General Impact Factor (GIF): 0.875
Scientific Journal Impact Factor: 1.205
e- ISSN: 2394-5532
p- ISSN: 2394-823X
International Journal of Applied And Pure Science and Agriculture
www.ijapsa.com

Solubilisation of Inorganic Phosphates by Microorganisms Isolated from Cold desert Habitat of *Populus alba* (White Poplar) in Trans Himalayas of Himachal Pradesh of India

Anshu S. Chatli[1], Swati Sood[2] and Viraj Beri[3]

[1]Department of Microbiology, Guru Nanak Girls College, Model Town, Ludhiana 141 002
[2]Department of Biotechnology, Guru Nanak Girls College, Model Town, Ludhiana 141 002
[3]Department of Soils, Punjab Agricultural University, Ludhiana 141 004

Abstract

Phosphate solubilising microorganisms (PSM) were isolated from the natural habitats of Populus alba from Lahaul and Spiti valleys of Himachal Pradesh by pour plating. The PSM formed yellow halos around their colonies on Modified Pikovskaya agar using bromo phenol blue, supplemented with tri calcium phosphate (TCP) as source of insoluble inorganic phosphate. Population enumeration showed the highest count of Phosphate solubilising bacteria (PSB) and fungi (PSF) in the rhizosphere than that of non-rhizosphere. The PSB isolated belonged to genera Bacillus and Micrococcus while the PSF isolated frequently belonged to species of Aspergillus and Penicillium. The isolates with high efficiency for tricalcium phosphate solubilisation, were further tested for their capacities to solubilise Mussoorie Rock Phosphae (MRP), Udaipur Rock Phosphate (URP) and North Carolina Rock Phosphate (NCRP). The bacterial isolate PBC2 (Bacillus) was reported to be highly efficient while amongst fungi PFC6 (Aspergillus niger) was solubilising maximum insoluble phosphate.

Key Words: PSM, Populus alba,Lahaul and Spiti Valley

I. Introduction

Phosphorus (P) is an essential soil nutrient which plays a key role in various important steps of cellular metabolism for accumulation and release of energy **(1).** A large amount of P introduced as a fertilizer enters into immobile pools through specific reactions **(2).** Mostly soils contain sufficient reserves of P, a major quantity of it remains relatively inactive while less than 10% of it enters plant-animal cycle **(3).** Phosphate solubilising microorganisms (PSM) affect the soil fertility by improving the P availability to plants by mineralizing complex organic P in soil **(4).** The cold desert in Lahaul and Spiti valleys, located between latitude 31° 42′ 33″ North and 77° 21′ 33″ East in Indian Trans Himalayas, is marked by stressful environment and their soils are neutral to slightly alkaline in nature. *Populus alba* (White Poplar) is a deciduous tree in this area and has a number of medicinal properties. The stem bark is anti-inflammatory, antiseptic, astringent, diuretic and tonic. The plantation of this tree has been taken to avoid soil erosion and degradation in the fragile mountainous system in these valleys. The highly efficient PSM can increase the P availability for the improvement of its growth rate. This paper reports on isolation and screening of PSM for the development of microbial additives for application in afforestation.

II. Materials and Methods

Soil samples (0-30 cms depth) were collected from the rhizosphere and non-rhizosphere of feeder roots of the natural populations of *Populus alba* at Kukumseri and Udaipur. These samples were examined for physico-chemical properties viz. pH, Electrical conductivity, Organic carbon, available P, K and Total nitrogen following Standard AOAC methods. Total bacterial and fungal populations were estimated by serial dilution pour plate method on Nutrient agar media (NAM) and Potato Dextrose agar (PDA), respectively.

Isolation of Phosphate Solubilising Microorganisms:

P solubilising bacteria (PSB) and fungi (PSF) were enumerated by using Pikovskaya **(5),** Modified Pikovskaya **(6)** and National Botanical Research Institute Agar **(7)** supplemented with Tricalcium Phosphate (TCP) as insoluble inorganic phosphate source. The population of these microbes was determined as a ratio of total number of P solubilising colonies to total number of colonies of bacteria and fungi isolated on NAM and PDA, respectively, using same serial dilution and expressed as percentage. The bacterial isolates were characterized on the basis of cultural, morphological and biochemical characteristics as described by Bergey's Manual. Identification of Fungal isolates was done on the basis of various cultural and microscopic characteristics **(8).** The PSM isolated from these locations are represented below.

