Indian J. Microbiol. (June 2008) 48:267–273

ORIGINAL ARTICLE

Isolation and characterisation of phosphate solubilising microorganisms from the cold desert habitat of *Salix alba Linn.* in trans Himalayan region of Himachal Pradesh

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Received: 5 May 2006 / Accepted: 15 January 2008

Abstract Phosphate solubilising microorganisms (PSM) (bacteria and fungi) associated with *Salix alba* Linn. from Lahaul and Spiti valleys of Himachal Pradesh were isolated on Pikovskaya (PVK), modified Pikovskaya (MPVK) and National Botanical Research Institute agar (NBRIP) media by spread plating. The viable colony count of P-solubilising bacteria (PSB) and fungi (PSF) was higher in rhizosphere than that of non-rhizosphere. The frequency of PSM was highest on MPVK followed by NBRIP and PVK agar. The maximum proportion of PSM out of total bacterial and fungal count was found in upper Keylong while the least in Rong Tong. The PSB frequently were Gram-positive, endosporeforming, motile rods and belonged to *Bacillus* sp. The PSF mainly belonged to *Penicillium* sp., *Aspergillus fumigatus*, *A. niger*, *A.* spp. and non-sporulating sterile. Amongst the isolates with high efficiency for tricalcium phosphate (TCP) solubilisation, seven bacterial and seven fungal isolates dissolved higher amount of P from North Carolina rock phosphate (NCRP) than Mussoorie rock phosphate (MRP) and Udaipur rock phosphate (URP). However, the organisms solubilised higher-P in NBRIP broth than PVK broth. SBC5 (*Bacillus* sp.) and SBC7 (*Bacillus* sp.) bacterial isolates exhibited maximun P solubilisation (40 and 33 μg ml^{-1} respectively) whereas FC28 (*Penicillium* sp.) isolate (52.3 μg ml^{-1}) amongst fungi while solubilising URP. The amount of P solubilised was positively correlated with the decrease in pH of medium. SBC5 (*Bacillus* sp.), SBC7 (*Bacillus* sp.) and SBC4 (*Micrococcus*) decreased the pH of medium from 6.8 to 6.08 while FC28 (*Penicillium* sp.) and FC39 (*Penicillium* sp.) isolates of fungi recorded maximum decrease in pH of medium from 6.8 to 5.96 in NBRIP broth.

Keywords Phosphate Solubilising Microorganisms · *Salix alba* Linn. · Lahaul and Spiti valleys · Rock Phosphate

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Introduction

Phosphorus, an essential plant nutrient is required by plants. It is generally present in soil in fixed forms. Most of it is not readily available to plants due to its low solubility in the soil [1]. Phosphate solubilising microbes (PSM) are known to bring about mobilisation of insoluble phosphates and this can stimulate plant growth even under the conditions of Phosphorus deficiency [2]. PSM were extensively studied by various scientists but a large body of research have been confined to dissolution of tricalcium phosphate [3]. Soil inoculation with PSM improves P solubilisation of fixed soil phosphate and result in higher crop yields [4]. Hence, P solubilising microbes can be used as biofertilisers. PSM are present in most soils [5], however, their population and activity vary with the region depending upon various environmental factors. The soils of trans-Himalayan region in Himachal Pradesh (India) are neutral to slightly alkaline in nature, low to medium in organic matter and remain under snow for 3-4 months for a year. *Salix alba* Linn. (White Willow) is a large tree growing in this area. It is highly useful as medicinal as well as other household goods. Efficient PSM may increase the availability of P for the enhancement of growth rate of this medicinally and commercially important tree of the region. The information related with

occurrence and activity of PSM in the typical rhizosphere is scanty. Moreover, in trans-Himalayan, cold desert Lahaul and Spiti, typical ecosystem PSM population estimates has never been attempted and the activity of these organisms has never been tested. The reliability of halo zone formation on agar plates for testing P solubilising microorganisms is questionable. However, this method is always used for initial screening of P solubilisers. The organism not forming halo on one media can produce clear zone on other media and can further be tested for P solubilisation in broth. Various workers have used different media for above studies [6, 7, 8]. It was of interest to screen the available media to show the halos clearly for easy identification of the PSM. Therefore, the present study was undertaken with the objective of isolation and characterisation of PSM in the soils of Lahaul and Spiti district of Himachal Pradesh using different media viz. Pikovskaya (PVK), modified Pikovskaya (MPVK) and National Botanical Research Institute (NBRIP) agar.

