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Dissolution of Inorganic Phosphates by Microorganisms isolated from the cold desert habitat of Seabuckthorn (*Hippophae rhamnoides*) in Trans-Himalayas of Himachal Pradesh of India

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ABSTRACT

Phosphate solubilisation efficacies of four bacterial and four fungal isolates were tested on different media after isolating from the soils of Lahaul and Spiti valleys. Amongest bacterial isolates, HBP1 and in fungal isolates, HFP1, represented appreciate solubilisation of TCP followed by MRP and URP and represented maximum fall in pH of filterate in NBRIP broth. The HBP1 solubilised maximum TCP at pH 9 and HFP1 at pH 5. Moreover, HBP1 proved to be the most efficient strain in solubilizing TCP in the presence of 2.5% w/v NaCl. HFP1 fungal isolate represented highest potential to solubilize TCP in control than all other concentrations. The HBP1 and HFP1 solubilised maximum P at 24^{o}C temperatures.

Key words: *Hippophae rhamnoides, Phosphate solubiliser, Lahul, Spiti.*

INTRODUCTION

Phosphorus (P) is one of the major essential macronutrient for biological growth and development. A large portion of inorganic P applied to soil as fertilizer is rapidly immobilized soon after application and becomes unavailable to plants. Plant growth promoting microbial formulations in the form of P solubilising microbes (PSM) can overcome this problem by dissoluting these immobilized products (Dey, 1988). The solubilisation of insoluble phosphates in soil is due to acidification, chelation and exchange reactions (Gerke, 1992; Vazquez *et al,* 2000). Organic acids which bind phosphate anions bring about phosphate in solution (Nautiyal *et al*, 2000) and improve soil fertility. Therefore the pH dependent release of insoluble and fixed form of P is an important aspect of increasing soil P availability.

The cold desert in Lahaul and Spiti valleys, located between latitude 31^o 42$^/$ 35$^{//}$ East in Indian trans-Himalayas, is marked by stressful environment and rugged terrain with poor availability of some essential mineral nutrients in soil. Consequently, these valleys have a sparse vegetation cover. Pre-transplant fortification of nursery plants with suitable native microbial inoculants appears imperative for restoring ecological balance by checking desertification through afforestation. Seabuckthorn (*Hippophae rhamnoides*) is ecologically the most important plant in the cold desert region with the highest Importance Value Index (Singh and Gupta, 1990). Plantation of the spinescent shrub-tree has been taken to avoid erosion and degradation in the fragile mountaneous system in these valleys. The paper reports on the screening and selection of phosphate solubilising bacteria and fungi for the development of microbial additives for application in afforestation. Information on occurrence and activity of these microorganisms in the soils of cold desert in Lahaul and Spiti valleys is lacking altogether.

MATERIALS AND METHODS

Soil sampling, physicochemical properties and Population Enumeration

Soil samples (0-15 cm. depth) collected from the rhizosphere and non-rhizosphere of *Hippophae rhamnoides* at Trilokinath, Jhalma, Theorat, Keylong, Kukumseri and Rong Tong in Lahaul and Spiti valleys of Himachal Pradesh were examined for physicochemical properties viz. pH, Electrical conductivity, organic carbon, available P, K and total N following standard AOAC methods. Total bacterial and fungal population were determined by serial dilutions on Nutrient Agar medium (NAM) and Potato Dextrose agar (PDA), respectively.

Isolation of Phosphate solubilising Microorganisms

Phosphate dissolving bacteria and fungi were isolated and enumerated by employing Pikovskaya (PVK) (Pikovskaya, 1948), modified Pikovskaya (MPVK) (Gupta *et al*, 1994) and National Botanical Research Institute (NBRIP) agar (Nautiyal, 1999), respectively to total number of colonies of bacteria and fungi obtained on NAM and PDA, respectively, using the same soil dilutions. The PSM isolated from different locations are represented below:

Location		Bacteria	Fungi
Trilokinath	R	-	HFP3 (*Penicillium* sp.)
	NR	-	-
Jhalma	R	-	-
	NR	-	HFP2 (Unidentified)
Theorat	R	HBP1 (*Bacillus* sp.)	-
	NR	-	-
Keylong	R	HBP2 (Unidentified)	HFP1 (*Penicillium* sp.)
	NR	-	-
Kukumseri	R	HBP3 (*Bacillus* sp.)	-
	NR	-	HFP4 (*Aspergillus* sp.)
Rong Tong	R	HBP4 (*Micrococcus*)	-
	NR	-	-

R = Rhizosphere, NR = Non-rhizosphere

Phosphate Solubilising Efficiency of Culture Isolates

10^6 bacterial cells and 3X 10^6 fungal spores/ml were inoculated in 100ml PVK and NBRIP broths supplemented with tri calcium phosphate (TCP) (18.5 % P_2O_5), Mussoorie rock phosphate (MRP) (21.1 % P_2O_5) and Udaipur rock phosphate (URP) (23.3 % P_2O_5), respectively. The phosphate sources were added @ 5 g/ l after 4-5 washings with 5N sodium bicarbonate and then dried at 40^0 C for 24 hours. These microbes were incubated for 5 days under shake at 250 rpm. Uninoculated broth served as control. The solubilised P was determined in clear filtrate using Ascorbic acid method (Watanabe and Olsen, 1965). The intensity of blue colour was measured on spectrophotometer at

730 nm and the quantity of P solubilised was expressed as µg/ml. The final pH of culture filtrate was also determined.

Analysis of organic acids

The organisms were grown in PVK broth supplemented with TCP for 5 days under shake at 28$\pm$2^{o}C. Qualitative analysis of organic acids was done by paper chromatography using solvent n-butanol: acetic acid: water (12:3:5) (Nordmann and Nordmann., 1960).

The P solubilising microbes were selected for P dissolution at various pH values (5, 6, 7, 8, 9), NaCl concentrations (2.5, 5, 7.5 and 10 % w/v) and temperature regimes (9^{0}, 12^{0}, 18^{0}, 24^{0}, 35^{0} and 40^{0} C). The highly efficient microbes were also used in combinations for testing their P dissolution abilities.

