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RESEARCH ARTICLE

Assessment of phosphate solubilizing activity of different fungal and bacterial isolates

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SUMMARY

In vitro studies were conducted to find out most efficient phosphate solubilizers. The 23 fungal and 9 bacterial phosphate solubilizing microorganisms were isolated from rhizospheric soil of different weeds occuring in sorghum and cotton crops by serial dilution method. Most efficient 'P' solubilizers were identified on the basis of halo zone formation on Pikovskaya's agar medium, reduction in pH, organic acid production, and P_2O_5 solubilized in broth culture. The result indicated that among fungi *Aspergillus niger*-20 and *Aspergillus niger*-5 and among all bacterial isolates Dr. PDKV strain of PSB and PSB-3 produced maximum halo zone (5.33 to 4.66 mm) and they solubilized more tricalcium phosphate *i.e.* 26.24 to 18 P_2O_5 µg/ml with reduction in pH (3.1 to 3.30) with increasing in titrable acidity *i.e.* 3.60 to 3.0. PSB-3, PSB- 4, PSB-6 and PSB-8 produced indole acetic acid (IAA).

Key Words : Phosphate solubilizing, Rhizospheric, Fungal, Bacteria

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Phosphorus is an important plant nutrient along with major nitrogen and potassium plant nutrient elements. It is associated with several vital functions and is responsible for several characteristics in plant growth such as utilization of sugar and starch, photosynthesis, nucleus formation and cell division, fat and albumin formation, cell organization and the transfer of heredity. It is also essential in seed formation and early maturity of crop particularly in cereals. It plays important role as higher energy phosphate bonds (ATP, ADP) in respiratory and photosynthetic process. Phosphorus is constituent of nucleic acid, phytin, phospholipids. Phosphorus stimulates root growth also reported by some workers. Phosphorus is essential in seed formation and present in large quantities of plant, seed and fruit (Gaur, 1990).

Increase in cost of fertilizers and worldwide energy crises, low purchasing power of farmers, increase in cost of production restricted the use of chemical fertilizers alone as a source of plant nutrient. Under such condition it has become alternative to use all available resources of plant nutrients including microorganisms like PSM for sustainable soil fertility and productivity. These PSM have ability to increase stress tolerant capacity in plant

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and induce disease resistance against soil borne pathogens. 'P' solubilizing capacity of PSM varies with soil and soil condition. Therefore, use of efficient isolates is necessary. Hence, here the attempts were made to isolates efficient 'P' solubilizing microorganisms from rhizosphere of various weeds occur in sorghum and cotton and studies were undertaken to test the efficiency of different PSM isolates in laboratory.

MATERIAL AND METHODS

In present study attempts were made to isolate various phosphate solubilizing micro-organisms from rhizosphere of some weeds occuring in sorghum and cotton crops. The soil samples were collected from Yeola taluka of Nashik district and Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Research Farm Akola, for studying their 'P' solubilizing efficiency so as to get efficient phosphate solubilizing micro-organisms.

Collection of soil samples :

Rhizosphere soil from different commonly occurring weeds of sorghum and cotton were collected from two different locations of Maharashtra *viz.*, Yeola dist. Nashik and Central Research Station, Dr. PDKV. Akola. Soil samples were drawn from 5-25 cm depth of rhizosphere region, air dried in laboratory at room temperature and then used for isolation. The soil samples were collected in June- July 2012.

Isolation of phosphate solubilizing microorganism (Sanjotha *et al.*, 2011) :

Efforts have been made to isolate PSM from rhizosphere region of different weeds of sorghum and cotton following serial dilution method using 10^{-3} and 10^{-7} dilution on Pikovskiya's medium (Gaur, 1990). The soil samples collected were stored for air drying at room temperature and one gram soil sample used for isolation. The proper dilution was obtained by following procedure.

Solubilization of tricalcium phosphate on Pikovskiya's agar medium :

Sterilized Pikovskaya's agar medium was poured in to sterilized Petri dishes after solidification of media a pin point inoculation of test fungal/ bacterial isolates was inoculated in centre of Petri dishes under aseptic condition. The plates were incubated at 28 ± 2^0C for 7 days and observed for colony diameter, diameter of solubilization (clear zone) regularly during incubation period. Solubilization index was evaluated accordingly (Clear halo zone = total zone – colony diameter) in mm.The incubation period for fungal isolates was 3, 5 and 7 days and 2, 3 and 4 days for bacterial isolates at 28 ± 2^0C.

