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Effect of potassium and phosphorus solubilizing bacteria on growth parameters of chilli (*Capsicum annuum* L.) under Kashmir climatic conditions

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Abstract
Soil contains substantial reserves of total Phosphorus and Potassium but majority is present in the form of insoluble form and hence can not be utilized by plants. To overcome this deficiency in soils, Phosphorus and Potassium fertilizers are applied but due to soon fixation and precipitation of P and K becomes unavailable to plants. The P and K solubilizing bacteria are ubiquitous in soils and capable of hydrolysing inorganic and organic forms of P and K. Use of PSB and KSB as inoculants increases P and K uptake by plants and thus play an important role in plant nutrition. In the present study the Phosphorus and Potassium solubilizing bacteria, *Bacillus sp and Pseudomonas sp* were examined for plant-growth-promoting characteristics and nutrient uptake on chilli plant in K and P deficient soil in field experiments. Inoculation with bacterial strains were found to increase height, number of branches and number of fruits in Chilli (*Capsicum annuum* L., Kashmir long 1).Both PSB and KSB were able to mobilize Potassium and Phosphorus efficiently in plants when inorganic P and K were added to the soil.

Keywords: *Bacillus, Pseudomonas*, Chilli, Potassium (K), Phosphorus (P), PSB and KSB.

1. Introduction

Phosphorus is a bio-critical element in short supply in nature. Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant (Gyaneshwar *et al.*, 2002) [8] Approximately 95–99% of soil Phosphorous is present in the form of insoluble phosphates and hence can not be utilized by the plants (Alok *et al.*, 2013) [3]. Out of added phosphorus fertilizer only 10-20% is available for the plants. The rest remains in the soil as insoluble phosphate in the form of rock phosphate and tricalcium phosphate. Phosphate solubilizing Bacteria (PSB) significantly help in the release of this insoluble inorganic phosphate and makes it available to the plants. PSB are a group of beneficial bacteria capable of hydrolysing organic and inorganic phosphorus from insoluble compounds. Phosphate-solubilizing bacteria (PSB) are one of the most interesting microorganisms concerned with plant P nutrition. The principal mechanism for mineral phosphate solubilization by these bacteria is the production of low-molecular weight organic acids, which have high potential as cations bound to phosphate; as a result of their conversion into soluble forms. PSB has the ability to dissolve tricalcium phosphate from an insoluble form into a soluble form, as has been reported by many researchers.

Potassium (K) is the third important plant nutrient. Potassium plays a role in enzyme activation, maintaining cell turgor pressure, transportation of sugars and starches, plays a role in improving crop quality, Protein synthesis, Photosynthesis, increasing resistance against pests and diseases, and helping crops on stress conditions (Meena *et al*., 2014) [10]. Most of potassium (K) in the soil is present in mineral forms or non-exchangeable K which are not available for plants. The use of potassium solubilizing bacteria (PSB) as a biological fertilizer was suggested as a solution to improve plant nutrition. Soil microorganisms influence the availability of soil minerals, playing a central role in ion cycling and soil fertility (Bin Lian *et al.*, 2010) [6]. Very little of potassium source is available for plant use. Silicate solubilizing bacteria were found to resolve potassium, silicon and aluminium from insoluble minerals (Aleksandrov *et al*., 1967) [1]. Many microorganisms in the soil are able to solubilize 'unavailable' forms of K-bearing minerals, such as micas and illite by excreting organic acids which either directly dissolve rock K or chelate silicon ions to bring the K into solution (Friedrich *et al*., 1991; Ullman *et al*., 1996; Bennett *et al*., 1998) [7, 12, 5].

Therefore, the application of K -solubilizing microorganisms (KSM) (Barker *et al*., 1998) [4] is a promising approach for increasing K availability in soils. The major objective of the research was to determine the ability of Potassium and phosphorus solubilizing bacteria to enhance the growth of chilli plants grown in nutrient-deficient soil.