RESULTS AND DISCUSSION

Total eighteen soil samples, three from each subclass of every location viz. Trilokinath, Jhalma, Theorat, Keylong and Kukumseri from Lahaul valley while Rong Tong from Spiti valley were examined for physico-chemical properties. The soils of Lahaul and Spiti valleys were reported to be neutral to slightly alkaline with pH values 6.9 to 7.6. The electrical conductivity (E.C.) values ranged in between 0.35-0.69 mmhos/cm indicating that the nature of soil is normal. The organic matter content varied in between 0.09-1.22%, available P 30.2-38.6 Kg/ha, available K 145.6-310.8 Kg/ha and total Nitrogen 0.026-0.082 % (Table 1).

Phosphate solubilising bacteria and fungi were detected in all the soils from rhizosphere and non-rhizosphere of *H. rhamnoides*. The population counts of PSM were higher in rhizosphere soils than those in non-rhizosphere soils. The results showed a positive rhizosphere influence on the population of phosphate solubilising bacteria (PSB) and fungi (PSF) by *H. rhamnoides*. Also the proportion of PSM was low to the total populations of these organisms. The contribution of PSM to the total population was reported to be more in MPVK and NBRIP agar than that on PVK agar. The occurrence of PSB to total bacterial population ranged from 5.17-9.03 on MPVK and 5.06-8.40 on NBRIP agar in rhizosphere. The frequency of PSF to total fungal population varied from 8.38-9.82 on MPVK and 8.61-10.00 on NBRIP agar in rhizosphere. The occurrence of P solubilisers in higher numbers in the rhizosphere is of direct significance to plants as it helps in mobilization of insoluble P, especially in P deficient soils. The highest occurrence of PSM to total microbial population was reported in Theorat (Table 2). The low total viable counts of bacteria and fungi as well as low viable counts of PSB and PSF in the soil samples from Lahaul and Spiti valleys as compared to the soils from other climosequences in Himachal Pradesh are possibly due to temperature and moisture factors coupled with soil fertility levels (Gupta *et al*, 1986; Venkateswarlu *et al*, 1984).

Four bacterial viz. HBP1 (*Bacillus* sp.), HBP2 (Unidentified), HBP3 (*Bacillus* sp.) and HBP4 (*Micrococcus*) and four fungal isolates viz. HFP1 (*Penicillium* sp.), HFP2 (Unidentified), HFP3 (*Penicillium* sp) and HFP4 (*Aspergillus* sp.) tested for P solubilisation using TCP, MRP and URP in PVK and NBRIP broths brought about drop in pH of culture medium (Table 3). Amongst bacterial isolates HBP1 (*Bacillus* sp.) represented appreciable solubilisation of TCP (111.3 µg/ml in NBRIP and 111.3 µg/ml in PVK) followed by MRP (70.3 µg/ml in NBRIP and 63.0 µg/ml in PVK) and URP (51.4 µg/ml in NBRIP and 48.9 µg/ml in PVK) while the HBP4 (*Micrococcus*) the least. This isolate represented maximum fall in pH of filtrate in NBRIP broth (from 6.8 to 5.87) than that in PVK broth (from 6.8 to 5.98) during MRP solubilisation amongst rock phosphates. Similarly among the fungal isolates HFP1 (*Penicillium* sp.) solubilised highest TCP (100.0 µg/ml in NBRIP and 91.3 µg/ml in PVK) followed by MRP (59.6 µg/ml in NBRIP and 54.2 µg/ml in PVK) and URP (35.2 µg/ml in NBRIP and 33.7 µg/ml in PVK). This isolate exhibited maximum fall in pH from 6.8 to 5.97 in NBRIP broth and from 6.8 to 6.06 in PVK broth during MRP solubilisation. In the process of solubilisation of inorganic P, the PSM might also be exerting influence on soil alkalinity by decreasing pH of soil. The screened microorganisms viz. HBP1 (*Bacillus* sp.) and HBP2 (unidentified) after incubation in PVK broth could solubilise TCP and produced mainly citric and oxalic acids while HFP1 (*Penicillium* sp.) produced oxalic acid (Table 4). These organic acids seem to be responsible for drop in pH. Our results are in consonance with that of Gaind and Gaur., 1989 and Narsian and Patel, 2000.

Effect of pH on P Solubilisation

Solubilisation of phosphate sources by bacterial isolates increased with change in pH from neutral to alkaline range, while higher solubilisation of phosphate by fungal isolates was obtained with decrease in pH from neutral to acidic range.

Out of four bacterial isolates two viz. HBP1 (*Bacillus* sp.) and HBP2 (Unidentified) and four fungal isolates two viz. HFP1 (*Penicillium* sp.) and HFP2 (Unidentified) were tested for their P dissolution abilities at different pH values varying from acidic (pH 5) to alkaline (pH 9). The bacterial isolate HBP1 (*Bacillus* sp.) solubilised maximum TCP (116.2 µg/ml in NBRIP and 112.2 µg/ml in PVK), followed by MRP (82.6 µg/ml in NBRIP and 79.0 µg/ml in PVK) and URP (53.1 µg/ml in NBRIP and 51.7 µg/ml in PVK) at pH 9 while minimum at pH 5. The bacterial isolates represented a positive correlation of P solubilisation on increase in pH values (r = 0.569*** in PVK and 0.570*** in NBRIP). The relationship of P solubilisation unit (Y µg/ml) with increase in pH values (X) can be represented in the form of regression equation.

In Bacterial isolates

i) During TCP solubilisation

$Y = 14.59X – 10.93, R^2 = 0.85$

ii) During MRP solubilisation

$Y = 11.52X – 23.61, R^2 = 0.86$

iii) During URP solubilisation

$Y = 7.21X – 10.24, R^2 = 0.83$

The equation shows that the slope is positive so at lower pH, P dissolution is less. The TCP is much more solubilised than that of MRP and URP as the value of slope is more in TCP as compared to that of MRP and URP. Moreover the value of intercept is highest in TCP, followed by RPs, representing highest dissolution of TCP. There is much variability amongst bacterial isolates during P solubilisation as the value of R^2 is more.

