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Research Paper

PLANT GROWTH PROMOTING EFFICIENCY OF *CHRYSEOBACTERIUM* SP. PSR 10 ON FINGER MILLET (*ELEUSINE CORACANA*)

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Abstract

Phosphate solubilizing bacteria (PSB) is an environment friendly and cost-effective approach as an alternative strategy to reduce chemical fertilizers usage. Phosphate solubilizing bacteria with multiple beneficial characteristics are important to increase the effectiveness of the inoculated host plant. Due to said interest the previously characterized PSB strain *Chryseobacterium* sp. PSR 10 was used to evaluate the effectiveness of the strain on finger millet (*Eleusine coracana* var. VLM-149). Finger millet is the third most important millet, next to sorghum and pearl millet. To evaluate the efficacy of the microorganism the pot experiments were designed with sterilized and unsterilized soil system. Seed inoculation of *Eleusine coracana* (finer millet) by *Chryseobacterium* sp. PSR 10 showed better plant growth promotion and increased plant growth agronomical parameters, chlorophyll content and nitrate reductase activity in both the soil systems. The better performance of the bacterium *Chryseobacterium* sp. PSR 10 was attributed to its greater P solubilization activity and positive correlation with plant growth promotion of finger millet. Furthermore, the result of the present study suggested that it is might be possible to select, bacterial strain *Chryseobacterium* sp. PSR10 as a good phosphate solubilizing bioinoculant which can be exploited as PSB.

Key words: *Chryseobacterium* sp., phosphate solubilizing bacteria (PSB), plant growth promotion, bacterial inoculation, chlorophyll content, nitrate reductase activity.

INTRODUCTION

Finger millet (*Eleusine coracana)* is grown mostly in arid region of eastern and southern Africa, India and Nepal. The seeds of the crop can be stored for longer duration without any insect damage. Finger millet locally named as *ragi*, which is the third most important millet, next to sorghum and pearl millet. Finger millet is nutritionally rich crop as it contains high level of calcium, iron, and manganese and their straw is also an important livestock feed. The crop is much important for diabetic's patient and people with digestive problems because it contains a low glycemic index and has no gluten [1]. 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 soil health and crop yields, in order to sustain the efficiency of soil, crop yield and reduce the dependency of chemical fertilizers, the combined use of organic manures, biofertilizers and fertilizers is very much essential [2].

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 the plant roots and enhanced plant growth and seed yield, 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 [3]. Among the plant growth promotion effect of PGPR, phosphate solubilizing bacteria (PSB) has taken great attention of the agriculturist now days, because most soil phosphate is usually as insoluble metal chelates [4]; 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 [5]. 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 [6]. Optimization of a biological phosphate solubilizer in the form of rhizospheric microorganisms seems to be a suitable tool to release some of the soil-bound phosphates and reduce the use of chemical fertilizers. In particular, soil microorganisms are effective in releasing phosphate from total soil phosphorus through solubilization and mineralization. Currently, the main purpose of managing soil phosphate is to optimize crop production and minimize phosphate loss from soils. The plant growth-promoting property of PSB can be considered as one of the most important trait associated with plant phosphate nutrition, which is advantageous in sustainable agricultural practices.

Therefore, the present study evaluated the influence of PSB (*Chryseobacterium* sp. PSR 10) on agronomical plant growth parameters (i.e. root length, shoot length, fresh and dry weight), total chlorophyll content and nitrate reductase activity of finger millet (*Eleusine coracana* var. VLM-149) plants under green house conditions. The main aim of the study to reduce fertilizer supply using selected PSB in the cultivation of finger millet (*Eleusine coracana* var. VLM-149), which also helps to impart in soil health, plant health and ultimately leads to better plant growth and crop yield.

MATERIALS AND METHODS

Plant and Soil

The experiment was carried out using sandy loam soil (pH 6.9 ± 0.2) from crop research centre (CRC), G. B. Pant University of Agriculture and Technology, Pantnagar, India. Plants of finger millet (*Eleusine coracana* var. VLM-149) were used as the test plants for the bacterial inoculation experiments. Seeds of finger millet were obtained from College of Forestry and Hill Agriculture, Hill Campus, Ranichauri, Uttarakhand, India.

