World J Microbiol Biotechnol (2011) 27:1055–1062
DOI 10.1007/s11274-010-0550-0

ORIGINAL PAPER

Slow and steady phosphate solubilization by a psychrotolerant strain of *Paecilomyces hepiali* (MTCC 9621)

Rinu K. · Anita Pandey

Received: 28 May 2010 / Accepted: 20 August 2010 / Published online: 3 September 2010

Abstract A psychrotolerant phosphate solubilizing fungus has been isolated from the rock soil of a cold desert site in Indian Himalaya. The fungus grows from 4 to 35°C (optimum 21°C), and from 2 to 13.5 pH (optimum 9) under laboratory conditions. Based on phenotypic characters and 26S rDNA analysis, the fungus is identified as *Paecilomyces hepiali*. In quantitative estimation that was carried out at 9, 14, and 21°C, the fungus solubilized maximum phosphate at 14°C. In view of the slow growth and persistence of the desired activity at low temperature, the estimation was carried out for a longer period, i.e., up to 6 weeks. The suboptimal conditions for growth and biomass production were found to be optimal for phosphate solubilization by the fungus. At 14 and 9°C, the solubilization touched its maximum on day 42. Decline in pH was found to be significantly correlated with the phosphate solubilization at all the temperatures, under consideration. The acid phosphatase activity was found to be more prominent than alkaline phosphatase in culture filtrate. High performance thin layer chromatography (HPTLC) analysis showed production of six organic acids, gluconic and α-keto glutaric acid being in maximum amount in the culture filtrate. The study has ecological significance in view of the nutrient cycling under low temperature environment, prevalent in Himalayan region.

Keywords Psychrotolerant fungus · Cold desert Himalaya · Suboptimal conditions · Phosphatase · Organic acids

Rinu K. · A. Pandey (✉)
Biotechnological Applications, G. B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora, Uttarakhand 263 643, India
e-mail: anita@gbpihed.nic.in

Introduction

Phosphorus (P) is an important component of macromolecules, present in the living system. While it plays an important role in the nutrient balance in the ecosystem, it is essential for the fertility of soils and the plant growth. Phosphates are intimately involved in the storage and transfer of energy in the cells. Despite high total soil concentrations of P, its concentration in the soil solution is very low (Barber 1995) compared to the requirement of plants. Water soluble P, applied in acidic soils is rapidly fixed to unavailable forms and accounts for low phosphate use efficiency (Sarkar and Uppal 1994). Most of the applied P accumulates in the fine soil fractions that are readily transported to surface waters through runoff, especially in hilly regions (He et al. 1995).

Phosphate solubilizing microorganisms (PSMs) as a group form an important part of the microbial community that benefits plant growth and development. Mineral phosphate solubilization by PSMs is caused by lowering of the pH of the medium either by H^+ extrusion (Illmer et al. 1995) or by the excretion of organic acids and chelating metabolites (Asea et al. 1988; Cunningham and Kuiack 1992). PSMs are known to produce phosphatases, which are hydrolytic enzymes, responsible for the breakdown of insoluble compounds (Tarafdar et al. 2003).

Study on phosphate solubilizing efficiency of cold tolerant bacteria and fungi have got attention in recent years (Pandey et al. 2002, 2008; Das et al. 2003; Gulati et al. 2008; Rinu and Pandey 2010). In the present study, a cold tolerant fungus isolated from the rock soil of cold desert of Indian Himalaya has been identified up to species level and investigated for its phosphate solubilization efficiency at three temperatures, with particular reference to production of biomass, organic acids and phosphatases.

Materials and methods

Fungal isolate

The fungus used in the present study was isolated from rock soil collected from the cold desert area, Mana (30° 46′ 24.8″ N; 79° 29′ 33.4″ E; 3,238 m above mean sea level), about 4 km away from Badrinath, in district Chamoli of Garhwal Himalaya, India. The pH of soil was 5.9. The site remains covered with snow from October to March, maintaining sub-zero temperature. The fungus was maintained at 4°C in a refrigerator in the culture collection of Microbiology Laboratory of the Institute. Fresh culture was raised on Potato Dextrose Agar (PDA) for conducting the experiments.