Amongst the fungal isolates HFP1 (*Penicillium* sp.) solubilised maximum TCP (88.1 µg/ml in NBRIP and 86.6 µg/ml in PVK) followed by MRP (60.1 µg/ml in NBRIP and 58.7 µg/ml in PVK) and URP (47.4 µg/ml in NBRIP and 45.7 µg/ml in PVK) at pH 5 while it recorded minimum P solubilisation at pH 9 (Table 5). A negative correlation of P dissolution with increase in pH values was reported (r = -0.581*** in PVK and -0.556*** in NBRIP). The relationship of P solubilisation with increase in pH during P solubilisation in fungal isolates in equation form can be represented as

During TCP solubilisation

I) $Y = -9.135X + 133.5, R^2 = 0.79$

During MRP solubilisation

II) $Y = -8.20X + 102.69, R^2 = 0.82$

During URP solubilisation

III) $Y = -6.22X + 77.28, R^2 = 0.74$

The equation shows that the slope is negative in fungal isolates so at lower pH values the P dissolution is more. The value of intercept is more in TCP, followed by MRP and URP, hence TCP is solubilised easily followed by MRP and URP. The value of R^2 is more indicating that there is much more variation among both the fungal isolates.

The results showed that the pH of nutrient medium influences the performance of PSM. Gaur, 1990 observed that bacteria represented maximum dissolution at pH 7 to 8 while the fungi between 5 to 6. The concentration of hydroxyl ions is one of the important factors determining the P solubilising activity of microorganisms and also the availability of P in soil (Gaind and Gaur, 1989). Organic acids produced by PSM chelate calcium ions to bring P into solution (Randall *et al* 2001, Gresshoff et al, 2014).

Effect of NaCl on P solubilisation

To study the effect of high salt on P solubilisation abilities of microbes, the screened two bacterial and two fungal isolates were grown in the presence of high salt (2.5, 5, 7.5 and 10 % w/v NaCl). All these strains demonstrated diverse level of P solubilisation. The P dissolution abilities of PSB were higher than control (0% w/v NaCl) in the presence of 2.5 % w/v NaCl. It seemed therefore that the PSB from slightly alkaline soils have the potential to solubilise P at 2.5 % w/v NaCl. HBP1 (*Bacillus* sp.) proved to be the most efficient strain in solubilising TCP individually in the presence of 2.5 % w/v NaCl (111.3 µg/ml in NBRIP and 108.5 µg/ml in PVK). The maximum decrease in the pH of filtrate was also reported at this salt concentration. A decreasing trend in P solubilisation was observed with increase in salt concentration from 5% w/v to 10% w/v NaCl, resulting in increase in pH of culture filtrate. During rock phosphate solubilisation, this bacterial isolate solubilised maximum MRP (71.6 µg/ml in NBRIP and 69.4 µg/ml in PVK) followed by URP at 2.5% w/v NaCl and further increase in salt concentration resulted in decrease in RP solubilisation. Our results are in consonance with that of Gaind *et al*, 1999. The bacterial isolate HBP2 (unidentified) could not solubilise URP at 10% w/v NaCl. With the increase in salt concentration the P dissolution was found to decrease (r = -0.669*** in PVK and –0.688*** in NBRIP). Similarly, a negative correlation of pH decrease of broth with increase in salt concentration was reported (r = -0.419** in PVK and –0.476*** in NBRIP). On the other hand, amongst fungal isolates HFP1 (*Penicillium* sp.) represented highest potential to solubilise P in control than all other concentrations. Both the fungal isolates, HFP1 (*Penicillium* sp.) and HFP2 (Unidentified) showed nil solubilisation at 10% w/v NaCl during rock phosphate dissolution in control than all other concentrations (Table 6). With increse in salt concentration, the P dissolution was decreased (r = -0.694*** in PVK and –0.704*** in NBRIP). The opposite correlation of salt concentration with drop in pH was reported (r = -0.617*** in PVK and –0.615*** in NBRIP).

Effect of Temperature on P Solubilisation

To determine the effect of temperature on P solubilisation abilities of microbes, the isolates were grown at various temperatures (9^0, 12^0, 18^0, 24^0, 35^0 and 40^0 C). A diverse level of P dissolution was observed at higher temperatures viz. 35^o and 40^o C. The dissolution potentials of microbes were found to be highest at 24^o C temperature (mesophilic conditions). The highly efficient bacteria HBP1 (*Bacillus* sp.) solubilised maximum P (106.5 µg/ml in NBRIP and 100.9 µg/ml in PVK) and represented maximum fall in pH of filtrate at 24^0 C. The solubilisation efficacy of this isolate was reported to be at par at 18^0 C and 35^0 C followed by 12^0, 9^o and 40^o C. The maximum solubilisation and highest decrease in pH of filtrate was reported during MRP solubilisation (61.2 µg/ml in NBRIP and 59.5 µg/ml in PVK) and least during URP solubilisation (51.4 µg/ml in NBRIP and 48.6 µg/ml in PVK) at 24^o C. Amongst PSF, the isolate HFP1 (*Penicillium* sp.) solubilised maximum P (94.1 µg/ml in NBRIP and 92.8 µg/ml in PVK) at 24^o C, followed by 18^0, 35^o, 12^0, 9^0 and 40^o C. This isolate solubilised maximum MRP (59.2 µg/ml in NBRIP and 57.3 µg/ml in PVK) and least URP (46.6 µg/ml in NBRIP and 44.5 µg/ml in PVK) at 24^o C. The highest fall in pH of filtrate was reported under mesophilic conditions in all the PSMs (Table 7). Illmer and Schinner, 1992 and Tariq et al, 2014 also observed that the highest solubilisation of P occurs at 30^o and 25^oC temperature, respectively .

Dissolution of Inorganic Phosphates by Interacting P solubilisers in Solution Culture

The relative P solubilisation efficacies of screened two PSB viz. HBP1 (*Bacillus* sp.), HBP2 (Unidentified) and one PSF viz. HFP1 (*Penicillium* sp.) were tested either singly or in combinations using different sources of inorganic P viz. TCP, MRP and URP in PVK and NBRIP broths. HBP1 (*Bacillus* sp.) and HBP2 (unidentified) bacterial isolates solubilised maximum P in combinations (112.4 µg/ml of TCP solubilised in NBRIP and 110.3 µg/ml in PVK, 70.0 µg/ml of MRP solubilised in NBRIP and 69.5 µg/ml in PVK, 54.9 µg/ml of URP solubilised in NBRIP and 53.2 µg/ml in PVK) than individually. The interaction of HBP1 (*Bacillus* sp.), HBP2 (Unidentified) and HFP1 (*Penicillium* sp.) resulted in highest P solubilisation indicating synergistic effect of microbes (122.5 µg/ml of TCP solubilised in NBRIP and 120.8 µg/ml in PVK, 72.9 µg/ml of MRP solubilised in NBRIP and 70.9 µg/ml in PVK, 57.9 µg/ml of URP solubilised in NBRIP and 54.6 µg/ml in PVK). A direct correlation was reported between increase in P solubilisation with decrease in pH of filtrate (Table 8). The interactive effect and P dissolution was proved to be better in NBRIP than that in PVK broth.