Microbial solubilization of insoluble phosphate in Pikovskiya's broth (Gaur, 1990) :

Phosphate solubilization potential of PSM was studied *in vitro* by estimating available phosphorus in Pikovskiya's broth medium with known amount of tri calcium phosphate (0.5 g 100 ml^{-1}) as a substrate before sterilization. Each of 5 mm mycelial bit of test fungal culture and 0.5 ml suspension of each bacterial culture was inoculated in 250 ml conical flask containing 100 ml sterilized Pikovskaya's broth medium and made triplicate. A control without any PSM was also maintained. The fungal and bacterial isolates were allowed to grow for seven and fourteen days at 28 ± 2^0C in BOD incubator. For PSF, broth was filtered through Whatman filter paper no. 42 and clear solution was collected in 100 ml volumetric flasks and volume was made upto 100 ml with sterilized distilled water. For estimation of PSB broth, culture centrifuged at 15,000 rpm for 30 min. in centrifuge. The supernatant was collected in 100 ml conical flask and volume made upto 100 ml with glass distilled water. Thus, extract of each test fungal and bacterial solution was prepared then the available phosphorus in broth culture were determined (Gaur, 1990).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Assessment of phosphate solubilizing activity of phosphate solubilizing microorganism in broth medium :

The fungal and bacterial isolates were tested for phosphate solubilizing activity *in vitro*. Test organisms were inoculated in Pikovskiya's broth medium containing tricalcium phosphate (0.5 g/100 ml) and incubated for 7^{th} and 14^{th} days at 28 ± 2^0C and observations were recorded for reduction in pH and amount of solubilized phosphate (P_2O_5µg/ml) in broth medium. Initial pH of the broth was 7.00 before

inoculation.

The data presented in Table 1 revealed that after 7th day of inoculation with different fungal isolates the change reduction in pH of medium ranged from 7.00 to 3.10 and amount of P_2O_5 solubilized measured between 12.00 to 23.33 P_2O_5 µg/ml was observed, the pH of the medium was decreased due to production of organic acids in the medium by the isolates. Among all *Aspergillus* spp. maximum reduction in pH recorded in *Aspergillus niger*-20 *i.e.* 3.50 with maximum P_2O_5 solubilization *i.e.*19.33 P_2O_5µg/ml followed by *Aspergillus niger*-5 (3.53 pH) with 19.00 P_2O_5µg/ml, *Aspergillus niger*-17 (3.56 pH) 19.00 P_2O_5µg/ml and *Aspergillus niger*-15 (3.56 pH) with 18.66 P_2O_5µg/ml, whereas minimum reduction in pH recorded in *Aspergillus flavus*- 1 (4.2 pH) with 12.00 P_2O_5µg/ml as compared to control (6.9 pH) with 00.0 P_2O_5µg/ ml. Other isolates of *Aspergillus* spp. recorded reduction in pH ranged from 4.13 to 3.50 and 18.33 to 13.00 P_2O_5µg/ ml.

After 14th day of incubation *Aspergillus niger*- 20 exhibited maximum reduction in pH *i.e.* 3.1 with maximum P_2O_5 solubilization *i.e.* 23.33 P_2O_5µg/ml followed by *Aspergillus niger*-5 (3.13 pH) with 23.00 P_2O_5µg/ml, while *Aspergillus flavus*-2 showed minimum reduction in pH (3.90) with minimum P_2O_5 solubilization *i.e.* 17.66 P_2O_5µg/ml as compared to control (6.76 pH) with 2.00 P_2O_5µg/ml. *Aspergillus niger*-20 and *Aspergillus niger*-5 recorded high organic acid production, maximum reduction in pH and maximum P_2O_5 solubilization in Pikovskaya's broth medium on 7th and 14th day of incubation.