Location		Bacteria	Fungi
Kukumseri	R	PBC1 (*Bacillus*)	PFC1 (unidentified)
		PBC2 (*Bacillus*)	PFC2 (*Aspergillus niger*)
	NR	PBC3 (Unidentified)	PFC3 (*Penicillium*)
			PFC4 (Unidentified)
Udaipur	R	PBC4 (*Micrococcus*)	PFC6 (*Aspergillus niger*)
		PBC5 (Unidentified)	PFC7 (Unidentified)
	NR	PBC6 (*Bacillus*)	

R= Rhizosphere
NR= Non- Rhizosphere

Determination of Phosphate Solubilisation

The three day old bacterial cultures and seven days old fungal cultures were inoculated with 4x4 mm cork borer on three different media viz. PVK, modified PVK and NBRIP agar (5) **(7)** supplemented with TCP as insoluble inorganic Phosphate source. The plates were incubated at 28±1°C for 5 days for bacterial cultures and 7 days for fungal cultures. The halo size around the colony was calculated by subtracting the colony diameter from the total diameter of the colony with the surrounding halo. The uninoculated plates with three different media were used as control.

Quantitative Estimation of P solubilisation in Broth

10^6 bacterial cells and $30X10^5$ fungal spores/ml were inoculated in 100 ml PVK and NBRIP broth, respectively and incubated for 5 days for bacteria and 6 days for fungi under shake at 250 rpm. Uninoculated broth served as control. The solubilised P was determined in clear filterate using Ascorbic acid method **(9)**. The intensity of blue colour was measured on spectrophotometer at 730 nm and the quantity of solubilised P was expressed as µg/ml. The final pH of culture filterate and the fungal biomass per 100 ml of broth was estimated. The highly efficient P solubilisers were tested for their efficacy to solubilise rock phosphates viz. North Carolina Rock Phosphate (NCRP), Mussoorie Rock Phosphate (MRP) and Udaipur Rock Phosphate (URP) in PVK and NBRIP broths, respecively. The fungal biomass was also determined.

III. Results and Discussion

PSB and PSF were detected in all the soils from rhizosphere and non-rhizosphere regions of *P. alba*. The population counts of PSM was higher in rhizosphere soil than those in non-rhizosphere soils. The contribution of PSB to total population ranged from 1.69 to 3.33 percent in rhizosphere soils while 1.00 to 3.15 percent in non-rhizosphere soil. The contribution of PSF to total population ranged from 6.00 to 11.40 percent in rhizosphere while 3.33 to 10.00 percent in non-rhizosphere soils (Table 1). The low viable counts of microorganisms as well as that of PSM in the soil samples from Lahaul and Spiti valleys are possibly due to temperature and moisture factors coupled with soil fertility levels **(10).**

All the PSB and PSF produced distinct yellow coloured zones on modified PVK agar, supplemented with TCP (Table 2).The development of halos around the colonies indicated the dissolution of phosphate source by microbes and it is due to acidification of medium **(11) & (12).** The variations of halo size in isolates indicates the difference in their efficiency of phosphate solubilisation **(6)**. Amongst PSB, PBC2 (*Bacillus*) produced bigger clear zones followed by PBC5 (Unidentified) (Table 2) while among PSF, PFC6 (*A. niger*) represented bigger halos followed by PFC5 (*A. fumigatus*) (Table 3). While studying their efficacy to solubilise TCP in PVK and NBRIP broth, the maximum efficiency of P solubilisation was observed in NBRIP broth than in PVK broth. Amongst PSB, PBC2 (*Bacillus*) solubilised maximum TCP (77 µg/ml) followed by PBC5 (Unidentified) (62.3 µg/ml) and PBC1 (*Bacillus*) (50.6 µg/ml) in NBRIP broth. The maximum decrease in pH was observed in PBC2 (*Bacillus*) (5.92) in NBRIP broth (Table 2). Among PSF, PFC6 (*A. niger*) solubilised maximum TCP (104.3 µg/ml) followed by PFC5 (*A. fumigatus*) (100 µg/ml) and FC3 (*Penicillium*) (92.3 µg/ml) in NBRIP broth. PFC6 (*A.niger*) represented maximum decrease in pH (4.51) in NBRIP broth. The maximum fungal biomass was also observed in PFC6 (*A. niger*) (0.564 g/100ml) in NBRIP broth (Table 3).