Table 1 Physicochemical properties of some Lahaul and Spiti Valley soils

Area	pH	EC (mmhos/cm)	Organic Carbon (%)	Available P (Kg/ ha)	Available K (Kg/ ha)	Total N (%)
Trilokinath	7.0	0.35	0.77	34.4	263.2	0.076
Jhalma	6.9	0.69	1.00	35.6	274.4	0.081
Theorat	7.0	0.44	0.89	35.4	266.0	0.079
Keylong	7.1	0.37	1.22	38.6	310.8	0.082
Kukumseri	6.9	0.42	0.72	33.1	254.8	0.076
Rong Tong	7.6	0.68	0.09	30.2	145.6	0.026

Table 2 Phosphate solubilising microorganisms *from* Lahaul and Spiti valleys

Location	Soil Sample	Bacteria							Fungi						
		Mean plate count /gram soil (10^5)							Mean plate count /gram soil (10^3)						
		Total	P-Solubilisers			Per cent P-Solubilisers			Total	P-Solubilisers			Per cent P-Solubilisers (Range)		
			PVK	MPVK	NBRIP	PVK	MPVK	NBRIP		PVK	MPVK	NBRIP	PVK	MPVK	NBRIP
Trilokinath	R	227.3	11.50	12.95	12.45	5.05	5.69	5.47	75.45	5.50	6.80	6.50	7.28	9.01	8.61
	NR	89.95	3.45	4.80	4.65	3.83	5.33	5.16	57.45	2.50	3.65	3.50	4.35	6.35	6.09
Jhalma	R	173.95	7.63	9.00	9.15	4.38	5.17	5.26	51.00	3.94	5.01	5.03	7.72	9.82	9.86
	NR	67.15	1.66	2.08	1.98	2.47	3.09	2.94	46.65	2.66	3.83	3.66	5.70	8.21	7.84
Theorat	R	246.8	20.15	22.30	20.75	8.16	9.03	8.40	83.60	6.65	7.80	7.50	7.95	9.33	8.97
	NR	94.30	5.15	6.45	6.45	5.46	6.83	6.83	63.95	4.45	5.45	5.15	6.95	8.52	8.05
Keylong	R	218.65	15.80	17.60	17.75	7.22	8.04	8.11	67.65	5.00	6.40	6.10	7.39	9.46	9.01
	NR	66.65	1.16	1.66	1.60	1.74	2.49	2.40	50.00	3.20	4.20	3.55	6.40	8.40	7.10
Kukumseri	R	164.5	8.80	10.15	9.80	5.34	6.17	5.95	54.95	4.00	5.15	5.50	7.27	9.37	10.00
	NR	72.30	2.65	3.60	3.00	3.66	4.97	4.14	45.50	2.80	3.65	3.55	6.15	8.02	7.80
Rong Tong	R	175.8	7.45	9.10	8.90	4.23	5.17	5.06	55.45	3.15	4.65	5.05	5.68	8.38	9.10
	NR	70.60	1.15	1.31	1.35	1.62	1.85	1.91	47.65	2.30	3.50	3.50	4.82	7.34	7.34

Table 3 Phosphate solubilisation (μg/ml) and pH of filtrate in Pikovskaya and NBRIP broth

Phosphate sources		TCP		MRP		URP	
Media / Isolates		PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
Bacteria							
HBP1 (*Bacillus* sp.)	P solubilisation	106.9 ± 1.30	111.3 ± 0.776	63.0 ± 0.251	70.3 ± 0.493	48.9 ± 0.321	51.4 ± 0.450
	pH	5.39 ± 0.015	5.17 ± 0.020	5.98 ± 0.011	5.87 ± 0.020	6.11 ± 0.015	6.07 ± 0.020
HBP2 (Unidentified)	P solubilisation	95.5 ± 0.458	100.5 ± 0.888	51.4 ± 0.450	60.4 ± 0.907	39.5 ± 0.500	43.3 ± 0.264
	pH	5.72 ± 0.025	5.18 ± 0.062	6.07 ± 0.015	6.01 ± 0.010	6.21 ± 0.010	6.12 ± 0.015
HBP3 (*Bacillus* sp.)	P solubilisation	89.9 ± 2.92	94.6 ± 0.642	46.6 ± 0.665	48.5 ± 1.11	35.7 ± 0.585	40.9 ± 0.529
	pH	5.82 ± 0.025	5.48 ± 0.020	6.11 ± 0.020	6.04 ± 0.020	6.32 ± 0.0251	6.20 ± 0.010
HBP4 (*Micrococcus*)	P solubilisation	85.4 ± 0.568	89.6 ± 0.556	41.5 ± 1.13	45.4 ± 0.404	35.1 ± 0.500	37.2 ± 0.305
	pH	5.92 ± 0.025	5.62 ± 0.026	6.13 ± 0.015	6.08 ± 0.017	6.4 ± 0.015	6.28 ± 0.010
Fungi							
HFP1 (*Penicillium* sp.)	P solubilisation	91.3 ± 1.17	100.0 ± 0.503	54.2 ± 0.321	59.6 ± 0.416	33.7 ± 0.264	35.2 ± 0.208
	pH	5.78 ± 0.030	5.41 ± 0.020	6.06 ± 0.025	5.97 ± 0.025	6.10 ± 0.010	6.05 ± 0.015
HFP2 (Unidentified Non-sporulating)	P solubilisation	85.6 ± 0.400	90.7 ± 0.264	50.5 ± 0.814	54.6 ± 0.602	30.3 ± 0.264	33.3 ± 0.321
	pH	5.90 ± 0.025	5.63 ± 0.010	6.10 ± 0.005	6.03 ± 0.015	6.15 ± 0.020	6.09 ± 0.020
HFP3 (*Penicillium* sp.)	P solubilisation	80.5 ± 0.700	86.1 ± 0.264	46.4 ± 0.404	50.2 ± 0.953	29.6 ± 0.472	31.9 ± 0.416
	pH	5.96 ± 0.025	5.71 ± 0.010	6.11 ± 0.015	6.08 ± 0.015	6.17 ± 0.015	6.11 ± 0.015
HFP4 (*Aspergillus* sp.)	P solubilisation	76.2 ± 0.251	83.2 ± 0.305	39.4 ± 0.305	41.7 ± 1.41	25.5 ± 0.568	27.2 ± 0.208
	pH	6.01 ± 0.020	5.91 ± 0.020	6.18 ± 0.017	6.10 ± 0.010	6.26 ± 0.020	6.17 ± 0.020

