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Characterization and Evaluation of Bacterial Isolates Solubilizing both Potassium and Phosphorus from Different Rhizosphere Soil

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Fifty isolates of bacteria solubilizing both potassium and phosphorus was isolated from rhizosphere soil collected from different locations on modified Aleksandrov medium containing potassium and phosphorus sources. All the isolates were characterized upto genus level based on morphological and biochemical characters. Thirty nine isolates were Gram positive, rod shaped and spore former were identified as *Bacillus* species and 11 isolates was Gram negative, rod shaped and non spore former were identified as *Pseudomonas* species. And functionally these isolates possessed the ability to solubilize both potassium and phosphorus minerals in medium. The maximum solubilization of potassium and phosphorous observed at 15 days after incubation ranged from 2.36 to 29.83 µg/ml and 3.44 to 14.25 per cent, respectively. They also produced growth promoting substance like IAA (3.38 to 8.90 µg/25ml) and GA (1.27 to 3.67 µg/25ml).

Key words: *Bacillus, Pseudomonas,* Aleksandrov medium,

Phosphorus and potassium are the major essential macronutrients required for the biological growth and development of crop. However, the concentrations of soluble P and K in soil are usually very low and the large proportions of P and K in the soil are insoluble rocks, minerals and other deposits. Microorganisms play a key role in the P and K cycle. There are considerable populations of P or K-solubilizing bacteria in soil and plant rhizosphere. Different bacterial species such as *Pseudomonas, Agrobacterium, Bacillus, Rhizobium* and *Flavobacterium,* have been tested for their ability to solubilize inorganic phosphate compounds such as tri-calcium phosphate, hydroxylapatite and rock phosphate.

A wide range of bacteria namely *Pseudomonas, Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, B. edaphicus, B. circulans* and *Paenibacillus* sp. have been reported to release potassium in accessible form potassium-bearing minerals in soils (Sheng, 2006). These potassium solubilizing bacteria were found to solubalize potassium, silicon and aluminum from insoluble K-bearing minerals such as micas, illite and orthoclases, by excreting organic acids which either directly dissolved rock K or chelated silicon ions to bring K into the solution.

The present study aims to isolate and characterize bacterial strains which are able to solubalize both potassium and phosphorus containing minerals and influence growth promoting activity of maize plant.

MATERIAL AND METHODS

The rhizosphere soil of different crop plants of sorghum, maize, chilli, cotton and banana were collected from Dharwad, Haveri and Davanagere districts of Karnataka during *kharif*

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2012. The bacteria were isolated by liquid modified Aleksandrov medium (Glucose, 5g; $MgSO_4.7H_2O$, 0.005g; FeCl3, 0.1g; Calcium carbonate, 0.1g; Calcium phosphate, 2g; Potassium aluminum silicate, 2g; tri calcium phosphate, 5g; Mica, 2g; Yeast extracts, 2g; agar, 20g; distilled water, 1000 ml and tri calcium phosphate and Mica as phosphorus and Potassium sources.) (Hu *et al.*, 2006). Rhizosphere soil (5gm) was added to 25 ml medium and shaken for 48 h at 150 rpm at 30 ^{0}C. Serial dilution (upto 10^{-6} dilution) were made, plated onto the same medium and incubated for 24 h at 30 ^{0}C. The colonies on the plate were picked and grown in the same broth for 48 h at 150 rpm at 30 ^{0}C and then re-isolated by streaking on fresh plates and colonies were selected based on colony colour and morphology.

The bacterial isolates were examined for the colony morphology, cell shape, Gram's reaction and ability to form spores as per the standard procedures given by Barthalomew and Mittewer (1950). The biochemical characterization including starch hydrolysis, casein hydrolysis, catalase test, Gelatin liquefaction and methyl red test.

The bacterial isolates examined for their ability to release K and P in modified Aleksandrov broth. One ml of overnight culture of each isolate was inoculated to 25 ml in nine replicates. All the inoculated flasks were incubated for two weeks at 28±2 °C. The amount of K^+ and Pi released in the broth was estimated at 5, 10 and 15 days of incubation from triplicate flasks at each stage in comparison with uninoculated control. The broth cultures were centrifuged at 10,000 rpm for 10 min in the micro centrifuge to separate the supernatant from the cell growth and insoluble potassium and phosphate. The available K content in the supernatant was determined by flame photometry method (Sugumaran and Janarthanam, 2007) and available P content estimated by phosphomolybdic blue color method (Jackson, 1973).

The isolates were subjected to qualitative analysis for the production of IAA (Bric *et al.*, 1991) and GA (Brown and Burlingham, 1968). Quantitative estimation of IAA (Gordon and Paleg, 1957) and GA (Paleg, 1965).

