form (Hilda and Fraga, 1999). This process leads to increased P availability in soils, which ultimately increase plant P uptake. There are several findings support that the seed or soil inoculation of plant solubilizing bacteria (PSB) increased plant growth promoting effect under field and greenhouse conditions (Nazarat and Gholami, 2009; Singh *et al*., 2010a and 2010b; Singh *et al*., 2013). In the present investigation, a PSB *Chryseobacterium* sp. PSR10 was used to check their efficacy of plant growth promotion in lentil with different doses of nitrogen (N) and phosphate (P) fertilizer under field conditions. The performance of all the treatments of *Chryseobacterium* sp. PSR10 has considerable positive influence on plant growth, seed yield and other contributing characters in lentil (Table 1 and 2). But, the performance of the bacterium with 50% of the recommended doses of N and P in treatment T_4 was more promising than other treatments in pooled values of two years. The plant growth parameters, i.e. root length, shoot length, fresh and dry weight were significantly enhanced in treatment T_4 by 56, 36.4, 52.1 and 63.75%, respectively, over uninoculated control treatment (T_1) but over fertilized control treatment T_2, plant growth parameters were increased by 27.8, 14.3, 27.4 and 36.3%, respectively in pooled values of two years (Table 1). However, enhancement of these all plant growth parameters in treatments T_3 with 100% N and P and in T_5 with 30% N and P were lesser in comparison with T_4, but showed significantly better plant growth promotion over uninoculated control treatment T_1. While over control treatment (T_2), treatment T_3 showed significant improvement but the treatment T_5 not showed any improvement on these plant growth promotory characters. These findings are agreement with Singh and Prasad (2014) and Singh *et al*. (2010 and 2013), who observed that after inoculation with PSB the plant growth promotory characters were significantly improved over control. Haque and Dave (2005) reported the availability of phosphate in soil is effectively increased through microbial production of metabolites leading to lowering down the pH and release the phosphate from organic and inorganic complexes. Saleemi *et al.,* (2017) studied the integrated effect of plant growth-promoting rhizobacteria and phosphate-solubilizing microorganisms on growth of wheat (*Triticum aestivum* L.) under rainfed conditions.

The effect of bacterial strain *Chryseobacterium* sp. PSR10 inoculation was also analysed for chlorophyll content, nitrate reductase activity, plant P content and grain yield of lentil and showed similar trends as reported for plant growth parameters. And increased these parameters significantly over control treatment (T_2) and enhanced by 48.5, 58.8, 104.8 and 38.6% in T_4 treatment with 50% N and P, respectively in pooled values of two years (Table 2). However, the other bacterial inoculation treatments with 100 and 30 % of N & P showed significant increment over both the control treatment (T_1 and T_2) in spite of T_5 treatment over fertilized but uninoculated control treatment (T_2), which showed non-significant improvement. The results of the study concluded that the presence of microbial inoculant *Chryseobacterium* sp. PSR10 were able to stimulate the plant growth promotory activities in the lentil plants. The increment of available P contents in the lentil plants may be due to the activities of introduced phosphate solubilizing *Chryseobacterium* sp. PSR10, which might have the capacity to dissolved chemically fixed inorganic phosphate compounds.

Table.1 Effect of *Chryseobacterium* sp. (PSR10) on growth parameters of lentil under field conditions