Identification and characterization of the fungus

Seven days old culture grown on PDA at 21°C was used for recording observations on the colony morphology. Microscopic observations were made on the fungus stained with lactophenol cotton blue under the microscope (Nikon-Eclipse 50i, Japan). The species level was given by 26S rDNA sequencing (courtesy: Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India). The nucleotide sequence has been deposited with NCBI. Temperature tolerance was checked by incubating the fungal isolate at different temperatures (4, 9, 14, 21, 28, 35 and 42°C), up to 2 weeks. The pH tolerance was measured at different pH levels (1.5–13.5, at an interval of 0.5) by incubating the culture agar plate/broth at 21°C for 1 week. Only 5 mm colony diameter on agar plates was considered as growth for both temperature and pH tolerance.

Estimation of phosphate solubilization, biomass and pH

For qualitative estimation, the Pikovskaya's agar plates inoculated with fungus were incubated at 4, 9, 14, 21, 28 and 35°C. The plates were observed for zone of solubilization around the colony up to day 42 of incubation at weekly intervals. The zone of solubilization was calculated by subtracting the total diameter of the colony from the colony diameter plus zone of clearance. The solubilization efficiency (E) was calculated based on the equation as described by Nguyen et al. (1992):

$$\text{Solubilization Efficiency (E)} = \frac{\text{Solubilization diameter (S)}}{\text{Growth diameter}} \times 100$$

For quantitative estimation, the fungal culture was inoculated in Pikovskaya's broth containing tricalcium phosphate (0.5 g/100 ml). 0.5 g of tricalcium phosphate was weighed into 250 ml Erlenmeyer's flask and 100 ml of Pikovskaya's broth (without phosphorus source) was poured into it. The initial pH of the media before autoclaving was 7.5. The autoclaved media was inoculated with 5 mm disc of fungal culture and incubated at 4, 9, 14 and 21°C for 6 weeks. The culture filtrate was withdrawn from respective flasks on every 7th day of incubation and filtered through Whatman No. 42 filter paper and then analyzed for P_2O_5 production by using chlorostannous reduced molybdophosphoric acid blue method (Jackson 1967). The pH (Systronics, India) of the culture filtrate was also recorded each time. The biomass of the culture was estimated on every 7th day of incubation from the same flasks. The mycelium was collected after filtration of the broth culture, and dried at 65°C for 72 h.

Estimation of extracellular phosphatase activity

Phosphatase enzyme activity was estimated every 7th day of incubation in Pikovskaya's broth culture at different temperatures, 9, 14 and 21°C. The preparation of culture filtrate and the analysis of acidic and alkaline phosphatase enzyme activity were done as described earlier (Pandey et al. 2008). One enzyme unit was defined as the amount of enzyme that catalyzed the formation of 1 μmol of end product (*P*-Nitrophenol) in 1 min under experimental conditions (Tabatabai and Bremner 1969). All the experiments were conducted in triplicate and repeated twice. The glassware used was free of detergents and other contaminants.

Preparation of sample solution for the detection of organic acids

The organic acid production was detected at 21°C in the Pikovskaya's broth culture filtrate. The culture medium was filtered on 7th day through Whatman No. 42 filter paper and the filtrate was then centrifuged (Hitachi, Himac CR 22G Japan/Rotor, R20A2) at 10000 g for 10 min. This aliquot was filtered through a syringe filter (pore size 0.45 μm). This solution was used for HPTLC analysis of organic acids.

HPTLC analysis of organic acids

Six organic acid standards namely, citric, gluconic, succinic, malic, α-keto glutaric and oxalic were used for the present study. The purified filtrate and standards were applied to the silica gel TLC aluminium sheets of size 20 × 20 cm (Merk, silica gel 60 F_{254}) by using a manual applicator (Camag nanomat-4) through a HPLC injector and capillary tube. Then it was developed with diethyl

ether: formic acid: water (70:20:10) (Altomare et al. 1999). Another set of the loaded samples and standards were developed in acetonitrile: water (70:30) (Singh et al. 2005). After 20–30 min of activation, the TLC sheets were allowed to dry for 1 h and scanned in TLC Scanner (Linomat 5, Camag TLC scanner-3) with speed of 100 mm/s and data resolution 100 μm/step. Initial scanning was done at 254 nm, followed by spectral analysis at 200–700 nm. The analysis of the scanned data was done by Win CATS software. The Rf value and spectra of standards and samples were used for the identification of organic acids in the fungal culture filtrate. The concentration of acids was calculated according to the area of standards.