Table 1 : Estimation pH and phosphate solubilization by different fungal isolates in Pikovskaya's broth medium

Sr. No.	'P' solubilizing fungal Isolates	7th day of incubation		14th day of incubation	
		pH	P_2O_5 µg/ ml	pH	P_2O_5 µg/ ml
1.	*Aspergillus niger*- 1	3.63	18.33	3.20	22.33
2.	*Aspergillus niger*- 2	3.83	16.00	3.56	20.00
3.	*Aspergillus niger*- 3	3.80	16.33	3.50	20.33
4.	*Aspergillus niger*- 4	3.90	15.66	3.63	19.33
5.	*Aspergillus niger*- 5	3.53	19.00	3.13	23.00
6.	*Aspergillus niger*- 6	3.73	17.33	3.33	21.33
7.	*Aspergillus niger*- 7	3.66	18.33	3.23	22.33
8.	*Aspergillus niger*- 8	3.73	17.00	3.43	20.66
9.	*Aspergillus niger*- 9	3.83	16.00	3.60	19.66
10.	*Aspergillus niger*-10	3.66	18.00	3.26	22.00
11.	*Aspergillus niger*- 11	3.76	16.66	3.40	20.66
12.	*Aspergillus niger*- 12	3.93	15.33	3.70	18.66
13.	*Aspergillus niger*- 13	4.00	14.66	3.76	18.33
14.	*Aspergillus niger*- 14	3.73	17.00	3.36	21.00
15.	*Aspergillus niger*- 15	3.56	18.66	3.16	22.66
16.	*Aspergillus niger*- 16	3.63	17.66	3.30	21.66
17.	*Aspergillus niger*- 17	3.56	19.00	3.26	22.00
18.	*Aspergillus niger*- 18	4.03	14.33	3.80	18.00
19.	*Aspergillus niger*- 19	3.96	15.00	3.76	18.33
20.	*Aspergillus niger*- 20	3.50	19.33	3.10	23.33
21.	*Aspergillus niger*- 21	3.70	17.66	3.40	21.00
22.	*Aspergillus flavus*-1	4.20	12.00	3.83	17.00
23.	*Aspergillus flavus*-2	4.13	13.00	3.90	17.66
24.	Control	6.90	00	6.73	2.0
	'F'- test	Sig	Sig	Sig	Sig
	S.E.±	0.04	0.38	0.03	0.29
	C.D.(P=0.01)	0.13	1.44	0.12	1.09

Initial pH 7

Arora and Gaur (1979) noticed that as pH decreases solubilization activity increases and also reported that *Aspergillus* spp. and *Penicillium* spp. effectively solubilized tricalcium phosphate in liquid medium. *Aspergillus awamori and Pseudomonas striata* were found to solubilize maximum amount of tricalcium phosphate. In a similar study it was reported that isolates of *Aspergillus* and *Penicillium* isolated from agricultural soil showed maximum level of phosphate solubilization activity *in vitro* when liquid medium was supplemented with both tricalcium phosphate and rock phosphate separately. Chakraborty *et al*. (2010), Alam *et al.* (2002) and Rashid *et al*. (2004) reported maximum solubilization of tricalcium phosphate by PSM ranged from 0.04 to 0.147 per cent. Among fungal isolates *Aspergillus niger* showed maximum solubilization *i.e.* (0.45%) P_2O_5 in broth.

Manivannan *et al*. (2011), Patil (2002) and Chakraborty *et al*. (2010) reported that *Aspergillu*s spp. and *Penicillium* spp. solubilized maximum tricalcium phosphate ranged from 799 to 856 mg/ l in Pikovskaya's broth medium due to organic acid production.

The data presented in Table 2 revealed that on 7th day of incubation PSB isolates produced organic acid which decreased the pH of the medium and solubilized tricalcium phosphate in broth medium. Maximum reduction in pH recorded in Dr. PDKV strain of PSB *i.e*. 3.53 with 18.66 P_2O_5 µg/ml solubilization. Among all PSB isolates, PSB-3 showed maximum reduction in pH *i.e*. 3.7 with 17.33 µg/ml P_2O_5 solubilization followed by PSB-1 (3.8 pH) with 16.00 P_2O_5µg/ml and minimum reduction in pH was recorded in PSB-2 *i.e.* 4.6 pH with 11.00 P_2O_5µg/ml as compared to control (6.9 pH) and 0.00 P_2O_5µg/ml.

On 14th day of incubation, Dr. PDKV. strain of PSB recorded maximum reduction in pH (3.2) with 22.66 µg/ml P_2O_5 solubilization. Among PSB isolates, PSB-3 showed maximum decreased in pH (3.30) with 22.00 µg/ml P_2O_5 solubilization, whereas minimum reduction in pH recorded in PSB- 2 *i.e.* 3.96 pH with 16.00 P_2O_5µg/ml solubilization. Other isolates having reduction in pH ranged from 3.9 to 3.4 with 11.0 to 20.00µg/ml P_2O_5 solubilization as compared to control. Among all PSB isolates PSB- 3 and PSB-1 were efficient phosphate solubilizers.

