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Isolation, Characterization and Screening of Phosphate Solubilizing Bacteria

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Thirty phosphate solubilizing bacteria (PSB) were isolated from rhizosphere of maize crop from different maize growing areas of Kashmir valley by serial dilution technique using Pikovskaya's agar medium and were compared with one another for P-solubilization, production of IAA and GA (growth promoting substances), siderophore production and ammonia production. The PSB (K_4) proved to be the most efficient strain and was identified as *Pseudomonas* sp. On screening the isolate recorded maximum phosphorus solubilization (60.00 μg ml^{-1}), produced highest amount of IAA (36.07 μ mol ml^{-1}) and GA (38.20 μ mol ml^{-1}) which was significantly superior over all other isolates and thus can be exploited as biofertilizer. The isolate also showed ammonia and high siderophore production.

Key words: characterization, Isolation, Kashmir, Phosphate solubilizing bacteria, Screening.

Phosphorus (P) is the second most important macro-nutrient required by plants, next to nitrogen. Compared to other essential macronutrients (with exception of nitrogen), P is one of the less-abundant (0.1% of total) elements in the lithosphere thus often regarded as a limiting nutrient in agricultural soils. Therefore, it becomes quite common to use chemical fertilizers in ensuring phosphorous requirement fertilizer to soil. Upon application as inorganic phosphorus rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils or with Ca in calcareous soils thus becomes unavailable to plants. Frequent application of chemical fertilizers, on the other hand, is recognized to be a costly affair and environmentally undesirable too. Microorganisms which are capable of solubilizing insoluble phosphate, also called phosphate solubilizing microorganisms (PSMs) not only provide plants with phosphorus, but also facilitate the growth of plants through (a) fixing atmospheric nitrogen (b) accelerating the accessibility of other trace elements (c) producing plant hormones such as auxins , cytokinins and gibberellins (d) releasing siderophores , hydrogen cyanide , enzymes and/ or fungicidal compounds such as chitinase, cellulose, protease which ensure antagonism against phytopathogenic microorganisms.The present study was undertaken to isolate phosphate solubilizing bacteria from rhizosphere of maize and screen them for their phosphorus solubilizing activity.

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MATERIALS AND METHODS

Soil Samples for isolation of phosphate solubilizing bacteria were collected from rhizosphere of maize crop .Total 30 isolates were obtained . Phosphate solubilizing bacteria were

Table 1. Screening of Isolates for Tricalcium Phosphate Solubilization

District	Isolates	Coloney Characters	Cell shape	Gram reaction	P-solubilized (µg ml^{-1}) Qualitative	Quantitative
Baramulla	PSB (B_1)	Cream,circular raised,moderate,smooth, opaque	Rod	Gram-ve	+	58.20
	PSB (B_2)	Cream, irregular, raised, Small, rough, opaque	Rod	Gram-ve	+	55.13
	PSB (B_3)	White, irregular, raised, pinpoint, rough,opaque	Rod	Gram-ve	+	49.00
	PSB (B_4)	Dull white, irregular, flat, small, smooth, opaque	Rod	Gram-ve	+	45.00
	PSB (B_5)	Dull white, irregular, flat, moderate, rough, opaque	Rod	Gram+ve	+	46.00
	PSB (B_6)	Dull white, irregular, raised, moderate, rough,opaque	Rod	Gram+ve	+	47.31
Budgam	PSB (Bu_1)	Dull white, circular, raised, moderate, smooth, opaque	Rod	Gram-ve	+	55.00
	PSB (Bu_2)	Dull white, circular, raised, small, smooth, opaque	Rod	Gram+ve	+	49.67
	PSB (Bu_3)	Glistening white, irregular, raised, moderate, rough, opaque	Rod	Gram+ve	+	56.93
	PSB (Bu_4)	Dull white, circular, raised, small, smooth, opaque	Rod	Gram-ve	+	44.93
	PSB (Bu_5)	Glistening white, irregular, flat, moderate, rough, opaque	Rod	Gram-ve	+	45.97
	PSB (Bu_6)	White, irregular, raised, pinpoint, rough, opaque	Rod	Gram-ve	+	47.97
Ganderbal	PSB (G_1)	Dull white, irregular, flat, large, rough, opaque	Rod	Gram-ve	+	55.27
	PSB (G_2)	Dull white, circular, raised, small, smooth, opaque	Rod	Gram+ve	+	51.00
	PSB (G_3)	Dull white, irregular, flat, small, smooth, opaque	Rod	Gram+ve	+	45.00
	PSB (G_4)	Cream, circular, raised, moderate, smooth, opaque	Rod	Gram+ve	+	49.00
	PSB (G_5)	Dull cream, irregular, flat, large, rough, opaque	Rod	Gram+ve	+	45.07
	PSB (G_6)	Dull white, circular, raised, moderate, smooth, opaque	Rod	Gram-ve	+	47.83
Kupwara	PSB (K_1)	Cream, irregular, raised, Small, rough, opaque.	Rod	Gram-ve	+	57.00
	PSB (K_2)	Glistening white, irregular, raised, moderate, rough, opaque	Rod	Gram+ve	+	44.20
	PSB (K_3)	Dull white, irregular, flat, moderate, rough, opaque	Rod	Gram-ve	+	46.90
	PSB (K_4)	Glistening white, irregular, flat, moderate, rough, opaque	Rod	Gram-ve	+	60.00
	PSB (K_5)	Dull white, circular, raised, small, smooth, opaque	Rod	Gram-ve	+	49.33
	PSB (K_6)	Dull white, irregular, flat, moderate, rough, opaque	Rod	Gram-ve	+	52.33
Srinagar	PSB (S_1)	Dull white, circular, raised, moderate, smooth, opaque	Rod	Gram+ve	+	55.00
	PSB (S_2)	Glistening white, irregular, raised, moderate, rough, opaque	Rod	Gram+ve	+	45.33
	PSB (S_3)	Dull white, circular, flat, small, smooth, opaque	Rod	Gram-ve	+	49.97
	PSB (S_4)	Cream, irregular, raised, Small, rough, opaque	Rod	Gram-ve	+	55.33
	PSB (S_5)	White, irregular, raised, large, rough, opaque	Rod	Gram-ve	+	47.00
	PSB (S_6)	Dull white, circular, raised, small, smooth, opaque	Rod	Gram-ve	+	51.32
CD ($p \leq 0.05$)					-	1.0