RESULTS AND DISCUSSION

Total 50 bacterial isolates were obtained from rhizosphere soil of different crop plants around Dharwad, Haveri and Davanagere districts which having ability to solubilizing both potassium and phosphorus and examined.

All the 50 isolates of K-PSB were identified and classified upto genus level based on their morphological and biochemical characters. Among them, 39 isolates were Gram positive, rod shape and spore forming identified as genus *Bacillus* and showed positive results on starch hydrolysis, casein hydrolysis, catalase test, gelatin liquefaction and methyl red test. But 11 isolates were Gram negative, rod shape, no spore former and showed negative results on all the above biochemical tests named as genera *Pseudomonas* sp.

The results were agreed with the findings of Hu *et al*. (2006) isolated two phosphate and potassium solubilizing *Bacillus* sp. from the soils in the modified Aleksandrov medium of containing phosphorite and potassium minerals like kaolinite and potassium feldspar. Norkina and Pumpynaskaya (1956) isolated two strains of *Bacillus* sp. and *Pseudomonas* from rhizosphere soils of various crops which soluiblize mineral potassium. Gaur *et al*. (1973) isolated 2-keto gluconic acid producing *Pseudomonas* strain solubilize minerals like quartz, silicates and phlogopite.

The qualitative analyses of the isolates for both potassium and phosphorus solubilization were examined by the diameter of solubilization zone formed by the isolates ranged from 3.0 to 11.5 mm at 72 h after incubation (Table: 1 and plat:1). The isolates K-PSB 32 recorded maximum solubilization (11.5 mm in diameter) followed by K-PSB 36 (11.0 mm). Such observation were made earlier that among the K and P bearing silicate minerals mica and TCP was found to be solubilize readily (Sugumaran and Janarthanam, 2007;).

The quantitative estimation of K and Pi solubilizing activity of K-PSB isolates released from muscovite mica and tri-calcium phosphate in a modified Alexandrov broth by the isolates were studied at 5, 10 and 15 days after incubation (DAI) (Table 2). The results were indicated that, amount

of K and Pi released from the different minerals mica and TCP. All the strain increased with increase in the incubation time and maximum at 15 DAI. The K released from mica by strain at 15 DAI ranged from 2.36 to 29.83µg/ml and Pi released from tri-calcium phosphate (TCP) ranged from 3.44 to 14.25 per cent.

Among the isolates K-PSB 32 released maximum amount of K from mica and P from TCP were 29.83 µg/ml and 14.25 per cent , respectively followed by K-PSB 21, 28.74 µg/ml and 13.50 per cent and K-PSB 36 (27.76 µg/ml and 13.66 per cent) these isolates were significantly superior over all other isolates. The findings are in agreed with the findings of Hu and Boyer (1996) reported that *Bacillus megaterium* was capable of solubilizing mica in appreciable amounts. The different efficiency of bacteria to solubilize insoluble form potassium and phosphate could be due difference in their ability to release organic acids (Sheng and He, 2006).

Hu *et al*. (2006) reported that *B. megatherium* and *B. mucilaginosus* were capable of solubilizing both rock phosphate and potassium. The co-inoculation of these two *Bacillus* sp. were potential in solubilizing potassium rocks. The present study, indicated that *Bacillus* sp. was also capable of releasing some amount of phosphorus from TCP (14.25%) but was comparatively very less (Badar, 2006).

All the 50 isolates were examined for the production of IAA and GA on Luria's Agar supplemented with SDS (0.01%) and glycerol (1%). Based on the development of red colour on the filter paper or green fluorescence under UV light. All the 50 isolates were positive for IAA and GA production. All the isolates were earlier identified as K and P solubilizers. Based on solubilization the selected isolates were further subjected to quantification of the IAA and GA.

The amount of IAA and GA produced by the selected nine isolates were determined at 10 DAI and the results are presented in Table 3. The amount of IAA produced by different strains ranged from 3.38 to 8.90 µg/25ml broth. Among the isolates examined K-PSB 50 was found to produce the highest amount of IAA 8.9 µg/25ml broth, which was on par with K-PSB 32 (8.41 µg/25ml broth), while five isolates showed more than

