Research Article

Response of Cold-Tolerant *Aspergillus* spp. to Solubilization of Fe and Al Phosphate in Presence of Different Nutritional Sources

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Three species of *Aspergillus*, namely, *A. niger*, *A. glaucus* and *A. sydowii*, isolated from soil samples collected from the Indian Himalayan Region (IHR), have been investigated for solubilization of aluminium phosphate and iron phosphate in the presence of different carbon and nitrogen sources. Preference of each fungal species varied for nitrogen and carbon sources, in terms of phosphate-solubilization. Among three species, *Aspergillus niger* gave the best results; it solubilized 32% and 8% of the supplemented aluminium phosphate and iron phosphate, respectively. The results indicated that the effect of carbon and nitrogen sources can influence the phosphate solubilizing efficiency of all the three *Aspergillus* spp. tested. All the three species were found to be plant-growth promoters in bioassays conducted under greenhouse conditions. The Al and Fe phosphate solubilization efficiency, investigated in the present study, is at the lower end of their previously reported tricalcium phosphate solubilization efficiency. The cultures are likely to have better field applications in agrobiotechnology, due to their potential towards solubilization of Al and Fe phosphates, which are known to have lower solubility through microbial activity.

1. Introduction

Microorganisms are known to play a fundamental role in biogeochemical cycling of P in natural and agricultural ecosystems. Gerretsen [1] initially demonstrated that microbiological activity in the rhizosphere can dissolve sparingly soluble inorganic P and increase plant growth. Several species of *Aspergillus* and *Penicillium* are known for solubilizing insoluble phosphates [2–4]. Usually, in vitro P solubilization activity is known to be associated with decline in pH [5]. Organic acids can greatly increase the rate of soluble P through chelation and ion exchange reactions [6]. In addition to organic acid production [7], phosphate solubilization can also occur due to proton extrusion [8, 9].

The widespread use of phosphate-solubilizing microbial inoculants remains limited due to inconsistent results under field conditions. This is mainly due to the functional efficiency of microorganisms, which is largely governed by environmental conditions. The forest and cropped soils in

the Indian Himalayan Region (IHR) are generally acidic [10, 11]. In acidic soils, free oxides and hydroxides of Al and Fe are known to fix phosphorus [12, 13]. Inoculation with phosphate-solubilizing microorganisms has been considered as a feasible approach to increase the amount of available P in soil. Fungal species, in this context, have been recognized as more appropriate compared to bacterial species [14]. In a recent study, tricalcium phosphate solubilization efficiency of ten cold- and pH-tolerant species of *Aspergillus* have been reported, with *A. niger* followed by *A. glaucus* and *A. sydowii*, respectively, being the best performers [15]. The suboptimal conditions (temperatures) were found to be optimal for solubilization of phosphate in the cited study. Al and Fe phosphates are known for their low solubility through microbial activity (bacteria and fungi), as compared to tricalcium phosphate. In the present study, response of these three cold-tolerant *Aspergillus* spp. (*A. niger*, *A. glaucus*, and *A. sydowii*) has been investigated with respect to solubilization of Al and Fe phosphates, in the presence

of different nutritional sources. The plant-growth promoting ability of these fungal species has also been carried out under greenhouse conditions, using maize and wheat as test species.

2. Materials and Methods

2.1. Fungal Cultures. The fungal cultures were originally isolated from soil samples collected from various forest locations in higher altitudes (1800–3610 m above sea level) of the IHR, and maintained on agar slants at 4°C in the culture collection developed in the microbiology laboratory of the GB Pant Institute of Himalayan Environment and Development, Almora. The fungal cultures have also been deposited and accessioned at the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi (Accession numbers ITCC2546- *A. niger* and ITCC4210- *A. sydowii*), and the Agharkar Research Institute Fungal Culture Collection (ARIFCC), Pune, India (Accession number ARIFCC771- *A. glaucus*), respectively [15].

2.2. Estimation of $AlPO_4$ and $FePO_4$ Solubilization

$AlPO_4$. 0.5 g/100 mL of $AlPO_4$ (HiMedia, India) was added as a P source in Pikovskaya's broth (yeast extract, 0.50 g; dextrose, 10.00 g; ammonium sulphate, 0.50 g; potassium chloride, 0.20 g; magnesium sulphate, 0.10 g; manganese sulphate, 0.0001 g; and ferrous sulphate, 0.0001 g) [16]. For estimation of the effect of different carbon and nitrogen sources on solubilization of $AlPO_4$, dextrose was replaced with D-fructose, starch, and sucrose as the carbon source (separate treatments). Similarly, ammonium sulphate was replaced with ammonium chloride, sodium nitrate, and potassium nitrate (separate treatments) as the nitrogen source.