Data analysis

The data were analyzed with the computer programme Excel (Microsoft Corp.) for the graphical representations and mean values. The correlation between phosphate solubilization, pH changes, biomass production and phosphatase was worked out by using the computer software, SPSS/PC (1986). The nucleotide sequences, used for the analysis of phylogenetic relationship of the fungal isolate, were obtained by searching through NCBI and EMBL databases. Analysis of 26S rDNA sequence was performed by using neighbor joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were conducted in Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. (MEGA4, Tamura et al. 2007).

Results

Phenotypic, genotypic and growth characters of the fungal isolate

The fungal colonies grew moderately fast with powdery and aerial mycelium. The fungal culture sporulated better on mycological agar and Czapek dox agar, as compared to potato dextrose agar. The mycelium was observed to be white turning cream, reverse cream to yellow, conidiophores erect bearing several whorl of flask shaped phialides, conidia ellipsoidal to fusiform, some times lemon shaped, smooth walled and hyaline. Watery drop like exudates were also observed. The size of the conidia and phialides was 1.62×1.22 μm and 4.05×1.62 μm, respectively (average of five replicates; Fig. 1). The phylogenetic tree confirmed the identity and its phylogenetic relationship with other fungi, available in public domain (Fig. 2). The fungus showed maximum similarity with *Paecilomyces hepiali*, followed by *P. farinosus*. The fungus has been accessioned as MTCC 9621 by Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The Gene Bank accession number for the nucleotide sequence provided by NCBI is GQ370817. The fungal culture showed growth at a wide range of temperature, from 4°C (on day 10) to 35°C (on day 14), being optimum at 21°C (on day 7). The fungus formed 8 mm colony at 4°C and 5 mm colony at 35°C, on day 14. The fungal culture also grew at a wide range of pH, from 2.0 to 13.5, with an optimum at 9.0.

Qualitative estimation of phosphate solubilization

In plate assays, the maximum zone formation occurred at 21°C (4 mm zone), followed by 14°C (3 mm), 9°C (2 mm)

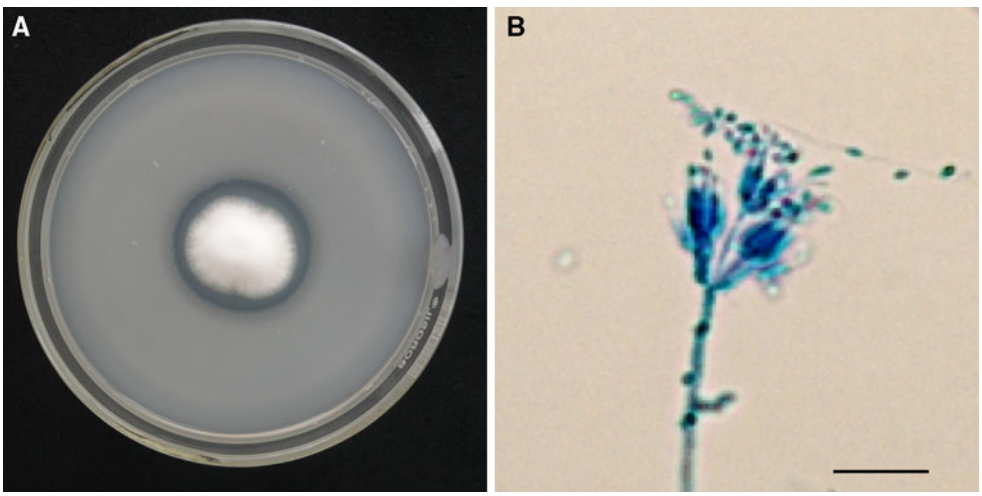

Fig. 1 Colony morphology and zone of solubilization **a** and microscopic characteristics **b** of *Paecilomyces hepiali*, *bar* = 5 μm