Bacterial isolates and Formulation

The bacterial isolate *Chryseobacterium* sp. PSR 10 was originally isolated from soybean rhizosphere and collected from the Department of Microbiology culture collection, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, India. The previous study of Singh *et al*. [7] confirmed that the bacterium *Chryseobacterium* sp. PSR 10 was a good phosphate (P) solubilizer. The bacterial isolate was maintained on nutrient agar slants at 4^{o}C and glycerol stock at -20^{o}C. Talc based formulation of the isolates was prepared according to Commare *et al*. [8] and at the time of application, the population of phosphate solubilizing bacteria in the formulation was 3×10^{8} cfug^{-1}. Soybean seeds were disinfected for 3 min with 0.1% mercuric chloride solution, disinfected again with 70% ethanol for 3 min. Subsequently, seeds were washed eight times with sterile distilled water. 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 [9].

Plant growth promotion

Plant growth promotion was studied under greenhouse conditions on finger millet (*Eleusine coracana* var. VLM-149). Bacterized finger millet seeds were sown in pots (20 cm

diameter) containing sterilized and unsterilized soils (sandy loam in texture, pH 6.9 ± 0.2) and reared in a greenhouse (temperature ranging 28 ± 2°C). The same treatment with nonbacterized seeds was also maintained to serve as a control. Sterilized distilled water was added daily to pots as necessary to maintain soil moisture levels near field capacity. Four plants were maintained in each pot and the plant growth parameters were recorded after 60th days of showing. Agronomical growth parameters (root length, shoot length, fresh and dry weight), chlorophyll content and nitrate reductase activity were recorded and analyzed for each replication of every treatment after 60th days of showing. Treatments were arranged in completely randomized design with three replications and data were analysed statistically at 5% level of significance.

Chlorophyll assay

The total chlorophyll content of plant flag leaves was measured according to Singh et al. [7]. 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 [10]. 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.

RESULTS AND DISCUSSION

Phosphate (P) solubilization potential has been attributed to the ability of the microorganisms to reduce the pH of the surrounding, either by releasing organic acids or protons [11]. This process leads to increased P availability, which ultimately increase plant P uptake. Phosphorous (P) is one of the essential element for growth and development of plants. Such microorganisms are called phosphate solubilizing microorganisms (PSM), those are capable of solubilizing the unavailable form of phosphate into available form [12]. In the present investigation a phosphate solubilizing bacterium *Chryseobacterium* sp. PSR10 was used to check their efficacy of plant growth promotion on finger millet under greenhouse conditions. The bacterial strain *Chryseobacterium* sp. PSR10 identified previously with accession number DQ-118018 [7].

In order to establish the relationship between phosphate solubilization capability and plant growth promotion, a pot experiment with sterilized and unsterilized soil was designed with *Chryseobacterium* sp. PSR10 under greenhouse conditions. Under sterilized and unsterilized soil system, the effect of PSR10 was significantly higher in unsterilized soil system in compare to sterilized system, except the result of plant dry weight, which showed better increment in sterilized soil system (Table 1). In unsterilized soil system the inoculated bacterium increased root length, shoot length, fresh eight and dry weight by 25.7%, 25%, 33.3% and 25.0%, while in sterilized soil system the growth is lesser in comparison to unsterilized soil system but significantly higher and increased by 23.2%, 9.7%, 22.9% and 47.0%, respectively, over control (Table 1). This finding supported by Singh *et al.* [13], in which they have reported the plant growth promotory effect of phosphate solubilizing bacterial isolates on *Cicer arietinum* in indigenous and alternate soil system under greenhouse conditions. However, the effect of PSB on mung bean (*Vigna radiata*) plants was observed by Walpola and Yoon [14]. Rafi *et al*. [15] reported that *Azospirillum* and PSB inoculants influence the growth and yield of foxtail millet.

PSB bring about the mobilization of insoluble phosphates in the soil and increase plant growth under conditions of poor phosphorus availability. These microorganisms also have the potential for ecological amelioration of P and thereby improve plant growth and the establishment of plants under low phosphorus availability. These beneficial bacteria enhance plant growth by improving soil nutrient status, secreting plant growth regulators and suppressing soil-borne pathogens [16]. Plant growth-promoting activities were found to be stimulated in the presence of microbial inoculant *Chryseobacterium* sp. PSR10. The increase in available P content may be due to the activities of introduced phosphate-solubilizing microflora, which might have dissolved chemically fixed inorganic phosphate compounds. This might be attributed to the greater uptake of this nutrient by the plant and chemical fixation of soluble P in soils [17].