Table 4 Detection of organic acids in culture filtrate of organism tested by paper chromatography

Test organism	Rf value	Organic acid
Bacteria		
HBP1 (*Bacillus* sp.)	0.170	Citric acid
HBP2 (Unidentified)	0.160	Oxalic acid
Fungus		
HFP1 (*Penicillium* sp.)	0.158	Oxalic acid

Table 5 Effect of pH on phosphate solubilisation (µg/ml) by microbial isolates in Pikovskaya and NBRIP broth

Isolates	Phosphate sources	pH Values									
		5		6		7		8		9	
		PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
Bacteria											
HBP1 (*Bacillus* sp.)	TCP	56.5 ± 0.950	59.8 ± 0.529	77.5 ± 0.360	79.3 ± 0.200	106.6 ± 3.01	110.4 ± 0.854	110.7 ± 0.721	115.9 ± 0.305	112.2 ± 0.360	116.2 ± 0.208
	MRP	30.5 ± 0.404	32.0 ± 0.152	49.1 ± 0.152	51.2 ± 0.251	68.3 ± 0.152	71.0 ± 0.953	73.2 ± 0.1	75.6 ± 0.400	79.0 ± 0.208	82.6 ± 0.458
	URP	22.5 ± 0.400	25.1 ± 0.500	33.4 ± 0.208	39.7 ± 0.264	47.5 ± 0.458	50.3 ± 0.200	50.2 ± 0.305	51.6 ± 0.200	51.7 ± 0.360	53.1 ± 0.971
HBP2 (Unidentified)	TCP	52.3 ± 0.264	54.5 ± 0.450	70.8 ± 0.351	73.4 ± 0.550	98.8 ± 0.305	99.6 ± 0.320	102.9 ± 0.360	109.2 ± 1.05	107.5 ± 0.458	110.2 ± 0.251
	MRP	27.3 ± 0.251	28.6 ± 0.435	40.3 ± 0. 264	42.3 ± 0.264	59.2 ± 0.251	62.3 ± 0.472	61.8 ± 0.7	65.2 ± 0.305	68.2 ± 0.152	72.5 ± 0.550
	URP	19.3 ± 0.152	20.2 ± 0.251	30.5 ± 0.351	32.7 ± 0.458	42.5 ± 0.351	45.3 ± 0.818	44.7 ± 0.351	45.8 ± 0.721	48.1 ± 0.152	50.4 ± 0.404
Fungi											
HFP1 (*Penicillium* sp.)	TCP	86.6 ± 0.458	88.1 ± 0.100	83.5 ± 0.458	84.6 ± 0.300	82.0 ± 0.346	86.2 ± 0.251	62.4 ± 0.450	64.2 ± 0.305	48.5 ± 0.351	51.4 ± 0.450
	MRP	58.7 ± 0.568	60.1 ± 0.100	55.3 ± 0.264	57.3 ± 0.305	52.9 ± 0.608	56.5 ± 0.461	40.4 ± 0.450	42.3 ± 0.472	23.8 ± 0.208	29.2 ± 0.916
	URP	45.7 ± 0.200	47.4 ± 0.208	43.7 ± 0.360	44.9 ± 0.650	40.1 ± 0.115	43.0 ± 0.781	33.7 ± 0.152	36.3 ± 0.404	20.2 ± 0.321	23.7 ± 0.568
HFP2 Unidentified Non-sporulating)	TCP	78.0 ± 0.057	81.5 ± 0.351	71.2 ± 0.251	74.5 ± 0.404	70.2 ± 0.251	73.3 ± 0.208	58.2 ± 0.305	61.2 ± 0.251	40.4 ± 0.378	45.1 ± 0.642
	MRP	52.5 ± 0.458	56.4 ± 0.611	51.1 ± 0.721	54.2 ± 0.200	49.4 ± 0.435	52.2 ± 0.208	33.7 ± 0.416	36.2 ± 0.100	20.1 ± 0.100	23.2 ± 0.321
	URP	39.3 ± 0.208	41.2 ± 0.200	38.1 ± 2.77	35.8 ± 0.721	32.1 ± 0.152	35.0 ± 0.850	20.5 ± 0.351	23.1 ± 0.832	13.5 ± 0.493	16.2 ± 0.305

Table 6 Effect of salt on phosphate solubilisation (µg/ml) by microbial isolates in Pikovskaya and NBRIP broth