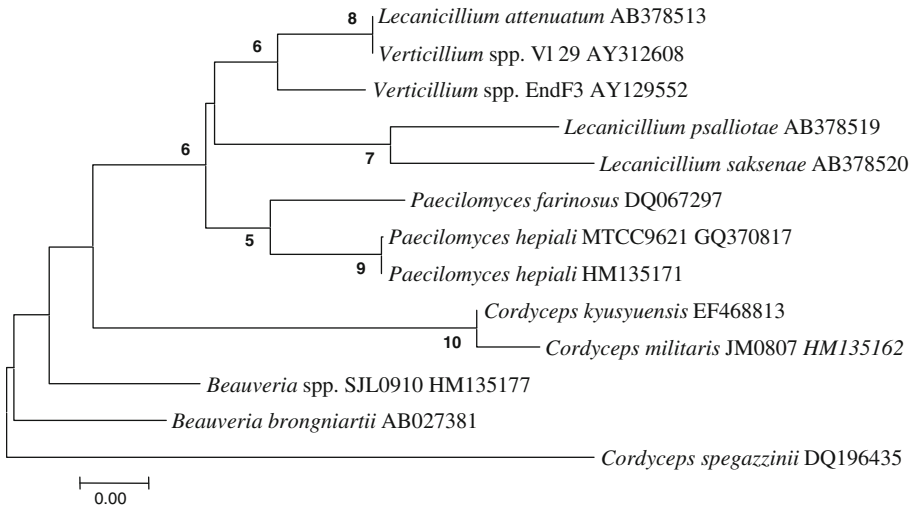

Fig. 2 The phylogenetic tree inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates), is shown next to the branches

and 28°C. However, the solubilization efficiency (E value) was maximum at 14°C.

Effect of temperature, pH, biomass production and phosphatase activity on tricalcium phosphate solubilization

At 21°C, maximum phosphate solubilization was estimated on day 35 of incubation (139.66 μg/ml); a decline was recorded afterwards on day 42 (Table 1). Maximum biomass production and decline in pH as well were also recorded in the culture media on day 35. On day 42 of incubation the fungus produced 7.4 Unit of acidic phosphatase in the culture filtrate (Fig. 3). The alkaline phosphatase was found to be lower (2.58 Unit on day 35), as compared to the acidic phosphatase (Fig. 4).

At 14°C, the fungal isolate solubilized maximum amount (164.21 μg/ml) of tricalcium phosphate on day 42. Maximum biomass production and minimum pH (4.18) of the culture media were recorded on the same day. Both acidic (11 Unit at day 42) and alkaline phosphatase (3.89 Unit on day 35) were found to be maximum at this temperature, as compared to other temperatures tested.

At 9°C, the phosphate solubilization efficiency was found to be at par (136.00 μg/ml on day 42) with that at 21°C, that persisted up to day 42 of incubation. Minimum pH and maximum biomass production was found on day 35 and day 42 of incubation, respectively. Acidic and alkaline phosphatase activity were found to be comparatively low (3.06 and 1.83 U, respectively on day 35) at 9°C.

At all the tested temperatures, during the entire incubation period, a negative correlation ($P \leq 0.01$) between reduction in pH and phosphate solubilization was obtained (Table 2). Similarly, a positive correlation ($P \leq 0.01$) between increase in biomass and the phosphate solubilization was obtained. Positive correlations ($P \leq 0.01$) between phosphatase activity with phosphate solubilization and biomass, and negative correlation ($P \leq 0.01$) with pH were also obtained.

Organic acid production by the fungus in Pikovskaya's broth

The definite peaks were not obtained in case of first mobile phase that was diethyl ether: formic acid: water. In case of the second mobile phase, that was acetonitrile: water, definite peaks were obtained repeatedly. Hence, the second case was used in the present study and analysis. The tested fungus was found to be more efficient to produce gluconic acid (4.77 μg/ml), than the other four acids namely, malic, succinic, α-keto glutaric and citric (Fig. 5).