The effect of *Chryseobacterium* sp. PSR10 on chlorophyll content and nitrate reductase activity of the plant leaves were also evaluated under both the soil system. The effect of the bacterium on chlorophyll content influencing in sterilized soil system, however, nitrate reductase activity increased in unsterilized soil system. In sterilized soil system the total chlorophyll content and nitrate reductase activity of plant leaves increase significantly by 35.5% and 28.0 %, respectively over control (Table 2). However, in unsterilized soil system increased by 23.4% and 38.0%, respectively over control (Table 2). Increased chlorophyll content and, subsequently, enhanced photosynthesis, is a known plant response to inoculation with several PGPBs [18, 19]. It was assumed that the increased production of photosynthesis enhanced plant growth and yield [18, 20]. The effect of *Chryseobacterium* sp. PSR10 inoculation on finger millet plants showed enhanced total chlorophyll content and nitrate reductase activity in comparison to uninoculated control (Table 2). The 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 [21].

Increased growth and P uptake of several plants due to PSB inoculation have been reported in a number of studies conducted under greenhouse and field conditions [7, 13, 22, 23, 24, 25]. Colonization of soil by non-indigenous microorganisms in unsterilized soils depends both on its interaction with indigenous microflora associated with plants and its ability to utilize diverse substrate in the soil [26]. In this study, it was observed that inoculation with P solubilizing strain of *Chryseobacterium* sp. PSR10 significantly promoted finger millet plant growth under different soil system. In general, inoculation resulted in enhancement of plant growth, chlorophyll content and nitrate reductase activity. These results are similar findings of Katiyar and Goel [27], who assessed inoculation effect of P solubilizing cold tolerant mutant of *Pseudomonas fluorescens* on growth of mung bean and resulted better growth. Enhancement of seedling growth due to seed treatment with phosphate solubilizing bacterial strain *Chryseobacterium* sp. PSR10 may be due to release of plant growth promoting substances. Plant growth promoting microbes are an important contributor to biofertilization of agricultural crops. Production of growth regulators by phosphate solubilizing bacteria has been studied by Ponmurugan and Gopi [28]. Chaykovskaya [29] reported that treatment with phosphate solubilizing bacteria resulted in increased yield of pea and barley.

Results of this study suggest that a PSB strain *Chryseobacterium* sp. PSR10 is a potential phosphate solubilizer and use of this bacterium as seed inoculants will improve seedling growth. This particular bacterial strain is effective in increasing plant growth viz., root & shoot length, fresh & dry weight, chlorophyll content and nitrate reductase activity under sterilized and unsterilized soil systems. The better performance of this bacterial strain could be attributed to its greater P solubilization activity. Therefore, present study suggested that it is might be possible to select bacterial strain *Chryseobacterium* sp. PSR10 as a good phosphate solubilizing bioinoculant which can be exploited as PSB.

Table 1: Effect of *Chryseobacterium* sp. PSR10 on plant growth parameters of finger millet.

Bacterial strain	Sterilized soil (SS)				Unsterilized soil (US)			
	Root length (cm)*	Shoot length (cm)*	Fresh Weight (gm) *	Dry Weight (gm) *	Root length (cm)*	Shoot length (cm)*	Fresh Weight (gm) *	Dry Weight (gm) *
Control	17.30	24.80	4.66	0.51	18.76	24.36	4.20	0.44
Chryseobacterium sp.	21.33	27.23	5.73	0.75	23.6	30.46	5.60	0.55
	(23.2)	(9.7)	(22.9)	(47.0)	(25.7)	(25.0)	(33.3)	(25.0)
SEm±	0.24	0.29	0.13	0.12	0.19	0.64	0.16	0.16
CD at 5%	0.96	1.15	0.54	0.48	0.75	2.50	0.63	0.63

Note: * Each value is the mean of three replicates. Values in parentheses indicate the percent increase over control. Data were analyzed statistically at the 5% ($p<0.05$) level of significance.