Materials and Methods

Soil samples (up to 15 cms. Depth) were collected from rhizosphere (with roots) and non-rhizosphere of *Salix alba* at Keylong, Kukumseri, Tandi, Sisu, Udaipur and Rong Tong in Lahaul and Spiti valleys of Himachal Pradesh at elevation ranges of 4000–4500 MSL in trans Himalayas. Soil samples of each soil were mixed thoroughly. Samples were air-dried and their physico chemical properties were determined using 2 mm sieved soil samples. Soil pH was determined in 1: 2 soil-water suspension using digital glass electrode pH meter. Electrical conductivity, organic matter, particle density, total nitrogen, available phosphorus and potassium were determined using standard AOAC methods (Table1). Total thirty-six soil samples three from each series were used for this investigation.

Isolation of PSM: Total bacterial and fungal population was determined using Nutrient Agar (NAM) and Potato Dextrose Agar (PDA) media, respectively. PSM were isolated from soil samples (Table 1) by serial dilution using spread plating on PVK [6], MPVK [7] and NBRIP [8] supplemented with tricalcium phosphate as insoluble inorganic phosphate source. The population of PSM was determined on the bases of ratio of number of phosphate solubilisers to total number of colonies and expressed as percentage. The isolated organisms were identified [9, 10]. The solubilisation capacity of different microbial isolates was tested by using tricalcium phosphate in PVK and NBRIP broth. The highly efficient microbes were screened on the basis of their capacities of tricalcium phosphate solubilisation. These microbes were tested for their potential to solubilise NCRP, MRP and URP in PVK and NBRIP broth. These sources of phosphate were added @ 5 g l^{-1} after 4–5 washings with 5N sodium bicarbonate to remove soluble P and subsequently these were dried at 40^0C for 24 hours. The isolated bacteria and fungi exhibiting clear zones were transferred on nutrient agar and potato dextrose agar slants, respectively, till further use.

Halo Size: A pinpoint inoculation of bacterial and fungal culture was placed on respective media with tricalcium phosphate as insoluble inorganic phosphate source. The microbial solubilisation of phosphates was exhibited with a clear zone formed around colony and its size was determined with measuring scale. The halo diameter around the colony was calculated by subtracting colony size from total size. All the observations were recorded in triplicate.

Phosphate solubilising efficiency: Bacterial cells @ 10^6 and fungal spores @ 30×10^5 ml^{-1} were inoculated in 100 ml PVK and NBRIP broth, respectively and incubated for 5 days in bacteria and 7 days in fungi under shake at 250 rpm. Triplicates were maintained for each treatment. Uninoculated broth served as control. The soluble phosphorus was determined in clear filtrate using Ascorbic acid method

Table 1. Physicochemical characters of different soils.

Area	pH	EC ($mmhoscm^{-1}$)	Particle Density (Mgm^{-3})	Organic Carbon (%)	Available P ($Kg\ ha^{-1}$)	Available K ($Kg\ ha^{-1}$)	Total N (%)
LAHAUL VALLEY							
Keylong	7.1	0.37	2.46	1.22	38.6	310.8	0.082
Kukumseri	6.9	0.42	2.62	0.72	33.1	254.8	0.076
Tandi	7.1	0.32	2.53	1.12	33.4	299.6	0.080
Sisu	7.0	0.58	2.67	0.34	32.9	196.0	0.062
Udaipur	6.8	0.16	2.57	0.66	33.7	252.0	0.073
SPITI VALLEY							
Rong Tong	7.6	0.68	2.73	0.09	30.2	145.6	0.026

[11]. The intensity of blue colour was measured on spectrophotometer at 730 nm and the quantity of solubilised phosphate was expressed as µg/ml. The pH of filtrate was recorded at the end of the experiment.

Rock phosphate solubilisation: Highly efficient microbial isolates solubilising tricalcium phosphate were screened and inoculated in PVK and NBRIP broth, respectively, supplemented with NCRP, MRP and URP. The rock phosphate solubilisation was determined using Ascorbic acid blue colour method [11].

