J. Mycopathol. Res. ***59(2)*** *: 123-127, 2021;* (ISSN 0971-3719)

Isolation of high altitude phosphate solubilizing microorganisms from Singalila range of Eastern Himalaya

SOMA PAL SAHA

Department of Microbiology, Lady Brabourne College, P-1/2, Suhrawardy Ave, Beniapukur, Kolkata 700017

Received : 26.01.2021 Accepted : 15.05.2021 Published : 28.06.2021

The Himalayan soil shows a poor content of bioavailable phosphorus. Phosphate solubilizing microbes (PSM) provide an ecofriendly and economically sound approach to overcome this P scarcity and its subsequent uptake by plants. In this study high altitude PSM have been isolated from eleven grass land soil samples of different altitude level of Singalila mountain range of Eastern Himalaya. Soil samples showed acidic nature and very low moisture content. Microbiological status reflected the colony forming unit (CFU) ranging between 7.1 X 10^7 and 43.6 X 10^7 per g of soil. The distribution of PSM appeared 0.78% - 5.2 % which decreased in number with elevation. Organisms with high phosphate solubilizing index were tentatively identified as the strains of *Bacillus, Micrococcus* and *Pseudomonas*.

Key words: Phosphate solubilizing microorganisms, high altitude microorganisms

INTRODUCTION

Phosphorus is the second most important macronutrient for growth and almost all kind of metabolic processes of plants. Though phosphorus is abundant in soil as organic and inorganic form, the plant root available forms of this element are present at low concentration and speceially in the soil of the Himalayan and sub Himalayan region. It is absorbed by plants in the form of $H_2PO_4^-$ and HPO_4^{2-} ions, called the labile phosphate.

Several plant growth promoting factors like IAA can also be synthesized by variety of biofertilizer including PSM (Saleemi *et al*. 2017). The main sources of this labile phosphate are chemical fertilizer, biofertilizer and via some naturally occurring ecological interaction and metabolism of phosphate solubilizing microorganisms, PSM (Tomer *et al*. 2017; Shi *et al*. 2017; Kumar *et al*. 2015). Phosphate solubilizing microorganisms have already been isolated from the soils of Central Himalaya (Gusain *et al*. 2015; Pandey *et al*. 2006), Western Himalayan soil (Tomer *et al*. 2017) and Sikkim Himalaya (Panda *et al*. 2016). Several phosphate solubilizing bacteria can also influence the indigenous rhizospheric microbial community by changing soil pH, available phosphorus and several kinds of trace elements (Liu *et al*. 2020).

The soils of Indian Himalayan region (IHR) are generally acidic in reaction, low in moisture status and soluble organic matter (Pal Saha *et al*. 2018). Among a variety of soil productivity constrains, phosphorus availability plays a very crucial role in crop production as well as for natural vegetation. Soluble phosphorus when applied in such acid soils is rapidly fixed to unavailable forms and accounts for the low phosphate use efficiency by the plants. Dwellers of Himalayan cold region mainly dependant on high altitude agro-ecosystem who rely on pristine natural farming methodologies, not on chemical fertilizers. In this context psychrotolerant PSM may be the treasure to provide enough bio-available phosphorus to soil (Selvakumar et al., 2009; Panda *et al*. 2016).

In this study the availability and frequency of phosphate solubilizing organism in the soil of Singalila Mountain range of Himalaya has been reported. Furthermore, the potential of the prevalent PSI (phosphate solubilizing index) has

Correspondence: spalsaha44@gmail.com

been measured and compared. Attempt has also been made to identify the efficient organisms tentatively following partial characterization.

MATERIALS AND METHODS

Soil sample collection and characterization

The soil sample used for bacterial isolation was collected from the grassland area grown at a high altitude location ranges between 6500 feet to 11929 feet of Singalila range of Eastern Himalaya. Altogether from 11 different altitudes samples were collected in triplicates and then mixed to make a single composite sample from each site during the period of April - May, 2018. Soil was taken in aseptic glass screw cap tubes and was preserved in laboratory at 15^{o}C for the experiments. Soil samples were characterized by their moisture contents, pH and the soil type depending on the moisture content.

Isolation of bacteria

The total number of viable and culturable colony forming unit (CFU) of soil has been estimated on nutrient agar plates following soil serial dilution and spread plate techniques. The phosphate solubilizing property of each isolate has been determined using replica plating method on Pikovskaya's agar plates. All the plates were incubated at 30^{o}C for 24h-48h.

Determination of phosphate solubilization index

To estimate the efficiency of phosphate solubilization, each organism was inoculated individually on Pikovskaya's agar plate and was incubated for up to 72h at 30^{o}C. The solubility index was measured after 48h and 72h of incubation following the formula: Phosphate solubilizing index = [Halo zone diameter (mm) - Colony diameter (mm)] / Colony diameter (mm)].