Table 2: Effect of *Chryseobacterium* sp. PSR10 on chlorophyll content and nitrate reductase activity of finger millet.

Bacterial strain	Sterilized soil (SS)		Unsterilized soil (US)	
	Chlorophyll Content (mg g^{-1} fr. wt)*	Nitrate reductase activity (mMol NO_2 g^{-1} fr. Wt. h^{-1})*	Chlorophyll Content (mg g^{-1} fr. wt)*	Nitrate reductase activity (mMol NO_2 g^{-1} fr. Wt. h^{-1})*
Control	1.35	1.00	0.94	1.00
Chryseobacterium sp.	1.83	1.28	1.16	1.38
	(35.5)	(28.0)	(23.4)	(38.0)
SEm±	0.41	0.19	0.28	0.10
CD at 5%	0.16	0.77	0.11	0.40

Note: * Each value is the mean of three replicates. Values in parentheses indicate the percent increase over control. Data were analyzed statistically at the 5% ($p<0.05$) level of significance.

CONCLUSION

The results of the study clearly showed that microbial inoculation as PSB improving the plant growth of finger millet. This practice is convenient and be cost-effective as well as ecofriendly. In summary, the final findings of this study of plant growth promotion showed that PSB can play an essential role in helping plants establish and grow under nutrient-deficient conditions. PSB are able to mobilize more P into plants, where hard soluble phosphates are present in soil and increase growth. Furthermore, the results of this study lead to the conclusion that bacterium *Chryseobacterium* sp. PSR10 might be used as a PSB for better crop production. Nonetheless, further studies are needed under field conditions with finger millet to confirm the present finding and their eventual commercial applications.

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REFERENCES

[1] Treen, H. 2005. Forging ahead with finger millet. New Agriculturist: Developments, pp. 1-2.
[2] Kumar, A. B. H., Sharanappa, K. T., Gowda, K. and Sudhir, K. 2003. Growth, yield and nutrient uptake as influenced by integrated nutrient management in dry land finger millet. *Mysore Journal of Agriculture Sciences*. 38(4): 487-495.
[3] Kloepper, J. W., Lifshitz, R. And Zablotowicz, R. M. 1989. Freeliving soil bacterial inocula for enhancing crop productivity. *Trends Biotechnology*. 7: 39-43.
[4] Vassileva, M., Vassilev, N., Fenice, M. and Federici, F. 2001. Immobilized cell technology applied in solubilization of insoluble inorganic (rock) phosphate and P plant acquisition. *Bioresource Technology*. 79: 263- 271.
[5] Bhattacharya, P. and Jain, R. K. 2000. Phosphorus solubilizing biofertilizers in the whirl pool of rock phosphate – challenges and opportunities. *Fertilizers News*. 45:45–52.
[6] Barroso, C. V., Pereira, G. T. and Nahas, E. 2006. Solubilization of $CaHPO_4$ and $AlPO_4$ by Aspergillus niger in culture media with different carbon and nitrogen sources. *Brazil Journal of Microbiology*. 37:434–438.
[7] Singh, A. V., Chandra, R. and Reeta, G. 2013. Phosphate solubilization by Chryseobacterium sp. and their combined effect with N and P fertilizers on plant growth promotion. *Archives of Agronomy and Soil Science*. 59(5): 641–651.
[8] Commare, R. R., Nandakumar, R., Kandana, A., Suresh, S., Bharathi, M., Raguchander, T. and Samiyappan, R. 2002. *Pseudomona ûuorescens* based bio-formulation for the management of sheath blight disease and leaf folder insect in rice. *Crop Protecection*. 21: 671-677.
[9] Lokesha, N. M. and Benagi, V. I. 2007. Biological Management of Pigeonpea Dry Root Rot Caused by *Macrophomina phaseolina. Karnataka Journal of Agricultural Sciences* 20(1): 54–56.
[10] Hageman, R. H. and Hicklesley, D. P. 1971. Nitrate reductase from higher plants. *Methods of Enzymology*. 23:491–503.
[11] Hariprasad, P. and Niranjana, S. R. 2009. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant and Soil*. 41: 95-98.
[12] Hilda, R. and Fraga, R. 1997. Phosphate solubilizing rhizobacteria and their role in plant growth promotion. *Biotechnological Advances*. 17: 319-359.
[13] Singh, A. V., Agarwal, A. and Goel, R. 2010a. Comparative phosphate solubilization efficiency of two bacterial isolates and their effect on *Cicer arietinum* seeds in indigenous and alternative soil system. *Environment and Ecology*. 28: 1979-1983.
[14] Walpola, B. C. and Yoon, M. H. 2013. Phosphate solubilizing bacteria: Assessment of their effect on growth promotion and phosphorous uptake of mung bean (*Vigna radiate* [L.]) R. Wilczek. 73(3): 275-28.
[15] Rafi, M. M., Varalakshmi, T. and Charyulu, P. B. B. N. 2012. Influence of *Azospirillium* and PSB inoculation on growth and yield of foxtail millet. *Journal of Microbiology and Biotechnology Research*. 2(4): 558-565.
[16] Gulati, A. Rahi, P. and Vyas, P. 2007. Characterization of phosphate solubilizing fluorescent *Pseudomonas* from the rhizosphere of seabuckthorn growing in the cold deserts of Himalayas. *Current Microbiology*. 56:73–79.
[17] Dadhich, S. K., Somani, L. L., Verma, A. 2006. Improved soybean yield, nutrient uptake and P enrichment in soil due to co-inoculation of phosphate solubilizing bacteria and VAM fungi in a clay loam soil. *Indian Journal of Microbiology*. 46:405–407.
[18] Alam, M. S, Cui, Z. J., Yamagishi, T., Ishii, R. 2001. Grain yield and related physiological characteristics of rice plants (Oryza sativa L.) inoculated with free-living rhizobacteria. *Plant Production Science*. 4:126–130.
[19] Sharma, A., Johri, B. N., Sharma, A. K. and Glick, B. R. 2003. Plant growth promoting bacterium Pseudomonas sp. strain GRP SUB 3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). *Soil Biology and Biochemistry*. 35:887–894.