Results and Discussion

Physicochemical properties of experimental soils

Thirty-six soil samples, three from each location were tested for physico- chemical properties. Most of the soils under study were neutral with pH varying from 6.8 to 7.1 in Lahaul and slightly alkaline (pH 7.6) in Spiti subdivision. The electrical conductivity values from different locations indicated that all the soils were non-saline in nature with electrical conductivity values ranged in between 0.16-0.68 mmhos cm^{-1}. The soils were medium to high in organic carbon content in Lahaul valley. In Lahaul, the organic carbon was maximum in Keylong (1.22 %) while the least in Sisu (0.34 %). In Spiti valley, the organic carbon was low (0.09 %). In contrary to organic carbon the maximum particle density was reported in Sisu (2.67 Mg m^{-3}) while the least in Keylong (2.46 Mg m^{-3}) in Lahaul valley. The highest available P (38.60Kg ha^{-1}), available K (310.8 Kg ha^{-1}) and total Nitrogen (0.082 %) were found in Keylong while minimum available P (30.20 Kg ha^{-1}), available K (145.6 Kg ha^{-1}) and total Nitrogen (0.026 %) in Rong Tong (Table 1).

P solubiliser population in different media

All the collected soil samples were evaluated for P solubilising bacteria and fungi. PSM were observed in all the soils from rhizosphere and non-rhizosphere of *Salix alba*, though wide variation was found in their population in two classes of soil samples. The population count of PSM was higher in rhizosphere than those in non-rhizosphere soil. The higher occurrence of P solubilisers in rhizosphere is of direct significance to the plants as it helps in mobilisation of insoluble phosphorus near the root, especially in phosphorus deficient soils. The positive rhizosphere effect of perennial plants on microbial activity have been widely reported, though limited information is available about the rhizosphere influence on P solubilising microorganisms [12]. The number of P solubilising bacterial and fungal

Table 2. Phosphate solubilising microorganisms from the natural habitat of *salix alba* from Lahaul and Spiti valleys.

Location	Soil Sample	Bacteria						Fungi					
		Mean plate count (X10^5) g^{-1} soil						Mean plate count (×10^3) g^{-1} soil					
**	P-Solubilisers	Total Bacterial count	P-Solubilisers		Per cent P-Solubilisers		Total Fungal count	P-Solubilisers			Per cent P-Solubilisers		
			PVK	MPVK	NBRIP	PVK	MPVK	NBRIP		PVK	MPVK	NBRIP	PVK	MPVK	NBRIP
Keylong	R	214.53 ± 4.67	21.83 ± 0.802	23.16 ± 1.02	23.16 ± 1.35	10.17	10.79	10.79	67.70 ± 4.10	9.03 ± 2.10	9.21 ± 1.81	9.86 ± 1.62	13.33	13.60	14.56
	NR	70.10 ± 3.50	4.05 ± 1.23	5.52 ± 1.61	5.75 ± 1.82	5.77	7.87	8.20	50.43 ± 1.40	5.22 ± 0.495	5.88 ± 0.509	6.19 ± 0.751	10.35	11.65	12.27
Kukumseri	R	174.13 ± 1.40	7.30 ± 1.12	8.53 ± 0.175	8.18 ± 0.162	4.19	4.89	4.69	51.73 ± 1.55	5.00 ± 0.871	6.53 ± 0.378	6.25 ± 0.217	9.66	12.62	12.08
	NR	66.30 ± 2.85	2.80 ± 0.624	3.03 ± 0.737	2.83 ± 0.665	4.22	4.57	4.26	45.20 ± 2.81	3.83 ± 0.568	5.50 ± 0.453	4.65 ± 0.312	8.47	12.16	10.28
Tandi	R	218.70 ± 30.93	16.50 ± 4.82	17.66 ± 5.13	17.40 ± 5.03	7.54	8.07	7.95	83.40 ± 1.05	8.76 ± 0.680	9.40 ± 0.721	8.71 ± 0.765	10.50	11.27	10.44
	NR	75.53 ± 12.60	4.53 ± 1.07	4.83 ± 1.15	4.53 ± 1.00	5.99	6.39	5.99	69.73 ± 14.80	4.46 ± 1.27	7.46 ± 1.66	7.06 ± 2.15	6.39	10.69	10.12
Sisu	R	172.53 ± 6.08	16.36 ± 0.709	17.63 ± 0.907	17.16 ± 1.04	9.48	10.21	9.94	59.96 ± 5.35	7.53 ± 2.80	8.43 ± 2.97	7.96 ± 2.32	12.55	14.05	13.27
	NR	75.00 ± 1.35	6.33 ± 0.907	7.36 ± 0.709	6.77 ± 0.692	8.44	9.81	9.02	45.53 ± 2.33	4.80 ± 1.58	6.36 ± 2.65	6.00 ± 2.12	10.54	13.96	13.17
Udaipur	R	208.66 ± 3.21	13.73 ± 0.378	14.76 ± 0.680	15.06 ± 0.680	6.58	7.07	7.21	76.23 ± 4.90	8.36 ± 1.30	9.63 ± 1.19	9.03 ± 1.26	10.96	12.63	11.84
	NR	87.50 ± 1.85	5.50 ± 0.500	5.83 ± 0.568	5.73 ± 0.550	6.28	6.66	6.54	59.66 ± 1.52	5.20 ± 1.65	4.73 ± 0.808	4.73 ± 1.02	8.71	7.92	7.92
Rong Tong	R	170 ± 3.30	15.32 ± 0.600	16.21 ± 0.698	16.15 ± 0.230	9.01	9.53	9.50	50.60 ± 1.33	7.19 ± 1.34	7.32 ± 2.55	7.28 ± 1.00	14.20	14.40	14.30
	NR	73 ± 1.10	6.13 ± 0.367	6.22 ± 0.453	6.90 ± 0.450	8.39	8.52	9.45	44.21 ± 1.35	3.50 ± 1.22	4.21 ± 1.17	4.10 ± 0/98	7.91	9.52	9.27