Discussion

In the present study an efficient phosphate solubilizing fungus isolated from rock soil of a cold desert site in Indian Himalaya has been identified. On the basis of its tolerance and activity at low temperature, the fungus *Paecilomyces hepiali* MTCC 9621, is referred as psychrotolerant. The fungus also showed tolerance for wide range of pH. Microorganisms are known to possess the ability to adapt to environmental changes that may be due to their physiological flexibility. Although phosphate solubilizing microorganisms are commonly found in most soils, their establishment and performance are severely affected by environmental factors, especially under stress conditions (Gupta et al. 1986; Tilak 1991).

In view of slow growth of the fungus and persistence of the desired activity at low temperature, phosphate solubilization efficiency was studied at different temperature levels at weekly intervals, following a relatively longer

Table 1 Phosphate solubilization efficiency, change in pH and biomass production by *Paecilomyces hepiali* at different temperatures

Incubation time (days)	9°C			14°C			21°C		
	PS (μg/ml)	pH	Biomass	PS (μg/ml)	pH	Biomass	PS (μg/ml)	pH	Biomass
7	3.53 ± 0.40	7.43 ± 0.03	200 ± 0.03	4.50 ± 0.00	7.13 ± 0.10	400 ± 0.02	43.33 ± 3.05	6.03 ± 0.18	480 ± 0.02
14	57.33 ± 1.15	6.12 ± 0.15	510 ± 0.02	62.66 ± 3.05	6.32 ± 0.08	550 ± 0.01	80.16 ± 2.08	5.71 ± 0.16	560 ± 0.04
21	75.26 ± 3.05	5.40 ± 0.06	520 ± 0.02	116.26 ± 1.15	4.96 ± 0.08	540 ± 0.04	135.35 ± 3.05	4.95 ± 0.12	580 ± 0.02
28	91.14 ± 5.77	4.95 ± 0.04	560 ± 0.03	136.66 ± 1.15	4.61 ± 0.18	610 ± 0.02	137.56 ± 1.15	4.80 ± 0.24	720 ± 0.02
35	123.08 ± 4.16	4.08 ± 0.06	640 ± 0.05	158.33 ± 1.15	4.34 ± 0.08	660 ± 0.03	139.66 ± 2.08	4.93 ± 0.23	760 ± 0.03
42	136.00 ± 4.00	5.00 ± 0.08	660 ± 0.07	164.21 ± 2.31	4.18 ± 0.10	660 ± 0.07	123.83 ± 5.86	5.27 ± 0.14	670 ± 0.08

PS Phosphate solubilized

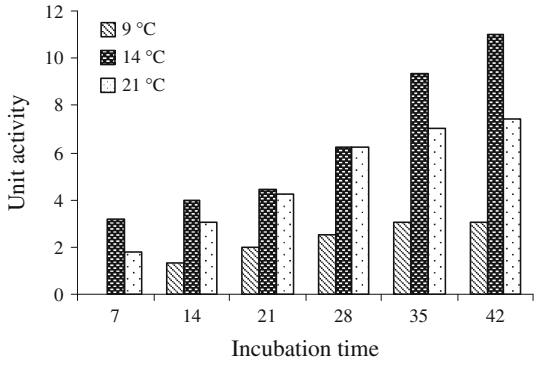

Fig. 3 Unit acidic phosphatase activity of *Paecilomyces hepiali* at different temperatures, up to day 42 of incubation

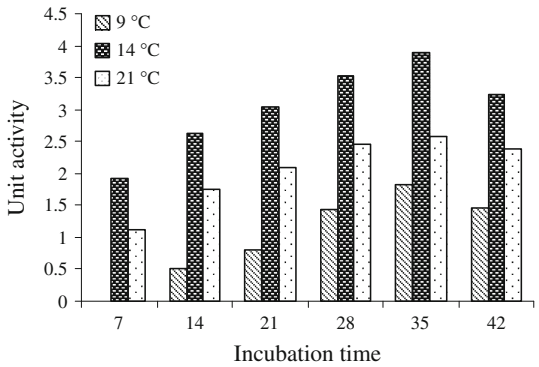

Fig. 4 Unit alkaline phosphatase activity of *Paecilomyces hepiali* at different temperatures, up to day 42 of incubation

period (up to 6 weeks) of incubation. The level of soluble P in solution culture is likely to fluctuate over time. Therefore, in such studies, it is desired to measure *P* level at various time intervals. The importance of this parameter has been demonstrated in case of *Penicillium radicum*, and some other phosphate solubilizing microorganisms (Illmer and Schinner 1992; Whitelaw et al. 1999; Wakelin et al. 2004), which is in tune of the findings of the present study.