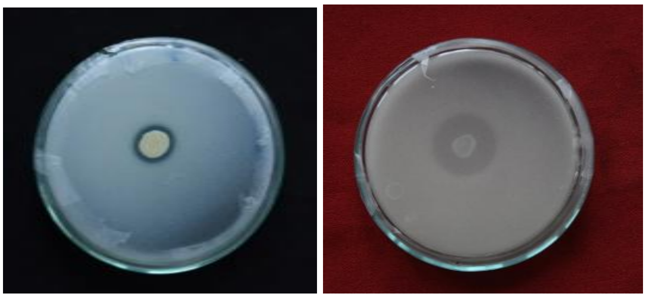
Materials and Methods

a) Collection of soil samples

The soil for the experiments was collected from a non-fertilized field site in Sopore, District Baramula, Jammu and Kashmir, India. The properties of the soil used in the experiments were: pH (1:1 w/v water) 6.80; organic carbon 1.03%; total N 0.11%; available P 20.77 kg/hac; total K2O 156 kg/ hac-v

Isolation and screening of PSB and KSB

The Soil samples were serially diluted and spread plated on Pikoviskayas agar (PVK) medium and Aleksandrov medium and incubated at 30 ^{0}C for 3–5 days. Colonies were selected on the basis of phosphate and potassium solubilization as indicated by clear halo around the bacterial colonies (FIG 1and Fig 2). Solubilization Efficiency (SE %) was calculated and those showing more than 50% SE were selected and further checked for their solubilization potential *in vitro*. The phosphorous and potassium solubilization potential of the selected PSB and KSB isolates was tested by estimating available phosphorous and potassium in the Pikoviskayas and Aleksandrov broth media amended with tricalcium phosphate and mica as a substrate. According to quantitative analysis results, PSB-2 and KSB-5 was the best phosphate and potassium solubilising bacteria among the various isolates obtained.



1. **2.**

Fig 1, 2: Phosphorus and Potassium Solubilizing bacteria on pikoviskayas and Aleksandrov media respectively.

Identification of PSB 2 and KSB 5

The bacterial isolate PSB2 and KSB5 was characterized for its morphological and biochemical characters as per the Bergey's manual. The isolate were subjected to testing of morphological characters by Gram staining (Gram nature, shape & arrangement) and Schauffer Fulton (spore) staining methods. A Hi assorted Biochemical Kit (Hi Media) was used to study the biochemical characters of the organism. The organism was isolated and purified and pure culture was inoculated into nutrient broth till it showed absorbance of 1.0 OD at 620 nm. Kit was opened aseptically. Each well was inoculated with 50µl of the inoculum by surface inoculation method. Temperature was maintained at 37 °C for 24 - 48 hours and the results were checked and compared with standard interpretation chart Potassium and phosphorus solubilizing bacterial strains were selected from 5 potassium solubilizing bacterial strains isolated from Sopore, wadura and Rafiabad, of District Baramula, Kashmir, India. Secondary screening was carried out from the different isolates by studying their ability of higher potassium solubilization by Khandeparkar's selection ratio. *Bacillus* – PSB 2 bacterial strain and *Pseudomonas* KSB 5 strain were selected and identified using standard cultural, morphological and biochemical methodology.

Colonies exhibiting clearance zone indicating P and K Solubilization were selected. The solubilization index was obtained by formula: PSI= Colony diameter + Diameter of zone/colony Diameter. Selected colonies are morphologically distinct. Total 10 bacterial isolates were selected as P and K solubilizers and named as PSB 1-5 and KSB 1-5. (Table 1)

Table 1: Details of solubilization index of PSB and KSB isolates

S. No.	PSB and KSB isolates	Solubilization index (*Si*)
1	PSB-1	1.20
2	PSB-2	5.33
3	PSB-3	0.50
4	PSB-4	2.20
5	PSB-5	1.00
6	KSB-1	1.00
7	KSB-2	1.25
8	KSB-3	2.50
9	KSB-4	2.64
10	KSB-5	4.66

Morphological and biochemical characterization of the five isolates of PSB and KSB, PSB-2 and KSB -5 exhibiting higher solubilization index (*Si*) for Phosphorus and Potassium solubilization were selected. The morphological and biochemical characterization of 2 best isolates are given in Table 2.

Table 2: Morphological and Biochemical characterization

Characteristics		
Colony characteristics	**PSB -2**	**KSB -5**
Size	Medium	Small
Shape	Round	Round
Margin	Entire	Entire
Elevation	Raised	Convex
Texture	Rough	smooth
Pigmentation	White	green
Morphological	PSB -2	KSB -5
Cell shape	Long rod	Short rod
Arrangement	Chain	singly
Gram reaction	+ve	+ve
Spore	+	-
Biochemical	PSB -2	KSB -5
MR	+	-
VP	-	+
CA	+	+
UH	+	+
SU	+	_
ML	+	+
Species	*Bacillus sp*	*Pseudomonas sp*