*- on NAM, ** - on PDA

Table 3. Tricalcium phosphate solubilisation by bacterial isolates in agar and broth using PVK and NBRIP medium.

Bacteria	Agar (Halo size (mm))			Broth (μg ml^{-1}P solubilised)		Final pH of broth *	
	PVK	MPVK	NBRIP	PVK	NBRIP	PVK	NBRIP
SBC1 (Bacillus sp.)	0.0 ± 0.00	4.0 ± 0.00	2.6 ± 0.57	57.0 ± 3.60	57.6 ± 2.51	6.06 ± 0.015	6.11 ± 0.025
SBC2 (Pseudomonas)	3.0 ± 1.00	4.3 ± 0.57	4.3 ± 1.52	82.3 ± 2.08	86.6 ± 1.52	5.99 ± 0.010	6.02 ± 0.030
SBC3 (Unidentified)	0.0 ± 0.00	2.6 ± 0.57	1.6 ± 0.57	30.0 ± 2.00	39.6 ± 1.15	6.15 ± 0.025	6.22 ± 0.020
SBC4 (Micrococcus)	3.3 ± 1.15	4.0 ± 1.00	5.6 ± 1.15	88.3 ± 1.52	94.3 ± 4.04	5.92 ± 0.020	5.82 ± 0.020
SBC5 (Bacillus sp.)	6.3 ± 1.15	9.0 ± 1.00	10.3 ± 1.52	100.6 ± 3.05	110.0 ± 5.00	5.79 ± 0.015	5.61 ± 0.030
SBC6 (Unidentified)	3.0± 1.00	4.3 ± 0.57	3.3 ± 1.15	70.3 ± 2.51	75.3 ± 2.51	6.01 ± 0.005	6.07 ± 0.011
SBC7 (Bacillus sp.)	5.3 ± 1.52	7.3 ± 0.57	7.6 ± 2.08	95.0 ± 2.00	101.6 ± 1.52	5.85 ± 0.040	5.70 ± 0.020
SBC8 SBC8 (Unidentified)	0.66 ± 0.57	3.3 ± 0.57	1.6 ± 1.15	38.0 ± 2.00	43.3 ± 1.52	6.12 ± 0.00	6.18 ± 0.011
SBC9 (Micrococcus)	0.33 ± 0.57	3.3 ± 1.52	2.0 ± 1.00	45.0 ± 3.00	53.6 ± 0.570	6.09 ± 0.005	6.16 ± 0.010