Isolates	Phosphate sources		Salt Concentration NaCl (w/v)									
			0		2.5		5		7.5		10	
			PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
Bacteria												
HBP1 (*Bacillus* sp.)	TCP	P solubilisation	106.9 ± 1.30	111.3 ± 0.776	108.5 ± 0.550	111.6± 0.568	78.6 ± 0.568	81.8 ± 0.700	25.7 ± 0.360	26.0 ± 0.100	1.9 ± 0.100	2.1 ± 0.100
		pH	5.39 ± 0.015	5.18 ± 0.062	5.38 ± 0.010	5.35 ± 0.015	5.8 ± 0.010	5.75 ± 0.015	5.97 ± 0.010	5.92 ± 0.020	6.32 ± 0.005	6.28 ± 0.015
	MRP	P solubilisation	63.0 ± 0.251	70.3 ± 0.493	69.4 ± 0.503	71.6 ± 0.208	19.9 ± 0.300	22.7 ± 0.152	8.3 ± 0.208	10.4 ± 0.208	1.1 ± 0.057	1.56 ± 0.152
		pH	5.98 ± 0.011	5.87 ± 0.020	6.10 ± 0.015	6.07 ± 0.015	6.20 ± 0.015	6.14 ± 0.010	6.22 ± 0.005	6.19 ± 0.010	6.54 ± 0.005	6.51 ± 0.010
	URP	P solubilisation	48.9 ± 0.321	51.4 ± 0.450	40.9 ± 1.02	43.9 ± 0.300	12.8 ± 0.208	16.7 ± 0.152	5.2 ± 0.152	7.9 ± 0.057	1.0 ± 0.057	1.33 ± 0.152
		pH	6.11 ± 0.015	6.07 ± 0.020	6.16 ± 0.010	6.13 ± 0.011	6.26 ± 0.026	6.18 ± 0.005	6.25 ± 0.005	6.22 ± 0.015	6.62 ± 0.005	6.58 ± 0.017
HBP2 (Unidentified	TCP	P solubilisation	95.5 ± 0.458	100.5 ± 0.888	98.5 ± 0.757	101.5 ± 0.400	69.2 ± 0.305	71.4 ± 0.321	23.6 ± 0.838	27.5 ± 1.53	1.6 ± 0.173	1.8 ± 0.115
		pH	5.72 ± 0.025	5.17 ± 0.020	5.71 ± 0.020	5.65 ± 0.005	5.98 ± 0.015	5.90 ± 0.015	6.07 ± 0.032	6.01 ± 0.015	6.36 ± 0.015	6.29 ± 0.005
	MRP	P solubilisation	51.4 ± 0.450	60.4 ± 0.907	56.0 ± 0.100	56.7 ± 0.321	16.5 ± 0.416	19.3 ± 0.264	5.1 ± 0.152	7.9 ± 0.251	1.0 ± 0.057	1.66 ± 0.251
		pH	6.07 ± 0.015	6.01 ± 0.010	6.14 ± 0.005	6.11 ± 0.015	6.21 ± 0.010	6.18 ± 0.020	6.24 ± 0.025	6.19 ± 0.015	6.58 ± 0.015	6.54 ± 0.020
	URP	P solubilisation	39.5 ± 0.500	43.3 ± 0.264	36.5 ± 0.550	39.7 ± 0.378	10.8 ± 0.305	12.7 ± 0.152	3.4 ± 0.152	4.5 ± 0.100	0.0 ± 0.00	0.0 ± 0.00
		pH	6.21 ± 0.010	6.12 ± 0.015	6.18 ± 0.005	6.14 ± 0.025	6.28 ± 0.005	6.26 ± 0.010	6.29 ± 0.011	6.25 ± 0.015	-	-
Fungi												
HFP1 (*Penicillium* sp.)	TCP	P solubilisation	91.3 ± 1.17	100.0 ± 0.503	86.2 ± 0.251	89.0 ± 0.503	49.1 ± 1.24	53.3 ± 1.15	22.7 ± 0.763	25.1 ± 0.702	2.0 ± 0.00	2.2 ± 0.152
		pH	5.78 ± 0.030	5.41 ± 0.020	5.85 ± 0.015	5.79 ± 0.015	6.02 ± 0.005	5.99 ± 0.015	6.14 ± 0.001	6.10 ± 0.010	6.29 ± 0.015	6.26 ± 0.005
	MRP	P solubilisation	54.2 ± 0.321	59.6 ± 0.416	17.8 ± 0.971	19.5 ± 0.404	8.8 ± 0.264	10.2 ± 0.208	3.8 ± 0.208	4.2 ± 0.152	0.0 ± 0.00	0.0 ± 0.00
		pH	6.06 ± 0.025	5.97 ± 0.025	6.17 ± 0.011	6.12 ± 0.020	6.21 ± 0.005	6.19 ± 0.010	6.27 ± 0.005	6.24 ± 0.015	-	-
	URP	P solubilisation	33.7 ± 0.264	35.2 ± 0.208	12.4 ± 0.264	13.5 ± 0.305	5.6 ± 0.35	8.1 ± 0.152	2.2 ± 0.208	3.9 ± 0.208	0.0 ± 0.00	0.0 ± 0.00
		pH	6.10 ± 0.010	6.05 ± 0.015	6.25 ± 0.005	6.22 ± 0.015	6.25 ± 0.010	6.21 ± 0.015	6.38 ± 0.015	6.34 ± 0.015	-	-
HFP2 Unidentified Non-sporulating)	TCP	P solubilisation	85.6 ± 0.400	90.7 ± 0.264	81.9 ± 1.40	84.9 ± 0.264	42.3 ± 0.737	45.4 ± 0.700	21.4 ± 1.05	22.9 ± 0.435	1.6 ± 0.115	2.0 ± 0.173
		pH	5.90 ± 0.025	5.63 ± 0.010	5.97 ± 0.015	5.93 ± 0.005	6.07 ± 0.015	6.01 ± 0.010	6.16 ± 0.0208	6.13 ± 0.010	6.35 ± 0.020	6.31 ± 0.011
	MRP	P solubilisation	50.5 ± 0.814	54.6 ± 0.602	15.4 ± 0.251	17.1 ± 0.115	5.9 ± 0.115	6.5 ± 0.200	2.5 ± 0.200	3.2 ± 0.251	0.0 ± 0.00	0.0 ± 0.00
		pH	6.10 ± 0.005	6.03 ± 0.015	6.21 ± 0.010	6.19 ± 0.010	6.25 ± 0.005	6.22 ± 0.020	6.31 ± 0.015	6.25 ± 0.011	-	-
	URP	P solubilisation	30.3 ± 0.264	33.3 ± 0.321	10.4 ± 0.208	12.9 ± 0.100	3.0 ± 0.264	4.4 ± 0.300	1.8 ± 0.057	2.0 ± 0.152	0.0 ± 0.00	0.0 ± 0.00
		pH	6.15 ± 0.020	6.09 ± 0.020	6.23 ± 0.020	6.20 ± 0.017	6.29 ± 0.010	6.36 ± 0.011	6.43 ± 0.010	6.38 ± 0.015	-	-

Initial pH = 6.8

Table 7 Effect of temperature on phosphate solubilisation (µg/ml) in Pikovskaya and NBRIP broth