Table 1. Zone of solubilization by K-PSB isolates at 72 hours of incubation

S. No	Isolate no	Zone of solubilization (Dia in mm)
1	K-PSB 1	4.0
2	K-PSB 2	9.5
3	K-PSB 3	6.5
4	K-PSB 4	6.0
5	K-PSB 5	7.2
6	K-PSB 6	7.0
7	K-PSB 7	5.2
8	K-PSB 8	8.0
9	K-PSB 9	5.2
10	K-PSB 10	7.1
11	K-PSB 11	5.0
12	K-PSB 12	7.2
13	K-PSB 13	6.0
14	K-PSB 14	7.0
15	K-PSB 15	6.2
16	K-PSB 16	5.0
17	K-PSB 17	4.2
18	K-PSB 18	5.5
19	K-PSB 19	6.2
20	K-PSB 20	10.0
21	K-PSB 21	9.2
22	K-PSB 22	8.1
23	K-PSB 23	6.2
24	K-PSB 24	9.1
25	K-PSB 25	7.1
26	K-PSB 26	7.4
27	K-PSB 27	4.5
28	K-PSB 28	8.2
29	K-PSB 29	10.0
30	K-PSB 30	4.5
31	K-PSB 31	6.0
32	K-PSB 32	9.5
33	K-PSB 33	7.0
34	K-PSB 34	5.4
35	K-PSB 35	10.0
36	K-PSB 36	11.0
37	K-PSB 37	7.2
38	K-PSB 38	7.0
39	K-PSB 39	11.5
40	K-PSB 40	6.2
41	K-PSB 41	6.8
42	K-PSB 42	7.2
43	K-PSB 43	5.2
44	K-PSB 44	4.2
45	K-PSB 45	3.0
46	K-PSB 46	5.2
47	K-PSB 47	5.5
48	K-PSB 48	7.0
49	K-PSB 49	10.0
50	K-PSB 50	10.0

Table 2. Release of K^+ ion and Pi from mica and TCP by bacteria solubilizing both potassium and phosphorus at three incubation period

S. No	Isolate no	5 DAI		10 DAI		15 DAI	
		Potassium (ppm)	Phosphorus (%)	Potassium (ppm)	Phosphorus (%)	Potassium (ppm)	Phosphorus (%)
1	K-PSB 1	5.09	2.38	7.28	3.38	9.76	5.39
2	K-PSB 2	7.54	4.72	16.50	9.48	23.87	12.05
3	K-PSB 3	3.35	3.14	4.51	3.65	6.04	4.39
4	K-PSB 4	1.48	5.38	2.96	6.39	4.33	8.69
5	K-PSB 5	2.85	2.15	5.53	5.49	6.63	7.27
6	K-PSB 6	3.60	1.27	4.68	3.45	7.38	5.55
7	K-PSB 7	4.17	1.28	7.77	4.73	9.90	6.54
8	K-PSB 8	2.56	3.39	4.89	5.50	7.51	6.59
9	K-PSB 9	1.53	6.34	2.52	9.93	3.55	10.84
10	K-PSB 10	2.35	7.17	3.53	9.39	4.46	9.22
11	K-PSB 11	6.71	1.38	10.26	3.48	11.71	5.49
12	K-PSB 12	5.58	2.59	7.38	3.46	9.53	4.45
13	K-PSB 13	1.92	6.54	3.39	7.36	5.93	8.87
14	K-PSB 14	2.32	4.43	4.50	5.63	7.48	6.47
15	K-PSB 15	3.35	1.38	5.54	3.40	7.66	4.48
16	K-PSB 16	1.87	5.30	2.63	6.46	4.66	8.60
17	K-PSB 17	1.00	3.34	1.56	5.29	3.49	7.36
18	K-PSB 18	1.28	7.02	2.40	7.66	5.48	9.07
19	K-PSB 19	4.65	3.43	5.78	4.45	7.35	6.19
20	K-PSB 20	8.01	4.95	18.50	10.49	26.83	14.13
21	K-PSB 21	7.09	6.35	17.55	9.63	28.74	13.50
22	K-PSB 22	5.62	0.72	9.49	2.58	8.05	3.46
23	K-PSB 23	3.57	4.78	6.17	5.42	8.41	6.56
24	K-PSB 24	1.68	5.45	2.44	7.45	3.67	9.37
25	K-PSB 25	6.30	1.30	7.19	2.76	9.60	4.46
26	K-PSB 26	3.85	3.46	5.50	4.20	8.35	6.39
27	K-PSB 27	1.83	5.56	2.61	6.41	4.42	7.63
28	K-PSB 28	7.36	7.04	15.09	9.46	26.15	13.93
29	K-PSB 29	4.41	2.60	5.81	3.32	7.46	5.57
30	K-PSB 30	8.31	1.51	10.80	2.28	13.36	3.44
31	K-PSB 31	8.08	0.68	9.42	3.69	11.76	5.55
32	K-PSB 32	7.95	6.60	20.17	7.45	29.83	14.25
33	K-PSB 33	3.59	2.51	5.38	3.67	8.34	5.57
34	K-PSB 34	1.62	5.44	2.83	6.64	4.62	7.34
35	K-PSB 35	2.64	6.38	3.47	7.37	5.39	7.39
36	K-PSB 36	6.77	4.51	17.55	9.10	27.76	13.66
37	K-PSB 37	2.45	5.84	3.51	7.63	5.42	10.59
38	K-PSB 38	4.13	3.61	5.35	6.40	7.80	7.59
39	K-PSB 39	9.39	6.38	21.27	8.57	27.74	12.36
40	K-PSB 40	3.52	4.34	5.36	6.19	7.42	6.59
41	K-PSB 41	4.83	5.49	7.32	6.19	8.53	7.53
42	K-PSB 42	1.40	5.47	2.55	7.50	3.72	8.28
43	K-PSB 43	0.91	3.56	1.35	4.59	2.36	5.53
44	K-PSB 44	3.67	2.60	4.53	3.80	6.44	5.24
45	K-PSB 45	0.71	6.05	1.57	7.57	3.94	8.55
46	K-PSB 46	2.85	3.52	3.59	5.47	5.54	6.49
47	K-PSB 47	1.62	7.34	1.61	8.54	3.56	9.46
48	K-PSB 48	2.49	3.37	3.59	5.55	5.63	7.47
49	K-PSB 49	3.31	2.68	4.43	6.55	7.49	8.35
50	K-PSB 50	6.08	4.83	13.02	12.31	23.87	14.10
	S.Em±	0.15	0.15	0.17	0.15	0.49	0.16
	CD at 1%	0.55	0.57	0.62	0.55	1.82	0.61