± SD (Standard Deviation)
*Initial pH = 6.8

colonies were invariably higher on MPVK and NBRIP agar than those obtained on PVK agar. The highest percentage of PSM was found in the soils of Keylong. The population of PSB in this sub area was 10.17, 10.79 and 10.79 percent on PVK, MPVK and NBRIP agar, respectively in rhizosphere soil and 5.77, 7.87 and 8.20 percent on PVK, MPVK and NBRIP agar, respectively in non-rhizosphere soil. The percent PSF was 13.33, 13.60 and 14.56 per cent, respectively on PVK, MPVK and NBRIP agar in rhizosphere soil and 10.35, 11.65 and 12.27 percent, respectively on PVK, MPVK and NBRIP agar in non-rhizosphere soil. (Table 2). Thus the MPVK and NBRIP agar are the media of choice over PVK agar in the enumeration of P solubilisers from soil. Majority of PSB belonged to genus *Bacillus* and *Micrococcus*. The dominance of genus *Bacillus* as a P solubilising bacteria in the rhizosphere of several crops have been reported [13]. In addition to *Bacillus*, *Pseudomonas*, *Serratia*, *Enterobacter*, *Micrococcus* and *Flavobacterium* have been found to be active in the solubilisation of inorganic phosphatic compounds [14]. The PSF mainly belonged to *Penicillium*, *Aspergillus fumigatus*, *Aspergillus niger, A.* spp. and non-sporulating sterile. Species of *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and several other fungi have been reported amongst the P solubilising fungi [15, 16].

The nine bacterial isolates formed solubilisation zones from 0.0 to 6.3 mm on PVK, 3.3 to 9.0 mm on MPVK and 1.6 to 10.3 mm on NBRIP agar (Table 3). The solubilisation zone varied from 0.0 to 8.0 mm on PVK, 1.0 to 8.6 mm on MPVK and 1.3 to 7.6 mm on NBRIP agar in the fungal isolates (Table 5). The halo size of 2.0 to 5.0 mm on PVK agar and 5.0 to 13.0 mm on MPVK agar was reported in bacterial isolates [7]. The halos were distinctively bright around fungal than bacteria. This could be due to the greater activity of phosphatase enzyme in fungi than bacteria. The experimental petri plates with phosphate solubilising microbes were stored at 4^{0}C to arrest their growth and activity. The halos formed by all the isolates of microbes remained static under low temperature conditions except SBC8 (Unidentified) isolate of bacterium where the halo diminished. This could probably be due to reversion of soluble phosphates into insoluble ones. The clear zone reappeared after six hours incubation of plates at higher temperature 28 ± 2^{0}C. It could be due to reactivation of phosphatase enzyme which converted insoluble phosphates into soluble forms.

P solubilisation activity on Agar media

The bacterial isolates SBC1 (*Bacillus* sp.), SBC3 (Unidentified) and fungal isolates FC38 (*Penicillum* sp.), FC20 (Non-sporulating sterile) did not produce halo on PVK but formed halo on MPVK and NBRIP agar and solubilised P in PVK and NBRIP broth (Table 3 and Table 5). It indicates that these isolates did not produce any visible halo on agar plates but have the ability to solubilise various types of insoluble inorganic phosphates in liquid medium. The efficiency of P solubilisers was assessed and observed that SBC5 (*Bacillus* sp.) and SBC7 (*Bacillus* sp.) bacterial isolates had maximum activity with 100.6 μg ml^{-1} and 95.0 μg ml^{-1} respectively in PVK broth and 110.0 μg ml^{-1} and 101.6 μg ml^{-1} respectively in NBRIP broth (Table 3) while FC28 (*Penicillum* sp.) amongst fungal isolates recorded highest P solubilisation (136.9 μg ml^{-1} in PVK and 139.4 μg ml^{-1} in NBRIP broth (Table 5).

Table 4. Rock phosphate solubilisation (μg ml^{-1}) by bacterial isolates in PVK and NBRIP broth.