Similar result were reported by Chen *et al.* (2006) who observed that isolate 36 strain of phosphate solubilizing bacteria solubilized tricalcium phosphate 1.5 to 519.7 mg/l with decreasing the pH of the medium. Phosphate solubilizing bacteria isolated from rhizosphere of tea, bacteria produced organic acid (3.6 to 7.9 ml) which decreased the pH (4.65 to 3.88) and solubilized tricalcium phosphate (33.9 to 46.6 ppm) reported by Balamurugan *et al.* (2010). Manivannan *et al.* (2011) reported PSB (*Pseudomonas* spp.) solubilized tricalcium 98.5 mg/ 100ml in broth culture. Five PSB solubilized tricalcium phosphate ranged from 6.00 to 16.7 µg/ml in broth medium with lowering the pH (6.9

Table 2: Estimation of pH and phosphate solubilization by different bacterial isolates in Pikovskaya's broth medium

Sr. No.	'P' solubilizing bacterial isolates	7th day of incubation		14th day of incubation	
		pH	P_2O_5 µg/ml	pH	P_2O_5 µg/ml
1.	PSB- 1	3.80	16.00	3.40	21.00
2.	PSB- 2	4.60	11.00	3.96	16.00
3.	PSB-3	3.70	17.33	3.30	22.00
4.	PSB-4	4.30	14.00	3.70	18.00
5.	PSB-5	4.00	15.00	3.50	20.00
6.	PSB-6	4.40	12.00	3.90	16.33
7.	PSB-7	4.20	14.33	3.66	18.33
8.	PSB-8	3.90	15.33	3.46	20.33
9.	PSB-9	4.30	14.00	3.80	17.66
10.	Dr. PDKV strain	3.53	18.66	3.20	22.66
	control	6.90	0.00	6.80	1.66
	'F'- Test	Sig	Sig	Sig	Sig
	S.E.±	0.04	0.40	0.03	0.37
	C.D.(P=0.01)	0.17	1.59	0.12	1.49

Initial pH 7

to 5.26) reported by Humaira and Bano (2011). It may be due to the production of organic acids by these strains that lowers the pH of medium. The inverse relationship between pH and soluble phosphate was reported by Rashid *et al.* (2004). Sharma *et al.* (2012) they reported that PSB isolates, isolated from tea rhizosphere of Darjeeling hills are capable of producing plant growth promoting substance IAA ranged from 10-30 mg/l organic acids and capable of solubilizing inorganic phosphate ranged from 40.62 ± 1.1 to 136.73 ±1.7 mg/l thereby decreasing the pH of the medium.

Table 3 : Estimation of change in titrable acidity and pH by 'P' solubilizing fungal isolates

Sr. No.	'P' solubilizing fungal isolates	7^{th} day of incubation	
		Titrable acidity (ml)	pH
1.	*Aspergillus niger*- 1	3.43	3.63
2.	*Aspergillus niger*- 2	3.00	3.83
3.	*Aspergillus niger*- 3	3.06	3.80
4.	*Aspergillus niger*- 4	2.90	3.90
5.	*Aspergillus niger*- 5	3.53	3.53
6.	*Aspergillus niger*- 6	3.33	3.73
7.	*Aspergillus niger*- 7	3.36	3.66
8.	*Aspergillus niger*- 8	3.20	3.73
9.	*Aspergillus niger*- 9	2.96	3.83
10.	*Aspergillus niger*-10	3.36	3.66
11.	*Aspergillus niger*- 11	3.16	3.76
12.	*Aspergillus niger*- 12	2.86	3.93
13.	*Aspergillus niger*- 13	2.70	4.00
14.	*Aspergillus niger*- 14	3.26	3.73
15.	*Aspergillus niger*- 15	3.46	3.56
16.	*Aspergillus niger*- 16	3.40	3.63
17.	*Aspergillus niger*- 17	3.56	3.56
18.	*Aspergillus niger*- 18	2.63	4.03
19.	*Aspergillus niger*- 19	2.83	3.96
20.	*Aspergillus niger*- 20	3.60	3.50
21.	*Aspergillus niger*- 21	3.30	3.70
22.	*Aspergillus flavus*-1	2.43	4.20
23.	*Aspergillus flavus*-2	2.50	4.13
24.	Control	0.56	6.90
	'F'- test	Sig	Sig
	S.E.±	0.04	0.03
	C.D.(P=0.01)	0.18	0.13