DAI- Days after inoculation

5 µg/25ml broth and four isolates produced 3.5 µg/25ml broth of IAA.

The amount of GA produced by the strain ranged from 1.27 to 3.67 µg/25ml broth. Among the isolates K-PSB 50 produced the highest amount of GA 3.67 µg/25ml broth, which was on par with K-PSB 32 (3.48 µg/25ml broth), while seven isolates produced more than 2 µg/25ml broth and two isolates produced less than 2 µg/25ml broth. Sheng and Huang (2001) reported growth enhancement of *Bacillus* may also relate to its ability to produce hormones.

Table 3. Quantification of IAA and GA by selected bacterial isolates solubilizing both potassium and phosphorus

S. No	Isolates	IAA (µg/25ml)	GA (µg/ 25ml)
1	Isolate K-PSB 2	4.50	2.72
2	Isolate K-PSB 20	2.43	1.27
3	Isolate K-PSB 11	4.53	1.57
4	Isolate K-PSB 21	3.38	2.75
5	Isolate K-PSB 28	7.53	2.55
6	Isolate K-PSB 32	8.48	3.48
7	Isolate K-PSB 36	6.43	2.87
8	Isolate K-PSB 39	5.53	2.65
9	Isolate K-PSB 50	8.90	3.67
	S.Em±	0.15	0.12
	CD at1%	0.53	0.39

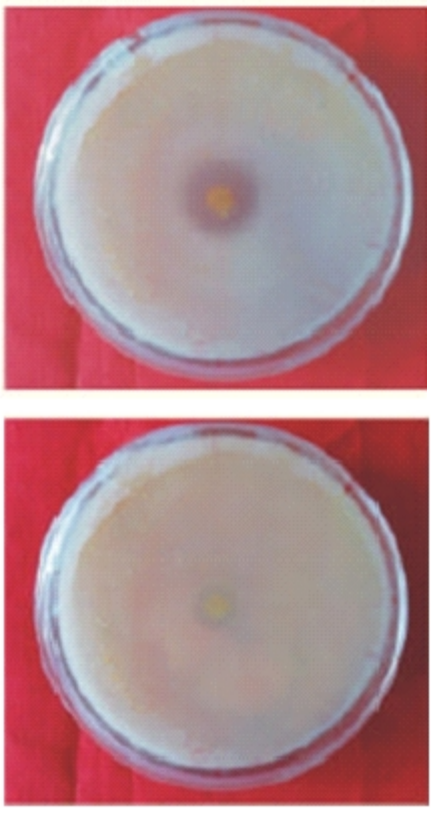

Plate 1. Zone of solubilization at 72 hours of incubation

CONCLUSION

The present study was successful in isolating efficient bacterial isolates capable of solubilizing both potassium and phosphorus and with capacity to solubilize tricalcium phosphate and mica are the source of phosphorus and porassium respectively in soil under *in vitro*. They produce plant growth promoting substances such as IAA and GA.

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