Rock phosphates		NCRP		MRP		URP	
Media / Bacteria		PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
SBC1 (Bacillus sp.)	P solubilisation	14.3 ± 2.08	20.0 ± 2.00	12.0 ± 2.64	15.6 ± 2.51	5.6 ± 0.570	6.0 ± 1.00
	pH*	6.31 ± 0.020	6.25 ± 0.020	6.42 ± 0.030	6.28 ± 0.017	6.43 ± 0.023	6.31 ± 0.015
SBC2 (Pseudomonas)	P solubilisation	32.6 ± 2.50	39.6 ± 1.52	28.3 ± 3.51	32.6 ± 2.88	15.66 ± 1.52	20.0 ± 1.00
	pH	6.16 ± 0.035	6.1 ± 0.010	6.26 ± 0.030	6.18 ± 0.011	6.31 ± 0.030	6.20 ± 0.015
SBC4 (Micrococcus)	P solubilisation	42.3 ± 3.50	48.3 ± 1.52	29.6 ± 0.570	34.3 ± 4.04	21.6 ± 2.08	25.3 ± 1.15
	pH	6.09 ± 0.015	6.01 ± 0.015	6.18 ± 0.015	6.12 ± 0.017	6.22 ± 0.020	6.18 ± 0.017
SBC5 (Bacillus sp.)	P solubilisation	52.0 ± 2.00	57.6 ± 2.51	41.3 ± 2.51	50.0 ± 2.00	35.3 ± 3.05	40.0 ± 1.00
	pH	5.92 ± 0.020	5.87 ± 0.020	5.08 ± 0.020	5.99 ± 0.020	6.11 ± 0.015	6.08 ± 0.005
SBC6 (Unidentified)	P solubilisation	28.3 ± 1.52	31.3 ± 1.52	19.6 ± 1.52	21.6 ± 1.52	12.6 ± 2.51	15.6 ± 2.08
	pH	6.20 ± 0.015	6.10 ± 0.015	6.30 ± 0.020	6.20 ± 0.025	6.30 ± 0.025	6.20 ± 0.011
SBC7 (Bacillus sp.)	P solubilisation	47.9 ± 2.30	51.6 ± 1.52	35.0 ± 3.00	39.0 ± 2.00	28.3 ± 1.15	33.0 ± 2.00
	pH	6.03 ± 0.020	5.95 ± 0.036	6.14 ± 0.020	6.10 ± 0.010	6.19 ± 0.005	6.12 ± 0.020
SBC9 (Micrococcus)	P solubilisation	8.6 ± 1.15	14.0 ± 2.00	5.6 ± 0.570	9.3 ± 0.57	1.6 ± 0.57	4.6 ± 1.52
	pH	6.37 ± 0.036	6.32 ± 0.030	6.44 ± 0.036	6.35 ± 0.035	6.47 ± 0.005	6.36 ± 0.020

± SD * Initial pH = 6.8

Table 5. Tricalcium phosphate solubilisation by fungal isolates in agar and broth using Pikovskaya (PVK) and NBRIP medium.

Fungi	Agar (Halo size (mm))			Broth (μg ml^{-1} P solubilised)		Final pH of broth*		Fungal biomass (g 100 ml^{-1}.)	
	PVK	MPVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
FC20 (Unidentified Non-sporulating sterile)	0.0 ± 0.00	1.3 ± 0.57	1.3 ± 0.57	46.5 ± 3.00	46.5 ± 0.00	5.50 ± 0.015	5.45 ± 0.010	0.306 ± 0.005	0.311 ± 0.001
FC25 (Unidentified Non-sporulating sterile)	3.3 ± 0.57	3.3 ± 0.57	4.3 ± 0.57	59.4 ± 2.23	65.8 ± 0.00	5.32 ± 0.049	5.23 ± 0.020	0.421 ± 0.001	0.434 ± 0.003
FC4 (Aspergillus fumigatus)	2.3 ± 0.57	3.3 ± 0.57	2.0 ± 0.00	52.9 ± 2.25	55.5 ± 2.23	5.47 ± 0.011	5.42 ± 0.010	0.315 ± 0.004	0.321 ± 0.001
FC38 (Penicillium sp.)	0.0 ± 0.00	1.0 ± 0.00	2.0 ± 1.00	43.9 ± 2.23	43.9 ± 2.23	5.59 ± 0.015	5.50 ± 0.020	0.296 ± 0.004	0.311 ± 0.001
FC31 (A. niger)	5.6 ± 0.57	6.3 ± 0.57	5.6 ± 0.57	87.8 ± 4.74	103.0 ± 4.46	5.22 ± 0.058	5.14 ± 0.010	0.459 ± 0.006	0.474 ± 0.004
AF (A. niger)	4.6 ± 1.52	5.0 ± 0.00	4.0 ± 1.73	83.9 ± 11.18	92.9 ± 20.5	5.29 ± 0.015	5.20 ± 0.010	0.442 ± 0.002	0.453 ± 0.004
FC39 (Penicillium sp.)	6.6 ± 0.57	8.6 ± 0.57	6.6 ± 0.57	103.3 ± 2.25	117.5 ± 0.76	4.45 ± 0.037	4.32 ± 0.010	0.528 ± 0.003	0.534 ± 0.003
FC28 (Penicillium sp.)	8.0 ± 0.00	8.3 ± 0.57	7.6 ± 1.15	136.9 ± 17.8	142.0 ± 4.45	4.40 ± 0.011	4.19 ± 0.010	0.533 ± 0.003	0.538 ± 0.004
FC37 (Aspergillus sp.)	7.6 ± 0.57	8.3 ± 0.57	7.6 ± 0.57	103.3 ± 0.21	109.7 ± 15.7	4.87 ± 0.100	4.69 ± 0.015	0.523 ± 0.001	0.534 ± 0.005