Phosphate solubilization by various fungal species of tropical origin, mainly belonging to *Aspergillus* and *Penicillium*, has been reported by several workers. In these studies, the solubilization is reported maximum during second week, followed by decline or fluctuations on further incubation (Vassileva et al. 1998; Goenadi et al. 2000; Wakelin et al. 2004). In our previous study, eight species of *Penicillium* of temperate origin showed maximum phosphate solubilization during 3rd week of incubation at 21°C (Pandey et al. 2008). In a recent study, Ahuja et al. (2007) reported the phosphate solubilization efficiency of a tropical species of *Paecilomyces*, *P. marquandii* AA1, matching to the activity reported in case of fungi of tropical origin. Gupta et al. (2007) have reported the phosphate

Table 2 Correlation coefficient (r) relating the amount of phosphate solubilized with pH of the medium, biomass (dry weight basis) and intracellular phosphatase

r value for the correlation of phosphate solubilized, with pH of the medium and biomass								
9°C			14°C			21°C		
pH		Biomass	pH		Biomass	pH		Biomass
−0.907**		0.937**	−0.983**		0.663**	−0.922**		0.798**
r value for the correlation of phosphate solubilized, with acidic and alkaline phosphatase								
9°C			14°C			21°C		
Phosphate solubilization	pH	Biomass	Phosphate solubilization	pH	Biomass	Phosphate solubilization	pH	Biomass
Extracellular acidic phosphatase								
0.954**	−0.942**	0.938**	0.687**	−0.643**	0.366	0.832**	−0.710**	0.860**
Extracellular alkaline phosphatase								
0.925**	−0.960**	0.875**	0.888**	−0.897**	0.842**	0.935**	−0.856**	0.887**

** Significant at $P \leq 0.01$ level

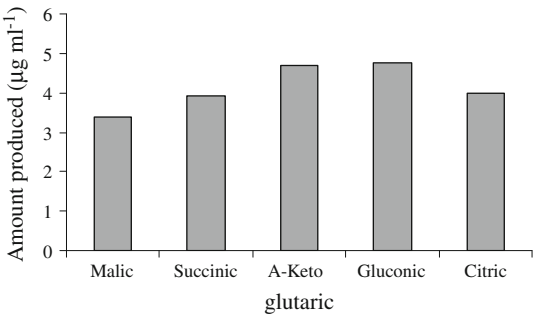

Fig. 5 Organic acids produced by *Paecilomyces hepiali* at 21 °C

solubilization efficiency of *Aspergillus* and *Penicillium* spp., isolated from the mines of Orissa (India), where the average temperature ranges between 32 and 40°C. These fungi preferred the pH of 7–9 for their best phosphate solubilization, and gave maximum solubilization (81.48 µg/ml) at day 12 of incubation. *P. hepiali* (MTCC 9621), in the present study, solubilized tricalcium phosphate at par after second and third week of incubation, at 14 and 9°C, respectively, and showed persistence of the activity up to day 42.

The suboptimal conditions recorded for growth and biomass production of *P. hepiali* were found to be optimal for the production of metabolites, mediating the phosphate solubilization process. While 21°C gave best results for biomass production, 14°C was found to be the best temperature for phosphate solubilization. In an earlier study, the phosphate solubilizing efficiency is reported to be higher at suboptimal carbon concentrated medium, as compared to the optimum requirement of the microbes (Illmer and Schinner 1992). Pandey et al. (2002) reported maximum phosphate solubilization at low temperature by a cold tolerant strain of *Pseudomonas corrugata*. Rinu and Pandey (2010) have recently reported the optimum phosphate solubilizing efficiency of different species of *Aspergillus* at suboptimal growth conditions. The elevated phosphate solubilization at low temperature can be attributed towards the involvement of cold active metabolites in the process. Microorganisms including fungi are known to produce cold active metabolites at low temperature (De Croos and Bidochka 2001).