Out of six PSB isolates, three PSB and out of seven PSF, three PSF were tested for their efficacy to solubilise NCRP, MRP and URP both in PVK and NBRIP broth. Amongst bacterial isolates, PBC2 (*Bacillus*) represented highest solubilisation of NCRP (47.5 µg/ml in NBRIP and 40.6 µg/ml in PVK broth), followed by MRP (38.9 µg/ml in NBRIP and 36.5 µg/ml in PVK broth) and URP (29.5 µg/ml in NBRIP and 26 µg/ml in PVK broth). PBC2 (*Bacillus*) represented maximum fall in pH of filtrate in NBRIP broth (from 6.8 to 6.12) than that in PVK broth (from 6.8 to 6.17) during NCRP solubilisation amongst rock phosphates (Table 4). Rock phosphate solubilisation by bacteria including *Bacillus*, *Pseudomonas* etc has also been reported by **(11)**. Similarly, among the fungal isolates, PFC6 (*A. niger*) solubilised highest NCRP (61.4 µg/ml in NBRIP and 58.5 µg/ml in PVK broth) followed by MRP (58.6

µg/ml in NBRIP and 55.9 µg/ml in PVK broth) and URP (41.9 µg/ml in NBRIP and 40.1 µg/ml in PVK broth). This isolate exhibited max fall in pH from 6.8 to 5.83 in NBRIP and from 6.8 to 5.9 in PVK broth during NCRP solubilisation. The fungal biomass was also reported to be highest in PFC6 (*A. niger*) in NBRIP broth (0.494 g/100 ml) than in PVK broth (0.491 g/100 ml) during NCRP solubilisation (Table 5). Some species of *Aspergillus* and *Penicillium* have been tested for solubilisation of different rock phosphates **(13) & (14).**

The quantities of rock phosphate solubilised both by bacteria and fungi were less than that of TCP solubilised. The fungal isolates proved to be more efficient phosphate solubilisers as compared to bacterial isolates.

Bibliography

[1] Tandon, H.L.S. (1987) Phosphorus Research and Agricultural Production in India. Fertilizer Development and Consultation Organization, New Delhi, pp 160.

[2] Hao, X; C.M. Cho, G.J. Racz and C. Chang 2002. Chemical retardation of phosphate diffusion in an acid soil as affected by liming. *Nutri. Cycl. Agroecosys.* 64: 213-224.

[3] Kucey, R.M.N., Janzen, H.H. and Leggett, M.E. 1989. Microbially mediated increases in plant available phosphorus. *Advances in Agronomy*. 42: 199-228 .

[4] Chen, Y.P., P.D. Rekha, A.B. Arunshan, W.A. Lai and C.C. Young 2006. Phosphate solubilising bacteria from subtropical soil and their tricalcium phosphate solubilising abilities. *Appl. Soil Ecol*. 34: 33-41.

[5] Pikovskaya, R. I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya* 17: 362-370.

[6] Gupta R, Singal R , Shankar A, Kuhad R.C. and Saxena R.K. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *J. Gen. Appl. Microbiol*. 40: 255-260.

[7] Nautiyal, C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett*, 170:265-270.

[8] Barnett, H.L. and Hunter, B.B. (1972) Illustrated Genera of Imperfecti Fungi. Burgess Publishing Company, Minneapolis, Minnesota, Pp. 241.

[9] Watanabe F.S and Olsen S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and Sodium bicarbonate extracts from soils. *Soil Sci Soc of America Proceedings*. 29: 677-678.

[10] Venkateswarlu, B, Rao, A.V., Raina, P. 1984. Evaluation of phosphorus solubilization by microorganisms isolated from Arid soils. *Journal of Indian Society of Soil Science*, 32:273-277.

[11] Halder, A. K., Mishra, A.K., Bhattacharya, P. and Chakrabartty, P.K. 1990. Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. *J. Gen. Appl. Microbiol*.. 36: 81-92.

[12] Yadav, K.S. and Dadarwal, K.R. 1997. Phosphate solubilization and mobilization through microorganisms. In: Dadarwal, K.R. (Ed), Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production, Scientific Publishers, Jodhpur, Pp. 293-308.

[13] Narsian, V. and Patel, H.H. 2000. Aspergillus aculeatus as a rock phosphate solubiliser. *Soil Biology and Biochemistry*, 32: 559-565.

[14] Reddy, M.S., Kumara, S, Babita, K and Reddy, M.S. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. *Bioresource Technology*, 84:187-189.