Determination of the best three strains and their characterization

Depending on the greater value of solubility index, three different strains have been chosen for characterization. Following the characterization of colony morphology, cell morphology, gram nature, sporulation and sugar utilization pattern (growing in carbohydrate, 1%, w/v- beef extract- peptone salt broth) of the each strain, the tentative genus identification has been attempted.

RESULTS AND DISCUSSION

Altogether eleven different grassland sites of Singalila range of Himalaya having altitudes ranges between 6,500ft to 11,929ft approx. have been chosen for soil sample collection. The data collected on the laboratory experiments on soils showed that all soils, irrespective of altitudinal variations were acidic in nature, pH 6.4 to 6.9 and the moisture content 9.3 to 14.78% and the soil type was mostly sandy loam (Table 1). The acidic nature and low moisture is the common characteristic of most of the Himalayan and sub Himalayan soil because of low availability of basic ions and excess of hydrogen (H^{+}), aluminium (Al^{3+}), and manganese (Mn^{2+}) in exchangeable forms (Choudhari and Kumar, 2015; Prakash, 2013). The microbiological status of the soil showed that soils were loaded with 7.1 X 10^{7} to 43.6 X 10^{7} per gram of soil, comparatively lower (Fig.1) than the grass land site of lower altitude site of Darjeeling Himalaya (Pal Saha *et al*. 2018). Phosphate solubilizing organism among the whole population appeared extremely low and ranges between 1 6- 5.2 %. The frequency of availability of PSM increased with decrease in altitude of the sites. The low concentration of PSM may be attributed by the soil acidic nature and other micro and macro nutrients.

The phosphate solubilization index of the best isolates from each site as showed in Fig. 2 revealed that it ranged between 0.046 to 0.392 after 48h of incubation and 0.099 to 0.581 after 72h of incubation (Fig.2). The solubilization index value appeared independent of altitude of the site. The three efficient strains, strain 1006, strain 301 and strain 202, according to the index value were isolated from Singalila National Park, Tonglu and Kalipokhri sites, respectively. The organisms were characterized partially by their colony morphology; cell morphology, gram nature, growth, temperature range (Table 2) and sugar utilization pattern (Table 3). Comparing the description made in Bargey's Manual of Determinative Bacteriology and Bergey's Manual of systematic bacteriology the strains were tentatively identified as the species of *Bacillus*, *Micrococcus* and *Pseudomonas* (Fig 3). A psychrotolerant *Pseudomonas fragi* strain CS11RH1 (MTCC 8984) has been isolated from

Table 1: Physical status of high altitude grass land soil

Site	Altitude, ft	GPS	Characterization of soil		
			pH*	% moisture#	Soil type
Sandakphu	11929	27 6° N88.0° E	6.42±0.2	9.3±0.57	Sandy loam
Kalipokhri	10400	27.04°N88.0°E	6.66±0.2	11.6±0.12	Sandy loam
Tonglu	10130	27.03°N88.09° E	6.81±0.1	10.9±1.6	Loam
Tumling	9600	27.031°N 88.06°E	6.92±0.2	12.6±1.5	Sandy loam
Meghma	9514	27.023°N88.083°E	6.55±0.5	12.4±2.0	Loam
Gairibas	8600	27.032°N88.035°E	6.71±0.2	13.6±1.8	Loam
Chitrey	8340	26.99°N88.11°E	6.42±0.5	12.44±1.18	Sandy loam
Gurdum	7150	27.12°N88.05°E	6.71±0.5	14.5±2.1	Sandy loam
Manebhanjang	7054	26.98°N88.12°E	6.54±0.2	13.8±2.3	Sandy loam
(Phatak) Singalila National Park	7000	27·03°N88·07°E	6.91±0.1	14.78±2.38	Sandy loam
Rimbik	6500	27.118°N88.1°E	6.82±0.1	13.5±1.6	Loam

*pH of the soil was determined from the soil suspension (1g/10ml) in double distilled water. #moisture content of soil was determined by deducting the weight of oven dried sample from the weight of fresh sample in a preweighed aluminium cup.