Isolates	Phosphate sources		Temperature (°C)											
			9		12		18		24		35		40	
			PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP	PVK	NBRIP
Bacteria														
HBP1 (*Bacillus* sp.)	**TCP**	**P solubilisation**	32.8 ± 0.251	35.8 ± 0.251	59.7 ± 0.416	60.2 ± 0.305	87.6 ± 0.838	90.5 ± 0.529	100.9 ± 0.818	106.5 ± 0.493	88.7 ± 1.04	89.4 ± 0.450	27.6 ± 0.550	28.6 ± 0.435
		pH	6.09 ± 0.020	6.06 ± 0.00	5.83 ± 0.010	5.80 ± 0.011	5.73 ± 0.030	5.70 ± 0.020	5.45 ± 0.010	5.41 ± 0.011	5.78 ± 0.011	5.74 ± 0.015	6.17 ± 0.005	6.15 ± 0.015
	MRP	**P solubilisation**	18.5 ± 0.409	19.8 ± 0.300	24.2 ± 0.251	26.2 ± 0.321	36.6 ± 0.416	39.2 ± 0.321	59.5 ± 0.2	61.2 ± 0.251	29.4 ± 0.264	32.4 ± 0.971	14.6 ± 0.208	16.6 ± 0.458
		pH	6.21 ± 0.011	6.17 ± 0.005	6.14 ± 0.025	6.11 ± 0.010	6.08 ± 0.011	6.05 ± 0.005	6.04 ± 0.020	6.00 ± 0.005	6.09 ± 0.015	6.07 ± 0.011	6.23 ± 0.015	6.19 ± 0.005
	URP	**P solubilisation**	11.9 ± 0.264	13.9 ± 0.264	21.8 ± 0.519	23.2 ± 0.264	29.5 ± 0.550	31.6 ± 0.458	48.6 ± 0.550	51.4 ± 0.208	21.2 ± 1.069	26.1 ± 0.832	10.3 ± 0.2	12.2 ± 0.264
		pH	6.27 ± 0.005	6.22 ± 0.00	6.19 ± 0.005	6.16 ± 0.015	6.11 ± 0.005	6.07 ± 0.015	6.08 ± 0.010	6.02 ± 0.015	6.12 ± 0.015	6.09 ± 0.010	6.32 ± 0.010	6.28 ± 0.015
HBP2 (Unidentified	**TCP**	**P solubilisation**	29.6 ± 0.556	33.2 ± 0.321	51.5 ± 0.351	52.9 ± 0.776	79.2 ± 0.888	80.6 ± 0.450	10.2 ± 0.251	93.3 ± 0.200	70.6 ± 0.450	74.6 ± 0.550	19.4 ± 0.400	23.3 ± 0.642
		pH	6.15 ± 0.015	6.10 ± 0.011	5.68 ± 0.010	5.61 ± 0.015	5.79 ± 0.025	5.75 ± 0.043	5.81 ± 0.010	5.77 ± 0.020	5.87 ± 0.015	5.82 ± 0.015	6.28 ± 0.010	6.21 ± 0.015
	MRP	**P solubilisation**	12.5 ± 0.351	13.2 ± 0.832	19.4 ± 0.36	21.06 ± 0.152	30.4 ± 0.208	34.2 ± 1.2	53.5 ± 0.208	54.2 ± 0.208	22.4 ± 0.152	26.1 ± 2.05	9.6 ± 0.264	10.7 ± 0.264
		pH	6.28 ± 0.005	6.24 ± 0.025	6.19 ± 0.015	6.18 ± 0.020	6.13 ± 0.020	6.08 ± 0.015	6.07 ± 0.020	6.03 ± 0.010	6.12 ± 0.025	6.08 ± 0.011	6.33 ± 0.025	6.29 ± 0.017
	URP	**P solubilisation**	9.5 ± 0.404	10.2 ± 0.208	17.3 ± 0.416	18.3 ± 0.264	26.4 ± 0.608	27.9 ± 0.152	42.7 ± 0.378	45.3 ± 0.818	18.3 ± 0.321	21.5 ± 0.602	8.53 ± 0.404	9.6 ± 0.458
		pH	6.32 ± 0.010	6.27 ± 0.015	6.24 ± 0.010	6.18 ± 0.015	6.17 ± 0.015	6.13 ± 0.020	6.12 ± 0.005	6.09 ± 0.010	6.20 ± 0.00	6.16 ± 0.015	6.38 ± 0.015	6.32 ± 0.020
Fungi														
HFP1 (*Penicillium* sp.)	**TCP**	**P solubilisation**	30.6 ± 0.503	34.3 ± 0.497	54.6 ± 0.450	59.3 ± 0.754	81.7 ± 0.493	82.9 ± 0.709	92.8 ± 0.802	94.1 ± 0.321	72.6 ± 0.435	75.6 ± 0.568	24.8 ± 0.360	26.3 ± 0.680
		pH	6.12 ± 0.015	6.08 ± 0.015	5.60 ± 0.010	5.57 ± 0.011	5.80 ± 0.015	5.78 ± 0.011	5.75 ± 0.015	5.71 ± 0.015	5.92 ± 0.005	5.89 ± 0.005	6.18 ± 0.015	6.15 ± 0.011
	MRP	**P solubilisation**	22.3 ± 0.3	24.2 ± 0.655	28.1 ± 0.907	29.9 ± 0.435	39.5 ± 0.351	40.5 ± 0.65	57.3 ± 0.3	59.2 ± 0.294	31.4 ± 0.585	34.5 ± 0.838	16.7 ± 0.404	18.4 ± 0.264
		pH	6.18 ± 0.070	6.14 ± 0.010	6.11 ± 0.025	6.08 ± 0.020	6.05 ± 0.015	6.02 ± 0.011	6.01 ± 0.010	5.98 ± 0.011	6.07 ± 0.010	6.03 ± 0.005	6.22 ± 0.025	6.17 ± 0.015
	URP	**P solubilisation**	17.7 ± 0.665	18.5 ± 0.493	23.2 ± 0.360	24.2 ± 0.723	30.5 ± 0.929	32.5 ± 0.450	44.5 ± 0.896	46.6 ± 0.665	27.4 ± 0.1	29.5 ± 0.608	12.6 ± 0.458	15.06 ± 0.152
		pH	6.23 ± 0.015	6.18 ± 0.015	6.18 ± 0.011	6.14 ± 0.015	6.1 ± 0.010	6.06 ± 0.010	6.05 ± 0.010	6.01 ± 0.015	6.09 ± 0.011	6.06 ± 0.025	6.30 ± 0.010	6.25 ± 0.010
HFP2 Unidentified Non-sporulating)	**TCP**	**P solubilisation**	27.7 ± 0.152	29.8 ± 0.624	49.6 ± 0.513	51.3 ± 0.3	73.6 ± 0.450	74.8 ± 0.550	88.5 ± 0.251	90.9 ± 0.737	66.3 ± 0.173	69.5 ± 0.608	22.3 ± 0.208	23.5 ± 1.40
		pH	6.21 ± 0.025	6.15 ± 0.15	5.93 ± 0.015	5.88 ± 0.017	5.83 ± 0.010	5.80 ± 0.010	5.78 ± 0.015	5.75 ± 0.005	5.85 ± 0.020	5.81 ± 0.057	6.32 ± 0.017	6.28 ± 0.015
	MRP	**P solubilisation**	10.7 ± 0.251	11.8 ± 0.251	14.2 ± 0.208	15.3 ± 0.472	25.7 ± 0.152	27.6 ± 0.498	52.5 ± 0.550	54.7 ± 0.044	10.5 ± 0.752	12.4 ± 0.208	9.86 ± 0.152	10.9 ± 0.251
		pH	6.3 ± 0.010	6.26 ± 0.010	6.28 ± 0.010	6.25 ± 0.010	6.19 ± 0.015	6.15 ± 0.015	6.07 ± 0.005	6.03 ± 0.010	6.11 ± 0.015	6.07 ± 0.005	6.31 ± 0.020	6.26 ± 0.005
	URP	**P solubilisation**	7.8 ± 0.351	8.3 ± 0.152	9.6 ± 0.5	11.03 ± 0.152	20.6 ± 0.781	23.0 ± 0.404	41.1 ± 0.458	43.5 ± 0.896	7.53 ± 0.404	8.93 ± 0.208	8.96 ± 0.152	9.73 ± 0.152
		pH	6.34 ± 0.010	6.28 ± 0.015	6.31 ± 0.015	6.27 ± 0.005	6.26 ± 0.011	6.21 ± 0.015	6.10 ± 0.010	6.06 ± 0.010	6.16 ± 0.015	6.10 ± 0.015	6.35 ± 0.011	6.31 ± 0.015