Table 2. Biochemical Characterization Of Isolates (+ = Positive Test; - = Negative Test)

District	Isolates	Starch hydrolysis	Casein hydrolysis	Lipid hydrolysis	Gelatin liquefaction	Citrate utilization	Hydrogen sulfide test	Urease test	Catalase test	Methyl Red test test	Voges Proskaur	Indole productin	Oxidase test
Baramulla	PSB (B_1)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (B_2)	-	+	+	-	+	-	-	+	-	-	-	-
	PSB (B_3)	-	+	+	-	+	-	-	-	-	-	-	-
	PSB (B_4)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (B_5)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (B_6)	-	+	+	+	+	-	-	+	-	-	-	-
Budgam	PSB (Bu_1)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (Bu_2)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (Bu_3)	-	+	+	-	+	-	-	+	-	-	-	-
	PSB (Bu_4)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (Bu_5)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (Bu_6)	-	+	+	+	+	-	-	-	-	-	-	-
Ganderbal	PSB (G_1)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (G_2)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (G_3)	-	+	+	-	+	-	-	+	-	-	-	-
	PSB (G_4)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (G_5)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (G_6)	-	+	+	+	+	-	-	+	-	-	-	-
Kupwara	PSB (K_1)	-	+	+	+	+	-	-	-	-	-	-	-
	PSB (K_2)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (K_3)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (K_4)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (K_5)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (K_6)	-	+	+	+	+	-	-	+	-	-	-	-
Srinagar	PSB (S_1)	+	+	+	+	+	-	+	+	-	-	-	-
	PSB (S_2)	-	+	+	-	+	-	-	+	-	-	-	-
	PSB (S_3)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (S_4)	-	+	+	+	+	-	-	+	-	-	-	-
	PSB (S_5)	-	+	+	+	+	-	-	-	-	-	-	-
	PSB (S_6)	-	+	+	+	+	-	-	+	-	-	-	-

isolated from collected soil samples by serial dilution technique (Pikovskaya, 1948) using Pikovskaya's agar medium which is a selective medium for isolation of phosphate solubilizers. All phosphate solubilizing bacteria isolates were examined for the colony features, cell shape, gram reaction, as per the procedures given by Anon (1957) and Bartholomew and Mittewer (1950).The biochemical characterization of the isolates was essentially done as per the procedures outlined by Cappuccino and Sherman (1992).The estimation of solubilization of tri calcium phosphate was done on Pikovskaya's agar medium by serial dilution technique (Pikovskaya, 1948). The amount of Pi released in the Pikovskaya's broth by the isolates was estimated after 10 days of inoculation by phosphomolybdic blue colour method (Jackson, 1973).All the isolates were subjected to qualitative analysis for the production of indole acetic acid (IAA) and gibberlic acid (GA) as per the procedures given by (Bric *et al.*, 1991) for IAA and (Brown and Burlingham,1968) for GA.Quantitative estimation of IAA was done by method given by (Gordon and Paleg, 1957) and that of GA was done by method given by (Paleg, 1965).Production of siderophore was assessed by plate assay Chrome Azurol S blue agar medium (CAS) was used to detect siderophore production by the isolates (Schwyn and Neilands, 1987).The production of ammonia by the isolates was detected by the procedure given by Dye (1962).