Although HPTLC is a powerful tool for analysis of various biological compounds, it has not been widely used for the detection of organic acids in microbial phosphate solubilization based studies. Out of the two mobile phases, the first one (diethyl ether: formic acid: water) was not found effective for detection of organic acids in the present study. The second mobile phase (acetonitrile: water) was found to be appropriate for the detection of organic acids repeatedly. No acids were determined in case of *Trichoderma harzianum* Rifai 1295-22 in an HPTLC analysis with diethyl ether: formic acid: water as mobile phase (Altomare et al. 1999). Singh et al. (2005) detected the concentration of gluconic acid produced by *Aspergillus niger* in different carbon sources containing media with the help of HPLC with acetonitrile: water as mobile phase. The production of various organic acids in the culture solution is considered the main mechanism for the solubilization of insoluble phosphate (Cunningham and Kuiack 1992).

Phosphate solubilizing microorganisms are known to produce phosphatases, which are hydrolytic enzymes, responsible for breakdown of insoluble compounds. The acidic phosphatase activity was found considerably greater at all the temperatures, probably due to the acidity of the

medium caused by the fungus. Both acidic and alkaline phosphatases were found to be maximum at 14°C. The acidic and alkaline phosphatases are classified due to their optimum activities in acidic or alkaline ranges, respectively. The temperature and pH dependency of soil immobilized phosphatases has also been explained by Pant and Warman (2000). The study of phosphatases, a family of enzymes involved in the acquisition of phosphate from the environment by fungi among other microorganisms is a powerful tool in the elucidation of gene expression (Metzenberg 1979). The synthesis and secretion of enzymes by eukaryotic cells in response to ambient factors, such as pH and carbon, nitrogen, sulphur and phosphorus sources, have been studied previously (Nahas et al. 1982; Caddick et al. 1986a, b; Arst 1994).

Conclusion

The slow and steady phosphate solubilizing efficiency of *P. hepiali* (MTCC 9621) seems to be a factor of ecological conditions, the low temperature, in particular, prevailing in the mountain ecosystem. The fungus used in this study was isolated from the rock soil of an Indian Himalayan location that remains snow covered for at least half of the year. While the documentation of such species is important in view of understanding the microbial diversity in diversified ecosystems, bioprospecting of such species needs attention for applied value. Moreover, the fungus may also be useful as a model for studying the ecological adaptability under low temperature environment. Microorganisms growing under extreme environments are likely to possess different active or passive mechanisms for their survival under extreme conditions. The slow and steady, but effective and prolonged activities, such as production of organic acids, enzymes, etc., are likely to have implications in the nutrient cycling under cold desert mountain ecosystem.

Acknowledgments Dr L.M.S. Palni, Director GB Pant Institute of Himalayan Environment and Development, Almora, is thanked for encouragement and extending the facilities. Dr M.G.H. Zaidi (Department of Chemistry, GB Pant University of Agriculture and Technology, Pantnagar) is thanked for extending the HPTLC facility. Department of Science and Technology and the Ministry of Environment and Forests, Govt. of India, New Delhi are acknowledged for financial support.

References

Ahuja A, Ghosh SB, D'Souza SF (2007) Isolation of starch utilizing, phosphate solubilizing fungus on buffered medium and its characterization. Bioresour Technol 98:3408–3411

Altomare C, Norvell WA, Bjőrkman T, Herman GE (1999) Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Appl Environ Microbiol 65:2926–2933

Arst HN Jr (1994) Regulation of gene expression by oxygen, phosphorous, and pH. In: Martinelli SD, Kinghorn JR (eds) *Aspergillus*: 50 years on. Elsevier Science, Amsterdam, pp. 369–380

Asea PEA, Kucey RMN, Stewart JWB (1988) Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Soil Biol Biochem 20:450–464

Barber SA (1995) Soil nutrient bioavailability, a mechanistic approach. Wiley, New York

Caddick MX, Brownlee AG, Arst HN Jr (1986a) Structural genes for phosphatase in *Aspergillus nidulans*. Genet Res (Cambridge) 47:83–91