[20] Panwar IDS. 1991. Effect of VAM and *Azospirillum brasilense* on photosynthesis nitrogen metabolism and grain yield in wheat. *Indian Journal of Plant Physiology*. 34:357–361.

[21] Solomonson LP, Barber, MJ. 1990. Assimilatory nitrate reductase functional properties and regulation. *Annual Review of Plant Physiology and Plant Molecular Biology*. 41:225–253.

[22] Singh, A. V., Prasad, B. and Shah, S. 2010b. Screening Plant growth promotory rhizobacteria for improving seed germination and seedling vigor of lentil (*Lens culinaris* Medik). *Environment and Ecology*. 28: 2055-2058.

[23] Singh, A. V., Prasad, B. and Shah, S. 2011. Influence of phosphate solubilizing bacteria for enhancement of plant growth and seed yield in lentil. *Journal of Crop and Weed*. 7(1). 1-4

[24] Reena, T., Dhanya, H., Deepthi, M. S. and Pravitha, D. 2013. Isolation of phosphate solubilizing bacteria and Fungi from rhizosphere soil from banana plants and its effect on the growth of *Amaranthus cruentus* L. *IOSR Journal of Pharmacy and Biological Sciences*. 5(3): 6-11.

[25] Singh, A. V. and Prasad, B. 2014. Enhancement of plant growth, nodulation and seed yield through Plant Growth Promoting Rhizobacteria in Lentil (*Lens culinaris* Medik cv. VL125). *International journal of current Microbiology and Applied Sciences*. 3(6): 614-622.

[26] Miethling R., Wieland, G., Backhaus, H. And Tebbe, C. C. 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin and inoculation with *Sinorhizobium meliloti* L33. *Microbial Ecology.* 41: 43-56

[27] Katiyar, V. and Goel, R. 2003. Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. *Microbiological Research*. 158:163–168.

[28] Ponmurugan P. and Gopi , C. 2006. In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. *African Journal of Biotechnology*. 5: 348-350.

[29] Chaykovskaya L. A., Patyka, V. P. and Melnychuk, T. M. 2001. Phosphorous mobilizing microorganisms and their influence on the productivity of plants. In: Plant Nutrition food security and sustainability of agroecosystem. Horst W.J. (Ed.) pp. 668-669.