Table 3. Production Of Plant Growth Promoting Substances By Isolates

District	Isolates	IAA (µ mol ml^{-1})		GA (µ mol ml^{-1})		Siderophore	Ammonia
		Qualitative	Quantitative	Qualitative	Quantitative		
Baramulla	PSB (B_1)	-	ND	+	11.60	+	+
	PSB (B_2)	+	11.47	-	ND	++	+
	PSB (B_3)	+	12.80	+	15.67	ND	+
	PSB (B_4)	+	11.83	+	21.23	+	++
	PSB (B_5)	+	14.20	+	17.33	++	+
	PSB (B_6)	-	ND	-	ND	ND	+
Budgam	PSB (Bu_1)	+	11.93	+	11.47	+	+
	PSB (Bu_2)	+	10.53	+	9.70	ND	ND
	PSB (Bu_3)	+	12.20	-	ND	ND	+
	PSB (Bu_4)	+	26.77	+	24.83	++	++
	PSB (Bu_5)	+	13.77	+	14.63	+	++
	PSB (Bu_6)	-	ND	+	10.80	+	+
Ganderbal	PSB (G_1)	+	12.40	-	ND	+	ND
	PSB (G_2)	+	11.67	+	12.77	+	+
	PSB (G_3)	-	ND	+	11.60	+	+
	PSB (G_4)	+	14.10	-	ND	+	+
	PSB (G_5)	+	12.00	+	11.50	++	+
	PSB (G_6)	-	ND	+	15.30	ND	+
Kupwara	PSB (K_1)	+	15.40	+	3.30	+	+
	PSB (K_2)	-	ND	-	ND	+	ND
	PSB (K_3)	+	11.50	+	15.40	ND	ND
	PSB (K_4)	+	36.07	+	38.20	++	+
	PSB (K_5)	+	9.70	+	10.30	+	+
	PSB (K_6)	+	10.17	+	20.07	+	+
Srinagar	PSB (S_1)	+	12.27	+	30.70	+	+
	PSB (S_2)	+	14.40	-	ND	+	++
	PSB (S_3)	-	ND	+	14.77	+	ND
	PSB (S_4)	+	13.63	+	15.27	+	+
	PSB (S_5)	+	15.57	+	25.47	ND	+
	PSB (S_6)	+	22.13	+	18.80	+	+
CD ($p \leq 0.05$)		-	1.26	-	1.25	-	-

+ = Low production; ++ = High production; ND = Not detected

RESULTS AND DISCUSSION

Characterization of phosphate solubilizing bacteria

All the 30 phosphate solubilizing bacterial isolates obtained were subjected to morphological (Table 1) and biochemical tests (Table 2) and were grouped into 2 genera according to Bergey's Manual of Determinative Bacteriology. Out of 30 bacteria, 19 isolates belonged to the genus *Pseudomonas*, 11 to *Bacillus*, indicating the predominance of pseudomonads among P-solubilizers. These results are in agreement with the findings of several workers (Maheshkumar, 1997; and Narsian and Patel, 2006), who have also observed predominant occurrence of P-solubilizing *Pseudomonas*, *Bacillus* and *Xanthomonas* in the rhizosphere of different crop plants.

Screening of isolates for beneficial traits

Phosphate solubilizing ability

Among the 30 isolates PSB (K_4) recorded maximum phosphorus solubilization (60.00 µg ml^{-1}) which was significantly superior over all other isolates (Table 1). Differences existed among the isolates with respect to the amount of phosphorus released from tri calcium phosphate. Such differences among the strains to solubilize inorganic phosphate have been reported earlier by Deepa (2000)) and were ascribed to genetic make of the strains Narsian and Patel (2006).

Production of IAA and GA (growth promoting substances)

23 PSB isolates were found to produce both IAA and GA. The amount of IAA produced by the isolates ranged from 9.70 to 36.07 µ mol ml^{-1} and GA ranged from 9.70 to 38.20 µ mol ml^{-1} .Isolate PSB (K_4) produced highest amount of IAA (36.07 µ mol ml^{-1}) and GA (38.20 µ mol ml^{-1}) which was significantly superior over other isolates (Table 3). Variation in the production of IAA among the isolates could be due to metabolic variability among the strains (Leinhos and Vacek, 1994).

Siderophore production

23 PSB isolates were positive for siderophore production and out of them 5 isolates showed high siderophore production (Table 3). The release of siderophores by phosphate solubilizing microorganisms was also reported by Panhwar *et al*. (2012).

Ammonia production

25 isolates were positive for ammonia production and out of them 4 isolates showed high ammonia production (Table 3). The production of ammonia by phosphate solubilizing bacteria was also reported by Kumar *et al*. (2012).

CONCLUSION

The most efficient strain PSB (K_4) isolated from maize rhizosphere of Khumriyal district Kupwara was identified as *pseudomonas* sp. and proved to be good phosphate solubilizer ,showed high production of growth promoting substances and other beneficial traits.Thus can be exploited as biofertilizer.

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