Table 2: Characterization of three microorganisms with high phosphate solubilizing index

Strain No.	Site	Phosphate* Solubilizing Index	Colony Morphology	Cell morphology	Temp. range for growth	Tentative genus
Strain 1006	Singalila National Park (Phatak)	0.581±0.04	Medium, white, with centre, flat smooth textured regular margin	Gram (+) ve, bacilli type cells in long chain, sporulating	6-35°C	*Bacillus*
Strain 301	Tonglu	0.43±0.01	Medium, yellowish smooth, regular margin, flat	Gram (+) ve cocci type cells, mostly in single	8-32°C	*Micrococcus*
Strain 202	Kalipokhri	0.406±0.02	Minute, opaque smooth, regular margin, raised	Gram (-) ve, small bacilli type cells, mostly in single	10-37°C	*Pseudomonus*

Table 3: Utilization of Sugar by the three efficient PSM strain

Sugar*	*Bacillus* (Strain 1006)	*Micrococcus* (Strain 301)	*Pseudomonas* (Strain202)
D-glucose	++	++	++
Galactose	+	±	-
Fructose	+	+	+
Mannitol	++	+	+
Lactose	+	+	-
Sucrose	+	+	+
Maltose	+	-	-
Ribose	±	±	-
Arabinose	±	-	-
Xylose	±	-	-
Rhamnose	-	±	-
Citrate	-	±	-

*Individual sugar was added to the broth medium at 1%, w/v level and one loop full of solid culture was inoculated to 10ml of broth in duplicate set and incubated for 24h. Growth is recorded by the culture opacity determination.

the soil of North West Himalayan region which was capable of phosphate solubilization and also showed synthesis of growth promoting factors (Selvakumar et al. 2009). *Pseudomonas palleroniana* N-26 and *Pseudomonas jessenii* MP-1 are the two efficient strains for phosphate solubilization were isolated from the Western Indian Himalayan rhizosphere of red kidney bean (Tomer *et al*. 2017). On the other hand Gusain et al. (2015) reported strains of *Klebsiella oxytoca* and

Arthrobacter nitroguajacolicus as PSM as well as IAA synthesizer from the acidic soil of Garhwal Himalaya. A variety of phosphate solubilizing *Bacillus sp*. and *Micrococcus sp* have been isolated along with fungal strains like *Penicillium* and *Aspergillus* from the cold desert of Himalaya of Himachal Pradesh region (Chatli et al., 2008). The efficiency of the strains isolated from Singalila range of Himalaya are comparable with those of other part of Indian Himalaya.

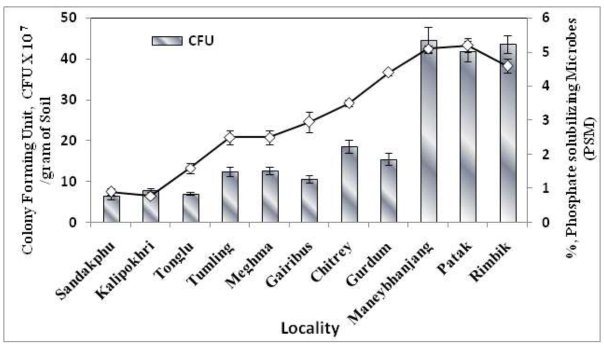


Fig. 1 : Microbiological status and content (%) of phosphate solubilizing microorganisms of high altitude grass land soil. Microorganisms were grown on nutrient agar medium for determination of total colony forming unit (CFU) after serial dilution of soil and % PSM was determined on Pikovskaya's agar plate following replica plate technique. All the plates were incubated for 72h at 30°C.

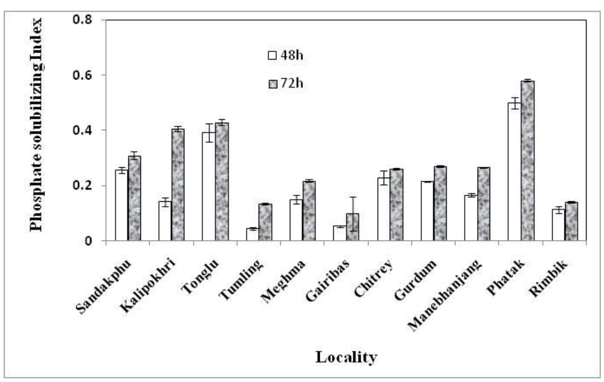
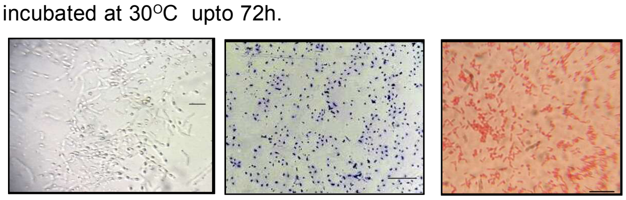


Fig. 2 : Phosphate solubilizing index* (after 48h and 72h) of the predominant phosphate solubilizing microorganism of the soil sample. *Phosphate solubilizing index = [Halo zone diameter (mm) - Colony diameter (mm)]/ Colony diameter (mm) Culture was incubated at 30°C upto 72h.