Table 1 Phosphate solubilising microorganisms from the natural habitat of *Populus alba* from Lahaul and Spiti valleys

Location	Soil Sample	Bacteria							Fungi						
		Mean plate count /gram soil (10^3)							Mean plate count /gram soil (10^3)						
		Total (Range)	P-Solubilisers (Range)			Per cent P-Solubilisers (Range)			Total (Range)	P-Solubilisers (Range)			Per cent P-Solubilisers (Range)		
			PVK	MPVK	NBRIP	PVK	MPVK	NBRIP		PVK	MPVK	NBRIP	PVK	MPVK	NBRIP
Kukumseri	R	118.00-125.00	2.00-3.00	2.00-4.00	2.00-3.00	1.69-2.40	1.69-3.20	1.69-2.40	50.00-53.00	3.00-5.00	4.00-5.00	3.00-5.00	6.00-9.43	8.00-9.43	6.00-9.43
	NR	80.00-95.00	1.00-2.00	1.00-3.00	1.00-2.00	1.25-2.10	1.25-3.15	1.25-2.10	30.00-45.00	1.00-2.00	2.00-3.00	2.00-3.00	3.33-4.44	6.66-6.66	6.66-6.66
Udaipur	R	130.00-150.00	3.00-4.00	3.00-5.00	3.00-5.00	2.30-2.66	2.30-3.33	2.30-3.33	30.00-35.00	2.00-3.00	3.00-4.00	2.00-3.00	6.66-8.57	6.66-11.4	6.66-8.57
	NR	100.00-120.00	1.00-3.00	2.00-3.00	1.00-3.00	1.00-2.50	2.00-2.50	1.00-2.50	25.00-30.00	1.00-2.00	1.00-3.00	2.00-3.00	4.00-6.66	4.00-10.00	6.66-10.00

Table 2 Tricalcium phosphate solubilisation by bacterial isolates in agar and broth using Pikovskaya medium and NBRIP medium in *Populus alba* (Initial pH = 6.8)

Bacteria	Treatment						
	Agar (Halo size (mm))			Broth (µg/ml P solubilised)		Final pH of broth	
	Pikovskaya	Modified Pikovskaya	NBRIP	Pikovskaya	NBRIP	Pikovskaya	NBRIP
PBC1 (*Bacillus* sp.)	4.60 ± 1.15	5.00 ± 0.00	4.30 ± 0.570	48.6 ± 2.08	50.6 ± 2.51	6.08 ± 0.015	6.00 ± 0.005
PBC2 (*Bacillus* sp.)	6.30 ± 1.15	8.00 ± 1.00	6.30 ± 0	72.3 ± 2.08	77.0 ± 3.00	5.95 ± 0.020	5.92 ± 0.015
PBC3 (Unidentified)	2.60 ± 0.570	4.00 ± 1.00	3.60 ± 1.00	38.3 ± 2.08	41.3 ± 1.52	6.16 ± 0.023	6.09 ± 0.010
PBC4 (*Micrococcus*)	3.30 ± 1.52	5.60 ± 0.570	3.60 ± 0.015	44.0 ± 2.00	45.6 ± 3.05	6.12 ± 0.015	6.03 ± 0.020
PBC5 (Unidentified)	5.30 ± 0.570	7.30 ± 1.52	5.30 ± 2.00	61.0 ± 1.00	62.3 ± 2.51	6.01 ± 0.020	5.98 ± 0.010
PBC6 (*Bacillus* sp.)	2.30 ± 0.570	4.30 ± 1.52	3.30 ± 0.570	27.3 ± 2.51	30.0 ± 2.00	6.25 ± 0.045	6.13 ± 0.030

Table 3 Tricalcium phosphate solubilisation by fungal isolates in agar and broth using Pikovskaya and NBRIP medium in *Populus alba* (Initial pH =6.8)

Fungi	Treatments								
	Agar (Halo size (mm))			Broth (µg/ml P solubilised)		Final pH of broth		Fungal biomass (gms/100ml)	
	Pikovskaya	Modified Pikovskaya	NBRIP	Pikovskaya	NBRIP	Pikovskaya	NBRIP	Pikovskaya	NBRIP
PFC1 (Unidentified)	2.6 ± 0.577	5.6 ± 0.577	5.6 ± 0.577	38.6 ± 2.30	55.0 ± 4.58	6.08 ± 0.010	6.00 ± 0.015	0.332 ± 0.002	0.343 ± 0.003
PFC2 (*A. niger*)	1.6 ± 0.577	4.0 ± 0.00	4.3 ± 1.15	38.6 ± 1.15	50.0 ± 1.00	6.07 ± 0.005	5.98 ± 0.011	0.330 ± 0.00	0.336 ± 0.002
PFC3 (*Penicillium* sp.)	7.0 ± 1.00	10.3 ± 0.577	9.6 ± 2.51	84.3 ± 4.04	92.3 ± 2.08	4.83 ± 0.032	4.77 ± 0.015	0.503 ± 0.004	0.517 ± 0.002
PFC4 (Unidentified)	5.6 ± 0.570	6.3 ± 1.15	9.0 ± 1.00	66.6 ± 5.85	80.6 ± 1.15	5.06 ± 0.005	4.99 ± 0.011	0.463 ± 0.003	0.487 ± 0.001
PFC5 (*A.fumigatus*)	9.0 ± 0.00	10.0 ± 2.00	11.0 ± 1.00	96.6 ± 2.08	100.0 ± 5.00	4.79 ± 0.017	4.71 ± 0.015	0.522 ± 0.003	0.541 ± 0.002
PFC6 (*A. niger*)	11.0 ± 1.00	12.6 ± 0.333	12.0 ± 2.00	101.6 ± 2.88	104.3 ± 5.13	4.56 ± 0.020	4.51 ± 0.020	0.548 ± 0.002	0.564 ± 0.004
PFC7 (Unidentified)	5.3 ± 0.333	5.6 ± 1.15	7.6 ± 0.577	60.6 ± 2.08	75.3 ± 2.51	5.11 ± 0.015	5.02 ± 0.005	0.456 ± 0.001	0.468 ± 0.002