Treatment	Root length (cm) [a]			Shoot length (cm) [a]			Fresh weight (gram) [a]			Dry weight (gram) [a]		
	2005-06	2008-09	Pooled	2005-06	2008-09	Pooled	2005-06	2008-09	Pooled	2005-06	2008-09	Pooled
T_1 (no N.P + no PSB)	8.80	6.66	7.73	28.13	28.33	28.23	16.40	17.33	16.86	1.36	1.63	1.49
T_2 (100% N.P. + no PSB)	11.20 (27.2)[b]	7.66 (15.0)[b]	9.43 (21.9)[b]	31.73 (12.7)[b]	35.66 (25.8)[b]	33.70 (19.3)[b]	21.36 (30.2)[b]	18.90 (9.05)[b]	20.13 (19.3)[b]	1.63 (19.8)[b]	1.96 (20.2)[b]	1.79 (20.1)[b]
T_3 (100% N.P. + PSR10)	13.06 (48.4)[b] (16.6)[c]	9.00 (35.1)[b] (17.4)[c]	11.03 (42.6)[b] (16.9)[c]	33.43 (18.8)[b] (5.3)[c]	37.33 (31.7)[b] (4.6)[c]	35.38 (25.3)[b] (4.9)[c]	24.90 (51.8)[b] (16.5)[c]	22.40 (29.2)[b] (18.5)[c]	23.65 (40.2)[b] (17.4)[c]	1.76 (29.4)[b] (7.9)[c]	2.16 (32.5)[b] (10.2)[c]	1.96 (31.5)[b] (9.4)[c]
T_4 (50% N.P. + PSR10)	14.13 (60.5)[b] (26.1)[c]	10.00 (50.1)[b] (30.5)[c]	12.06 (56.0)[b] (27.8)[c]	35.06 (24.6)[b] (10.4)[c]	42.00 (48.2)[b] (17.7)[c]	38.53 (36.4)[b] (14.3)[c]	25.50 (55.4)[b] (19.3)[c]	25.83 (49.0)[b] (36.6)[c]	25.66 (52.1)[b] (27.4)[c]	2.13 (56.6)[b] (30.6)[c]	2.76 (69.3)[b] (40.8)[c]	2.44 (63.75)[b] (36.3)[c]
T_5 (30% N.P. + PSR10)	11.16 (26.8)[b] (-0.3)[c]	7.66 (15.0)[b] (0.0)[c]	9.41 (21.7)[b] (-0.2)[c]	32.00 (13.7)[b] (0.8)[c]	34.33 (21.1)[b] (-3.7)[c]	33.16 (17.4)[b] (-1.6)[c]	21.00 (28.0)[b] (-1.6)[c]	19.26 (11.1)[b] (1.9)[c]	20.13 (19.3)[b] (0.0)[c]	2.13 (56.6)[b] (30.6)[c]	1.88 (15.3)[b] (-4.0)[c]	2.00 (34.2)[b] (11.7)[c]
SEm±	0.187	0.532	0.309	0.268	1.011	0.517	0.307	0.690	0.424	16.40	0.107	0.820

Note: [a]Each value is mean of three replicates. [b]Values in parentheses indicate percent increase over T_1. [c]Values in parentheses indicate percent increase over T_2. Data were analyzed statistically at the 5% ($p<0.05$) level of significance.

Table.2 Effect of *Chryseobacterium* sp. (PSR10) on chlorophyll content, nitrate reductase activity, P content of plant and yield of lentil under field conditions