Estimation of change in titrable acidity and pH by different phosphate solubilizing fungal and bacterial isolates by titration method

Isolated phosphate solubilizing microorganisms were tested to measuring their change in pH and titrable acidity by titration method *in vitro*. The Pikovskiya's broth medium was inoculated with test organism and incubated for 7 day at 28± 2^0 C and observations were recorded for change in titrable acidity and reduction in pH in broth medium. Initial pH of the broth medium was recorded at 7.00 before inoculation.

Data presented in Table 3 revealed that on 7th day of incubation all the fungal isolates produced various amount of organic acid. Among fungi *Aspergillus niger*-20 recorded maximum titrable acidity *i.e.* 3.6 ml with reduction in pH *i.e.* 3.5 as compared to control, followed by *A. niger*-17 (3.56 ml) with reduction in pH (3.56) and *A. niger*-5 (3.53 ml) with pH (3.53), whereas *A. flavus*-1 showed minimum titrable acidity *i.e.* 2.43 ml with pH 4.2 and other isolates of *Aspergillus niger* having titrable acidity ranged between 2.50 to 3.43 ml with pH ranged from 3.63 to 4.13. The result showed that all the fungal isolates produced organic acid with decrease in pH.

The similar results were reported by Patil (2002) who reported that *Aspergillus flavus, Aspergillus niger* and *Penicillium* spp. produced organic acid and reduced the pH of the medium with P_2O_5 solubilization which ranged from 65.40 to 137.4 P_2O_5 mg/100ml. Alam *et al.* (2002) observed that *Aspergillus* and *Penicillium* produce organic acid which reduced the pH of medium and solubilized tricalcium phosphate (0.22%) in liquid medium on 7th day of incubation.

The data presented in Table 4 revealed that on 7th day of incubation PSB isolates produced organic acid which decreased the pH of the medium. Maximum reduction in pH (3.53) with maximum titrable acidity (3.13 ml) showed by Dr. PDKV strain of PSB and among all PSB isolates, PSB-3 showed reduction in pH upto 3.7 with 3 ml titrable acidity followed by PSB- 8 pH (3.9) with 2.83 ml titrable acidity, while minimum reduction in pH (4.6) recorded in PSB-2 with 2.30 ml titrable acidity as compared to control and other PSB isolates (PSB-1, PSB-4, PSB-5, PSB-6, PSB-7 and PSB-9) having titrable acidity, ranged from 2.30 to 2.80 ml with pH reduction ranged from 4.8 to 3.8.

This result is similar to the results reported by Lal (2002) and Humaira and Bano (2011), they reported that reduction in pH and increase titrable acidity might be due to secretion of organic acids by PSB. Balamurugan *et al*. (2002) isolated the PSB from rhizosphere soil of tea and reported that these isolates reduced pH 3.88 to 4.62 of medium with increase in titrable acidity ranged from 3.9 to 7.9 ml. The result is also similar to Ponmurugan and Gopi (2006) who isolated the PSB from groundnut and reported that these isolates solubilized tricalcium phosphate which reduce pH (5.9 to 5.0) and increases in titrable acidity (2.4 to 3.9) due to organic acid production.

Table 4 : Estimation of change in titrable acidity and pH by PSB isolates

Sr. No.	'P' solubilizing bacterial isolates	7th day of incubation	
		Titrable acidity (ml)	pH
1.	PSB- 1	2.90	3.80
2.	PSB- 2	2.30	4.60
3.	PSB-3	3.00	3.70
4.	PSB-4	2.60	4.30
5.	PSB-5	2.80	4.00
6.	PSB-6	2.50	4.40
7.	PSB-7	2.66	4.20
8.	PSB-8	2.83	3.90
9.	PSB-9	2.60	4.30
10.	Dr. PDKV strain	3.13	3.53
	Control	0.53	6.90
	'F'- test	Sig	Sig
	S.E.±	0.04	0.05
	C.D.(p=0.01)	0.16	0.18

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