Table 4 Rock phosphate solubilisation (µg/ml) and pH of filtrate by bacterial isolates in Pikovskaya and NBRIP broth in *Populus alba*

Rock phosphates		NCRP		MRP		URP	
Media / Isolates		PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
PBC1 (*Bacillus* sp.)	P solubilisation	18.1 ± 0.378	21.3± 0.568	12.6 ± 1.15	15.1 ± 0.305	7.5 ± 0.503	10.5 ± 0.624
	pH	6.52 ± 0.025	6.44± 0.035	6.57 ± 0.025	6.49 ± 0.075	6.61 ± 0.011	6.59 ± 0.020
PBC2 (*Bacillus* sp.)	P solubilisation	40.6 ± 0.568	47.5 ± 1.55	36.5 ± 0.608	38.9 ± 0.208	26.0 ± 0.305	29.5 ± 0.513
	pH	6.17 ± 0.005	6.12 ± 0.00	6.2 ± 0.005	6.14 ± 0.037	6.25 ± 0.026	6.21 ± 0.040
PBC5 (Unidentified)	P solubilisation	19.6 ± 0.635	25.0± 0.100	16.4 ± 0.568	18.2 ± 0.305	13.3 ± 0.360	16.2 ± 0.404
	pH	6.23 ± 0.027	6.19± 0.010	6.27 ± 0.00	6.22 ± 0.028	6.31 ± 0.037	6.27 ± 0.025

Table 5 Rock phosphate solubilisation (µg/ml), pH of broth and fungal biomass (gms./100 ml) in Pikovskaya and NBRIP broth in *Populus alba*

Rock phosphates		**NCRP**		**MRP**		**URP**	
Media / **Isolates**		**PVK**	**NBRIP**	**PVK**	**NBRIP**	**PVK**	**NBRIP**
PFC3 (*Penicillium* sp.)	P solubilisation	48.1 ± 0.611	50.2 ± 0.832	39.7 ± 2.23	43.1 ± 0.360	29.9 ± 0.208	37.7 ± 0.493
	pH	6.04 ± 0.017	5.08 ± 0.020	6.15 ± 0.020	6.08 ± 0.568	6.25 ± 0.023	6.18 ± 0.020
	Fungal biomass	0.405± 0.004	0.421 ± 0.001	0.41 ± 0.001	0.420 ± 0.00	0.371 ± 0.00	0.381± 0.001
PFC5 (*A.fumigatus*)	P solubilisation	56.7 ± 0.585	60.7 ± 0.680	51.2 ± 0.665	55.6 ± 0.832	38.1 ± 1.10	40.1 ± 1.05
	pH	5.96 ± 0.025	5.85 ± 0.035	6.11 ± 0.020	6.01 ± 0.010	6.21 ± 0.025	6.11 ± 0.015
	Fungal biomass	0.453± 0.003	0.476 ± 0.003	0.447± 0.004	0.474 ± 0.005	0.428± 0.001	0.449± 0.001
PFC6 (*A. niger*)	P solubilisation	58.5 ± 1.15	61.4 ± 1.20	55.9 ± 1.00	58.6 ± 1.22	40.1 ± 0.200	41.9 ± 1.15
	pH	5.90 ± 0.010	5.83 ± 0.00	6.00 ± 0.020	5.95 ± 0.015	6.09 ± 0.020	6.06 ± 0.020
	Fungal biomass	0.491± 0.001	0.494 ± 0.003	0.480 ± 0.00	0.488 ± 0.003	0.473± 0.003	0.475± 0.001