Treatment	Chlorophyll content (mg g^{-1} fr. wt) [a]			Nitrate reductase activity (mMol NO_2 g^{-1} fr. Wt. h^{-1})[a]			P content of the plant (mg kg^{-1}) [a]			Yield (Quintal / hectare) [a]		
	2005-06	2008-09	Pooled	2005-06	2008-09	Pooled	2005-06	2008-09	Pooled	2005-06	2008-09	Pooled
T_1 (no N.P + no PSB)	1.42	1.39	1.40	0.86	0.736	0.793	5.57	2.60	4.09	3.06	3.63	3.34
T_2 (100% N.P. + no PSB)	1.58 (11.2)[b]	1.70 (22.3)[b]	1.63 (16.4)[b]	1.02 (18.6)[b]	1.05 (42.6)[b]	1.03 (29.88)[b]	6.39 (14.7)[b]	4.41 (69.6)[b]	5.39 (31.7)[b]	3.78 (23.5)[b]	4.22 (16.2)[b]	4.00 (19.7)[b]
T_3 (100% N.P. + PSR10)	1.85 (30.2)[b] (17.0)[c]	1.88 (35.2)[b] (10.5)[c]	1.86 (32.8)[b] (14.1)[c]	1.15 (33.7)[b] (12.7)[c]	1.14 (54.8)[b] (8.5)[c]	1.14 (43.7)[b] (10.6)[c]	8.17 (46.6)[b] (27.8)[c]	5.83 (124.2)[b] (32.1)[c]	7.00 (71.1)[b] (29.8)[c]	4.35 (42.1)[b] (15.0)[c]	4.45 (22.5)[b] (5.4)[c]	4.40 (31.7)[b] (10.0)[c]
T_4 (50% N.P. + PSR10)	1.91 (34.5)[b] (20.8)[c]	2.27 (63.3)[b] (33.5)[c]	2.08 (48.5)[b] (27.6)[c]	1.33 (54.6)[b] (30.3)[c]	1.20 (63.0)[b] (14.2)[c]	1.26 (58.8)[b] (22.3)[c]	9.73 (74.6)[b] (52.2)[c]	7.03 (170.3)[b] (59.4)[c]	8.38 (104.8)[b] (55.4)[c]	4.50 (47.0)[b] (19.0)[c]	4.75 (30.8)[b] (12.5)[c]	4.63 (38.6)[b] (15.7)[c]
T_5 (30% N.P. + PSR10)	1.72 (21.1)[b] (8.8)[c]	1.96 (41.0)[b] (15.2)[c]	1.84 (31.4)[b] (12.8)[c]	1.12 (30.2)[b] (9.8)[c]	1.03 (39.9)[b] (-1.9)[c]	1.08 (36.1)[b] (4.85)[c]	6.32 (13.4)[b] (-1.0)[c]	5.19 (99.6)[b] (17.6)[c]	5.75 (40.5)[b] (6.6)[c]	4.03 (31.6)[b] (6.6)[c]	4.25 (17.0)[b] (0.7)[c]	4.14 (23.9)[b] (3.5)[c]
SEm±	0.339	0.240	0.163	0.161	0.163	0.101	0.186	0.194	0.114	0.183	0.577	0.105

Note: [a]Each value is mean of three replicates. [b]Values in parentheses indicate percent increase over T_1. [c]Values in parentheses indicate percent increase over T_2. Data were analyzed statistically at the 5% ($p<0.05$) level of significance.

Increased chlorophyll content is a known plant response towards the PGPB inoculation, which subsequently enhances photosynthesis (Alam *et al*., 2001; Sharma *et al*., 2003). This can be correlated with enhanced plant growth and yield due to better photosynthesis capability of the plants (Singh *et al*., 2013). In the present study all treatments of *Chryseobacterium* sp. PSR10 inoculation able to enhanced chlorophyll content and simultaneously increased crop yield of lentil. This bacterium inoculation also increase the nitrate reductase activity of the lentil plant leaves compared with control treatments. Singh *et al*. (2015) confirmed the plant growth promoting efficiency of *Chryseobacterium* sp. PSR10 on finger millet under greenhouse conditions and analyzed chlorophyll content and nitrate reductase activity of the plants. However, Solomonson and Barber (1990) confirmed that reduced nitrogen input to the plant is determined by the activity of nitrate reductase, which catalyses the first step and determines the rate of this assimilating process that acts as a limiting factor of plant growth and development.

Statistical analysis confirmed that inoculation of *Chryseobacterium* sp. PSR10 bacterium with 50% recommended dose of nitrogen and phosphorous support maximum enhancement for plant growth parameters of lentil when compared with all other treatments.

In conclusion, the present study may be concluded that the reduction of 50% recommended doses of N and P fertilizers along with the bacterium *Chryseobacterium* sp. PSR10 able to reduce the input cost of inorganic fertilizers and support to formulate sustainable agriculture as well as precision farming. This practice may be very much convenient and cost effective as well as eco friendly. In summary, the final conclusion of the study of plant growth promotion showed that the PSB *Chryseobacterium* sp. PSR10 can play an essential role for helping plant establishment and growth under nutrient deficient conditions. Therefore, the use of such bacterium as PSB bioinoculant with 50% doses of N and P will increase the availability of phosphorous in soils and help to judicial use of phosphatic fertilizers.

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