MR = Methyl red test, VP = V.P Test, UH = Urea hydrolysis, CA = Casein test, SU = Sucrose, ML= Maltose

Results and Discussion

Table 3. Impact on growth parameters of chilli

The results of the inoculation assays are shown in Tables 3. Significant increases of number of branches, height and number of fruits were observed when the soil was inoculated with phosphorus and Potassium Solubilizing bacteria, compared to the soil without inoculum. The significant increases were obtained for plants grown in soil with strain PSB-2 and KSB - 5 as compared to rest and uninoculated soil. Soil inoculation with PSB-2 and KSB- 5 significantly increased plant growth in terms of plant height and number of branches. Further significant increases in available K and P content of the plants were generally found in the all-integrated.

Seedling, root dipping + soil application - seedlings were treated with microbial inoculants for 30 minutes before plantation and microbial inoculants were inoculated in soil. Plants were grown in field for 95 days under Kashmir climatic conditions with a temperature of 25–35 ^{0}C.The soil was moistened with water and maintained at 60% of its holding capacity. After 95 days of plantation shoots and roots were separated and dried at 105 ^{0}C before that the height, number of branches and number of fruits were determined. The criteria for growth promotion were studied as total P and K content of plants (Abul Fadl, 1948) [2].

Increased K Uptake by Plants

Six seedlings (chilli plants) were planted at 2 inch depth in each experimental block. Bacterial cells in the exponential phase were collected by centrifugation at 7000 rpm for 10 min at 60 ^{0}C, washed with distilled water and recentrifuged. Bacterial inoculum was prepared by resus pending pelleted cells in sterile distilled water. Seedlings of chilli were inoculated with 1 ml of bacterial suspension which resulted in an inoculum.

Table 3: Effect on growth of chilli plants

Treatments	Number of Branches	Plant height	Number of fruits per plant
	Mean ± SD	Mean ± SD	Mean ± SD
CONTROL	10.60 ± 0.22	22.60 ± 0.97	27.16 ± 2.30
INSOLUBLE K	10.73 ± 0.41	23.26 ± 1.69	27.90 ± 3.46
PSB	14.03 ± 0.98	35.33 ± 1.97	49.56 ± 1.55
KSB	14.43 ± 0.76	38.70 ± 1.67	59.03 ± 3.80
PSB+KSB	23.17 ± 1.36	56.97 ± 1.55	92.46 ± 6.44
CD = (P <0.05)	1.111	1.106	1.128
CV%	5.669	2.132	1.753

Discussion

Phosphorus and potassium are major essential macronutrients for plant growth and development. Potential bacteria such as phosphate Solubilizing bacteria and Potassium solubilizing bacteria are successfully found from rhizospheric soils of apple from district Baramula, Jammu and Kashmir, India. The bacteria could dissolve non-readily available forms of soil phosphorus and also potassium and play an essential role in providing it to plants. Therefore, direct application of phosphate and potassium solubilizing bacteria may be more useful and environmentally safer than chemical P and K fertilizers (Parmar & Sindhu 2013) [11]. Thomas and Bertsch *et al*., 1985) reported that most of the potassium in soil exists in the form of silicate minerals. The phosphorus and potassium can become available to the plants when minerals are solubilized. Schrocder (1978) pointed out that due to their different lattice structure of potassium and other minerals are released with different speeds from the silicate minerals. In uninoculated soils, the total K per plant was greatly increased if soluble potassium was added, but this increment was higher when the soil was inoculated with the strain PSB-2 and KSB - 5. Lin *et al.* (2002) [9] demonstrated that bacterial inoculation could resulted in growth promotion and higher K contents of plant components. The results showed that the survival of strain *Bacillus (PSB -2) and pseudomonas (KSB -5)* was much better in soil of Kashmir long 1 variety.

Conclusion

Phosphorus (P) and potassium (K) solubilizing bacteria were isolated from the rhizospheric soils of apple trees from district Baramula, Jammu and Kashmir, India. After secondary Screening two isolates were selected from 10 isolates able to solubilize higher amount of phosphorus and potassium minerals. Among them PSB-2 was gram positive long rod identified as *Bacillus species* while as KSB-5 was gram negative short rod identified as *pseudomonas sp.* When the soil was inoculated with these two Potassium and Phosphorus solubilizing strain (PSB-2 and KSB- 5). The total P and K content per plant was increased than those which are uninoculated (control) and the plant height, number of branches and number of fruits were increased.

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