A. Strain 1006 B. Strain 301 C. Strain202

Fig. 3 : [A] Sporulating gram positive Bacilli type microorganism (Without stain) Strain 1006, tentatively identified as a strain of *Bacillus* from Singalila National Park (Phatak) [B]. Cocci type gram positive microorganism Strain 301, tentatively identified as strain of *Micrococcus* from Kalipkhri. [C]. Non-sporulating gram negative bacilli type microorganism Strain 202, tentatively identified as a strain of *Pseudomonus* from Tonglu. All scales represent 2ìm.

CONCLUSION

The total viable colony forming units and frequency of phosphate solubilizing microorganisms is quite low in high altitude of Singalila mountain range of Himalaya. However, few efficient isolates, tentatively identified as the strains of *Bacillus*, *Micrococcus* and *Pseudomonas* are available in the grass land area of different altitude. Application of these bioinoculants could be beneficial for high altitude agronomy as commercially and ecologically viable.

ACKNOWLEDGEMENT

Author is grateful to the Officer-in Charge, Darjeeling Government College for providing the all laboratory facilities and financial support. Author is also thankful to Mr. Dinesh Tamang for helping in collection of soil samples.

REFERENCES

Chatli, S., Anshu, B.V., Sidhu, B. S. 2008. Isolation and characterisation of phosphate solubilising microorganisms from the cold desert habitat of *Salix alba Linn.* in trans Himalayan region of Himachal Pradesh. *Ind. J. Microbiol.* **48**: 267–273. doi: 10.1007/s12088-008-0037-y .

Choudhari, V.K., Kumar, P.S. 2015. Amelioration of Acidic Soil and Production Performance of Cowpea by the Application of Different Organic Manures in Eastern Himalayan Region, India. *Comm soil Sci. Plant Analysis.*.**46**: 2523-2533.

Gusain, Y.S., Kamal, R., Mehta, C.M., Sing, U.S., Sharma, A.K. 2015. Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himalaya aimed to improve the growth of rice. *J. Environ Biol*. **36**: 103-107.

Kumar, A., Guleria, S., Mehta, P., Walia, A., Chauhan, A., Shirkot, C.K. 2015. Plant growth promoting traits of phosphate solubilizing bacteria from Hippophae. Rhamnoides L (Sea buckthorn) growing in cold desart trans Himalayan Lahul and Spiti regions of India. *Acta Physiologiae Plantarum.* 37: 48-55.

Liu, J., Qi, W., Li, Q, Wang, S.G., Song, C., Yuan, X. 2020. Exogenous phosphorus- solubilizing bacteria changed the rhizosphere microbial community indirectly. *3 Biotech.* **10:** 164

Pal Saha, S., Chhetri, S., Rai, S., Chakraborty, H. 2018. Evaluation of ant nest microenvironment from Darjeeling Himalaya. *J. Mycopathol. Res.* **56**: 21-27.

Panda, B., Rahman, H., Panda, J. 2016. Phosphate solubilizing bacteria from the acidic soils of Eastern Himalayan region and their antagonistic effect on fungal pathogens. *Rhizosphere.* **2**:62–71.

Pandey, A. P., Trivedi, B. K., Palni, L.M.S. 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine location in the Indian Central Himalaya. *Curr. Microbiol.* **53**: 102–107

Prakash, P. 2013. Grazing management in temperate grassland of Kumaun Himalaya for soil water conservation. *J.Appl. Nat.Sci.*. **5**: 345-349. doi.org/10.31018/jans.v5i2.330

Saleemi, M., Kianiz, M.Z., Sultan, T.K., Mahmood, S. 2017. Integrated effect of plant growth promoting rhizobacteria and phosphate solubilizing microorganisms on growth of wheat (*Triticum aestivum* L.) under rain fed condition. *Agric & Food Sec.* 6:46

Selvakumar, G., Joshi, P., Nazim, S., Mishra, K. P., Bisht, K.J., Gupta, H. S. 2009. Phosphate solubilization and growth promotion by *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium isolated from a high altitude Himalayan rhizosphere. *Biologia.* **64**: 239–245.

Shi, X.K., Ma, J.J., Liu, L.J. 2017. Effects of phosphate-solubilizing bacteria application on soil phosphorus availability in coal mining subsidence area in Shanxi. *J. Plant Interact.*. **12**:1, 137-142.

Tomer, S., Suyal1, D. C., Shukla, A., Jyoti, R., Yadav, A., Shouche, Y., Goel, R. 2017. Isolation and characterization of phosphate solubilizing bacteria from Western Indian Himalayan soils. *Biotech.*. **7:** 95-100.