± SD * Initial pH = 6.8

Rock phosphate solubilisation by different culture in different broth and change in pH:

The pH of of the broth declined with the growth of microbial cultures (Table 3 and Table 5). This is due to production of acids with the increase in period of incubation that enhanced the breakdown of insoluble P into soluble ones. It was observed that a greater decrease in pH was recorded due to fungi. The lowest pH of the medium was observed in SBC5 (*Bacillus* sp.) isolate of bacterium (5.79 in PVK and 5.61 in

Table 6. Rock phosphate solubilisation (μg ml^{-1}), pH of broth and fungal biomass (g 100 ml^{-1}) in PVKand NBRIP broth.

Rock phosphates		NCRP		MRP		URP	
Media / Isolates		PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
FC25 (Unidentified Non-sporulating sterile)	P solubilisation	20.0 ± 2.00	26.0 ± 1.00	15.0 ± 2.00	20.3 ± 0.577	10.6 ± 1.15	16.3 ± 2.08
	pH*	6.24 ± 0.020	6.11 ± 0.011	6.27 ± 0.011	6.12 ± 0.015	6.41 ± 0.012	6.39 ± 0.010
	Fungal biomass	0.330 ± 0.001	0.338 ± 0.004	0.308 ± 0.002	0.314 ± 0.004	0.294 ± 0.004	0.302 ± 0.002
FC4 (Aspergillus fumigatus)	P solubilisation	15.0 ± 3.00	22.6 ± 2.51	10.0 ± 2.00	14.3 ± 1.15	0.0 ± 0.00	3.33 ± 0.577
	pH	6.29 ± 0.015	6.22 ± 0.020	6.35 ± 0.025	6.27 ± 0.015	6.67 ± 0.015	6.51 ± 0.040
	Fungal biomass	0.307 ± 0.006	0.324 ± 0.004	0.299 ± 0.001	0.301 ± 0.003	0.265 ± 0.005	0.292 ± 0.002
FC31 (A. niger)	P solubilisation	36.3 ± 1.52	43.3 ± 2.51	32.0 ± 2.00	38.3 ± 2.08	17.6 ± 2.51	26.6 ± 1.52
	pH	6.08 ± 0.015	6.05 ± 0.050	6.18 ± 0.020	6.09 ± 0.015	6.19 ± 0.015	6.20 ± 0.015
	Fungal biomass	0.352 ± 0.002	0.358 ± 0.003	0.340 ± 0.001	0.346 ± 0.003	0.330 ± 0.002	0.338 ± 0.002
AF (A. niger)	P solubilisation	32.3 ± 2.51	37.3 ± 2.51	28.3 ± 2.88	34.3 ± 1.52	14.3 ± 1.52	20.6 ± 2.08
	pH	6.11 ± 0.010	6.07 ± 0.017	6.21 ± 0.017	6.08 ± 0.011	6.33 ± 0.020	6.23 ± 0.020
	Fungal biomass	0.342 ± 0.002	0.353 ± 0.002	0.321 ± 0.001	0.326 ± 0.001	0.309 ± 0.003	0.320 ± 0.002
FC39 (Penicillium sp.)	P solubilisation	50.0 ± 2.00	55.6 ± 2.51	46.0 ± 2.00	53.3 ± 2.51	39.3 ± 1.15	41.3 ± 2.08
	pH	5.96 ± 0.015	5.80 ± 0.020	5.97 ± 0.015	5.91 ± 0.011	5.99 ± 0.005	5.96 ± 0.010
	Fungal biomass	0.391 ± 0.003	0.395 ± 0.003	0.374 ± 0.005	0.385 ± 0.000	0.361 ± 0.001	0.364 ± 0.003
FC28 (Penicillium sp.)	P solubilisation	61.3 ± 1.52	66.6 ± 2.51	58.3 ± 2.08	64.0 ± 1.73	50.3 ± 1.52	52.3 ± 2.51
	pH	5.85 ± 0.025	5.74 ± 0.020	5.92 ± 0.017	5.91 ± 0.015	5.97 ± 0.015	5.93 ± 0.030
	Fungal biomass	0.407 ± 0.006	0.412 ± 0.008	0.381 ± 0.003	0.390 ± 0.000	0.367 ± 0.002	0.376 ± 0.003
FC37 (Aspergillus sp.)	P solubilisation	42.6 ± 3.05	48.0 ± 1.00	39.3 ± 1.15	43.6 ± 2.08	36.3 ± 1.52	38.0 ± 2.64
	pH	5.97 ± 0.015	5.87 ± 0.015	6.02 ± 0.020	5.98 ± 0.015	6.09 ± 0.005	6.03 ± 0.035
	Fungal biomass	0.365 ± 0.005	0.372 ± 0.003	0.362 ± 0.001	0.369 ± 0.003	0.348 ± 0.003	0.355 ± 0.000