Caddick MX, Brownlee AG, Arst HN Jr (1986b) Regulation of gene expression by pH of the growth medium in *Aspergillus nidulans*. Mol Gen Genet 203:346–353

Cunningham JE, Kuiack C (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium billai*. Appl Environ Microbiol 52:1451–1458

Das K, Katiyar V, Goel R (2003) 'P' solubilization potential of plant growth promoting *Pseudomonas* mutants at low temperature. Microbiol Res 158:359–362

De Croos JNA, Bidochka MJ (2001) Cold induced proteins in cold-active isolates of the insect-pathogenic fungus *Metarhizium anisopliae*. Mycol Res 105:868–873

Goenadi DH, Sugiarto, Siswanto Y (2000) Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. Soil Sci Soc Am J 64:927–932

Gulati A, Rahi P, Vyas P (2008) Characterization of phosphate solubilizing fluorescent Pseudomonads from the rhizosphere of Seabuckthorn growing in the cold deserts of Himalayas. Curr Microbiol 56:73–79

Gupta RD, Bhardwaj KR, Morwan BC, Tripathi BR (1986) Occurrence of phosphate dissolving bacteria in soils of North West Himalayas under varying biosequence and climosequence. J Ind Soc Soil Sci 34:498–504

Gupta N, Sabat J, Parida R, Kerkatta D (2007) Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. Acta Bot Croatia 66:197–204

He ZL, Wilson MJ, Campbell CO, Edwards AC, Chapman SJ (1995) Distribution of phosphorus in soil aggregate fractions and its significance with regard to phosphorus transport in agricultural runoff. Water Air Soil Poll 83:69–84

Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biol Biochem 24:389–395

Illmer P, Barbato A, Schinner F (1995) Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganisms. Soil Biol Biochem 27:265–270

Jackson ML (1967) Soil chemical analysis. Prentice-Hall, New Delhi

Metzenberg R (1979) Implications of some genetic control mechanisms in *Neurospora*. Microbiol Rev 43:361–383

Nahas E, Terenzi HF, Rossi A (1982) Effect of carbon source and pH on the production and secretion of acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) in *Neurospora crassa*. J Gen Microbiol 128:2017–2021

Nguyen C, Yan W, Le Tacon F, Lapayrie F (1992) Genetic variability of phosphate solubilizing activity by monokaryotic and dikaryotic mycelia of the ectomycorrhizal fungus *Laccaria bicolar* (Maire) P. D. Orton. Plant Soil 143:193–199

Pandey A, Palni LMS, Mulkalwar P, Nadeem M (2002) Effect of temperature on solubilization of tricalcium phosphate by *Pseudomonas corrugata*. J Sci Ind Res 61:457–460

Pandey A, Das N, Kumar B, Rinu K, Trivedi P (2008) Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. World J Microbiol Biotechnol 24:97–102

Pant HK, Warman PR (2000) Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. Biol Fert Soils 30:306–311

Rinu K, Pandey A (2010) Temperature dependent phosphate solubilization by cold and pH tolerant species of *Aspergillus* isolated from Himalayan soil. Mycoscience 51:263–271

Sarkar MC, Uppal KS (1994) Phosphorus research in India. Potash and Phosphate Institute of Canada, India Programme, Gurgaon, Haryana, India

Singh OV, Kapur N, Singh RP (2005) Evaluation of agro-food byproducts for gluconic acid production by *Aspergillus niger* ORS-4.410. World J Microbiol Biotechnol 21:519–524

SPSS/PC (1986) SPSS/PC for the IBM PC/XT/AT. SPSS Inc., Illinois, USA

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenol phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tarafdar JC, Bareja M, Panwar J (2003) Efficiency of some phosphatase producing soil-fungi. Ind J Microbiol 43:27–32

Tilak KVBR (1991) Bacterial fertilizers. Technology Bulletin ICAR, New Delhi

Vassileva M, Vassilev N, Azcon R (1998) Rock phosphate solubilization by *Aspergillus niger* on olive cake based medium and its further application in a soil-plant system. World J Microbiol Biotechnol 14:281–284

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol Fertl Soils 40:36–43

Whitelaw MA, Harden TJ, Helyar KR (1999) Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. Soil Biol Biochem 31:655–665