Initial pH = 6.8

Table 8 Effect of P-solubilisers on the solubility of inorganic sources of phosphates

Medium	**Treatment**	**P solubilised (µg/ml)**	**Final pH of broth**
PVK	TCP		
	HBP1 (*Bacillus* sp.)	106.5 ± 1.30	5.39 ± 0.015
	HBP2 (Unidentified)	95.5 ± 0.458	5.72 ± 0.025
	HFP1 (*Penicillium* sp.)	91.3 ± 1.17	5.78 ± 0.030

	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified)	110.3 ± 0.709	5.31 ± 0.015
	HBP1 (*Bacillus* sp.) + HFP1 (*Penicillium* sp.)	107.7 ± 0.862	5.38 ± 0.017
	HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	99.6 ± 0.763	5.7 ± 0.030
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	120.8 ± 0.802	5.24 ± 0.010
	MRP		
	HBP1 (*Bacillus* sp.)	63.0 ± 0.251	5.98 ± 0.011
	HBP2 (Unidentified)	51.4 ± 0.450	6.07 ± 0.015
	HFP1 (*Penicillium* sp.)	54.2 ± 0.321	6.06 ± 0.025
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified)	69.5 ± 0.600	5.93 ± 0.015
	HBP1 (*Bacillus* sp.) + HFP1 (*Penicillium* sp.)	65.9 ± 0.680	5.97 ± 0.020
	HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	56.9 ± 0.152	6.00 ± 0.020
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	70.9 ± 0.737	5.90 ± 0.015
	URP		
	HBP1 (*Bacillus* sp.)	48.9 ± 0.321	6.11 ± 0.015
	HBP2 (Unidentified)	39.5 ± 0.500	6.21 ± 0.010
	HFP1 (*Penicillium* sp.)	33.7 ± 0.264	6.10 ± 0.010
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified)	53.2 ± 0.763	6.09 ± 0.030
	HBP1 (*Bacillus* sp.) + HFP1 (*Penicillium* sp.)	50.0 ± 0.020	6.06 ± 0.015
	HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	45.2 ± 0.808	6.18 ± 0.030
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	54.6 ± 1.55	6.04 ± 0.015
NBRIP	TCP		
	HBP1 (*Bacillus* sp.)	111.3 ± 0.776	5.17 ± 0.020
	HBP2 (Unidentified)	100.5 ± 0.888	5.18 ± 0.062
	HFP1 (*Penicillium* sp.)	100.0 ± 0.503	5.41 ± 0.020
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified)	112.4 ± 0.264	5.28 ± 0.010
	HBP1 (*Bacillus* sp.) + HFP1 (*Penicillium* sp.)	109.0 ± 0.152	5.32 ± 0.015
	HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	102.6 ± 0.568	5.63 ± 0.015
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	122.5 ± 0.503	5.20 ± 0.011
	MRP		
	HBP1 (*Bacillus* sp.)	70.3 ± 0.493	5.87 ± 0.020
	HBP2 (Unidentified)	60.4 ± 0.907	6.01 ± 0.010
	HFP1 (*Penicillium* sp.)	59.6 ± 0.416	5.97 ± 0.025
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified)	72.0 ± 0.173	5.89 ± 0.005
	HBP1 (*Bacillus* sp.) + HFP1 (*Penicillium* sp.)	68.5 ± 0.416	5.93 ± 0.030
	HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	58.8 ± 0.585	5.97 ± 0.026
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	72.9 ± 0.264	5.85 ± 0.011

	URP		
	HBP1 (*Bacillus* sp.)	51.4 ± 0.450	6.07 ± 0.020
	HBP2 (Unidentified)	43.3 ± 0.264	6.12 ± 0.015
	HFP1 (*Penicillium* sp.)	35.2 ± 0.208	6.05 ± 0.015
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified)	54.9 ± 0.305	6.06 ± 0.020
	HBP1 (*Bacillus* sp.) + HFP1 (*Penicillium* sp.)	52.3 ± 0.251	6.02 ± 0.005
	HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	48.0 ± 0.200	6.13 ± 0.005
	HBP1 (*Bacillus* sp.) + HBP2 (Unidentified) + HFP1 (*Penicillium* sp.)	57.9 ± 0.152	6.01 ± 0.010

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