$FePO_4$. 0.5 g/100 mL of $FePO_4$ (HiMedia, India) was added as the P source in Pikovskaya's broth. For estimation of the effect of different carbon and nitrogen sources on solubilization of $FePO_4$, dextrose was replaced with D-fructose, starch, and sucrose as the carbon source (separate treatments). Similarly, ammonium sulphate was replaced with ammonium chloride, sodium nitrate, and potassium nitrate as the nitrogen source (separate treatments).

The initial pH of the media, before autoclaving, was 7.50. The autoclaved medium was then inoculated with 5 mm disc cultures of the respective fungi and incubated at 21°C for 42 days. The culture filtrate was withdrawn from selected flasks on every 7th day of incubation by vacuum filtration through Whatman numbers 42 filter paper. The filtrate was then analyzed for P_2O_5 production following the chlorostannous reduced molybdophosphoric acid blue method [12]. The absorbance was recorded at 700 nm using Uvikon spectrophotometer (Kontron Instruments, UK). The pH (Systronics, India) of the culture filtrate was also recorded upon each sampling event. The biomass of the cultures was estimated every 7th day of incubation following drying at 65°C for 72 h, from the same flasks that were used for the above experiments.

2.3. Microcosm Studies. Plant-growth-promoting abilities of the three fungal species were performed following

a pot-based assay under greenhouse conditions (temp.: 25 ± 0.5°C; humidity: 75–85%). Maize (*Zea mays*) and wheat (*Triticum aestivum*) were used as test crops. The seeds were obtained from local farmers at a nearby village, Kosi-Katarmal. Plastic pots (10 × 15 cm) for each treatment were filled with 300 g soil (sandy loam with pH_{H₂O} 6.7, 40% (w/w) moisture content). The treatments were arranged in a randomized block design with 25 replicates.

For taking measurements on growth parameters (shoot and root length and dry weight of shoot, root, and seed), plants were harvested at peak flowering time (approx. 3 months after sowing, $n = 25$). The plants were randomly uprooted from treated and control pots and washed several times with running water. First, the shoot and root length were measured then the roots were cutoff, and following drying at 65°C for 72 h, the measurements of biomass of root and shoot parts were performed, separately. For yield parameters (biological and economic yield), ten plants were collected randomly from control and inoculated plots, at the time of senescence. The plants were dried at 70°C for 72 h for calculating the biological yield. Ten seeds from each plant were used for measurement of seed weight (an estimate of economic yield). Harvest index was calculated following the formula: harvest index = economic yield/biological yield × 100.

2.4. Statistical Analysis. The data were analyzed with the computer programme Excel (Microsoft Corp.) for graphical representation, and the mean values and variance among the means (fixed effects or model I one-way ANOVA), SPSS/PC [17], were used to explore the correlations between phosphate solubilization, biomass production, and pH.

3. Results

3.1. Aluminium Phosphate Solubilization

3.1.1. *A. glaucus*. *A. glaucus* solubilized 17% of the aluminium phosphate on day 21, which was found to be significantly correlated to decline in pH ($r = -0.987$, $P \leq 0.01$) and production of biomass ($r = 0.993$, $P \leq 0.01$). Less solubilization ($P \leq 0.01$) was recorded in the case of fructose (15.34%)-containing medium as compared to the normal Pikovskaya's broth medium (Figures 1 and 3(a)). Among nitrogen sources (Figure 3(b)), sodium nitrate gave the best results for solubilization of aluminium phosphate (11.56% on day 21). A statistically significant ($P \leq 0.01$) negative correlation was developed between solubilization of aluminium phosphate and a decline in pH. Significant positive correlation ($P \leq 0.01$) between production of biomass and solubilization of aluminium phosphate was also developed in the case of both media.