± SD * Initial pH = 6.8

NBRIP broth) which is in agreement with the results of rate of P solubilisation (Table 3). Amongst fungal isolates, the maximum fall in pH was observed with FC28 (*Penicillum* sp.) (4.4 in PVK and 4.19 in NBRIP broth) followed by FC39 (*Penicillum* sp.) (4.45 in PVK and 4.32 in NBRIP broth) and FC37 (*Aspergillus* sp.) (4.87 in PVK and 4.69 in NBRIP broth) (Table 5). Majority of fungi in general proved to be better solubilisers as compared to bacteria. While comparing P solubilisation capacities of different bacterial and fungal isolates in PVK and NBRIP broth, it was observed that the organisms proved to be better solubilisers when inoculated in NBRIP broth than when inoculated in PVK broth. Higher solubilisation of tricalcium phosphate has been observed in NBRIP than PVK medium [8]. The NBRIP medium lacks yeast extract and contains lesser amount of Ammonium sulphate as compared to PVK medium, which are considered as the non-essential components in P solubilising medium. It was also reported that the P solubilisation ability of *Pseudomonas* sp. 2 was increased by about 30% in the absence of either yeast extract or ammonium sulphate and the efficacy of organism was enhanced by 12.5 % in the absence of both yeast extract and ammonium sulphate [8].

Six out of nine PSB and seven out of nine PSF, were tested for their efficacy to solubilise complex insoluble sources of inorganic phosphates viz. NCRP, MRP and URP. The results on the solubilisation of three rock phosphates by the efficient isolates of inorganic P solubilising bacteria and fungi showed that these microorganisms differed in their abilities to solubilise rock phosphates. These microbial isolates solubilised maximum NCRP, followed by MRP and URP in NBRIP broth in a better way than that in PVK broth. Among the bacterial isolates, the bacteria SBC5 solubilised maximum NCRP (57.6 μg ml^{-1}) followed by MRP (50.0 μg ml^{-1}) and URP (40.0 μg ml^{-1}) in NBRIP broth. The bacterium solubilised NCRP (52.0 μg ml^{-1}), MRP (41.3 μg ml^{-1}) and URP (35.3 μg ml^{-1}) in PVK broth. This bacterial isolate represented 6.24% increase over PVK broth while solubilising URP in NBRIP broth (Table 4). Similarly, amongst the highly efficient PSF, the fungus FC28 (*Penicillium* sp.), solubilised maximum NCRP (66.6 μg ml^{-1}), followed by MRP (64 μg ml^{-1}) and URP (52.3 μg ml^{-1}) in NBRIP broth. In PVK broth, this fungal isolate solubilised NCRP (61.3 μg ml^{-1}), followed by MRP (58.3 μg ml^{-1}) and URP (50.3 μg ml^{-1}). It exhibited 1.94 % increase over PVK

during URP solubilisation in NBRIP broth. The minimum P solubilisation was observed in fungal isolate FC4 (Table 6).

It is concluded from the above discussion that rhizosphere soils has higher population of *Bacillus* amongst PSB and *Penicillium*, *Aspergillus fumigatus*, *Aspergillus niger, A.* spp. and non-sporulating sterile amongst PSF. The microbes from soil can be screened in NBRIP broth assay in a better way for identification of most efficient P solubilisers. North Carolina rock phosphate can be solubilised to higher extent even by microbes than Indian rock phosphates.

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