3.1.2. *A. niger*. *A. niger* exhibited maximum efficiency for solubilization of aluminium phosphate on day 35 (32%) with maximum decline in pH (2.39) and production of fungal biomass (Figure 1). A significant correlation was developed between the decline in pH of the medium ($r = -0.870$, $P \leq 0.01$) and the production of biomass ($r = 0.928$). With all

FIGURE 1: Solubilization of aluminium phosphate and biomass production by *Aspergillus* spp.

media compositions, except the medium supplemented with ammonium chloride, the solubilization of aluminium phosphate persisted even after day 28 of incubation (Figure 1). Significantly higher aluminium phosphate solubilization ($P \leq 0.01$) occurred in the medium supplemented with ammonium chloride (Figure 3(d), 37.78%) as compared to the normal Pikovskaya's broth (32%, Figure 1). Fructose was found to be the best among the carbon sources (Figure 3(c), 32.90% on day 28) in terms of enhancing phosphate solubilization. In the presence of starch, the solubilization was the lowest, and the decline in pH (up to 2.74) was also found to be comparatively low persisting even after day 42 of incubation. The decline in pH in the presence of fructose, ammonium chloride, sodium nitrate, and potassium nitrate was found to be equivalent to the decline in pH in the normal Pikovskaya's medium supplemented with aluminium phosphate. The correlations between phosphate solubilization, the decline in pH, and the production of biomass were found to be significant ($P \leq 0.01$) for all the media tested.

3.1.3. *A. sydowii*. Maximum aluminium phosphate solubilization (18%, Figure 1), pH decline (2.84), and biomass production were recorded on day 35 of incubation. The decline in pH and biomass production were found to be significantly correlated with solubilization ($r = -0.989$, $P \leq 0.01$; $r = 0.929$, $P \leq 0.01$, resp.). Solubilization in the medium supplemented with ammonium chloride was (17.66% on day 35, Figure 3(f)) equivalent to the maximum solubilization found in the Pikovskaya's medium supplemented with

aluminium phosphate. The decline in pH was also found to be 2.92 on day 42 in this medium. Among the carbon sources, fructose was the least efficient (solubilized 11.6% of supplied aluminium phosphate on day 45, Figure 3(e)), while the media supplemented with starch and sucrose solubilized up to 17% of the added aluminium phosphate. The production of biomass and reduction in pH were found to be minimum in the medium supplemented with fructose; however, the fungal growth and the solubilization activity persisted even after day 42. The correlations between phosphate solubilization, the decline in pH, and the production of biomass were found to be statistically significant ($P \leq 0.01$) for all of the media tested.

3.2. Iron Phosphate Solubilization

3.2.1. *A. glaucus*. Maximum (6%) solubilization of iron phosphate, decline in pH, and production of biomass (522 mg) were recorded on day 28 (Figure 2). Solubilization of iron phosphate exhibited a significant correlation with a reduction in pH ($r = -0.866$, $P \leq 0.01$) and production of biomass ($r = 0.848$, $P \leq 0.01$). Medium supplemented with starch performed best in terms of solubilization of iron phosphate (Figure 4(a)), resulting in significantly higher values ($P \leq 0.05$), as compared to the normal Pikovskaya's broth (Figure 2) containing iron phosphate. The maximum decline in pH (3.07) and production of biomass were also recorded in this medium. Slightly higher solubilization (statistically not significant) was also recorded in the medium

FIGURE 2: Solubilization of iron phosphate and biomass production by *Aspergillus* spp.

supplemented with ammonium chloride (Figure 4(b)) as compared to normal Pikovskaya's medium containing iron phosphate.

3.2.2. *A. niger*. Maximum solubilization of iron phosphate (8%), reduction in pH (4.03), and production of biomass were recorded on day 21 (Figure 2). Correlation between phosphate solubilization and acidification was not found to be statistically significant ($r = -0.870$), while the production of biomass exhibited significant ($P \leq 0.001$) correlation with phosphate solubilization. Significantly higher ($P \leq 0.009$) phosphate solubilization (8.32%) was found in the medium supplemented with sodium nitrate (Figure 4(d)) as compared to the normal iron-phosphate-containing Pikovskaya's broth (Figure 2). This was recorded as the best among the tested compositions where pH was reduced to 3.21. Among the carbon sources, fructose was found to be the best (6.58% on day 35), followed by sucrose (6.5% on day 28), and starch (5.6% on day 28) (Figure 4(c)).

3.2.3. *A. sydowii*. Maximum iron phosphate solubilization (4%), the minimum pH (4.73) and the maximum production in biomass were recorded on day 42 (Figure 2). Reduction in pH and biomass production were found to be significantly correlated with iron phosphate solubilization ($r = -0.993$, $P \leq 0.01$; $r = 0.974$, $P \leq 0.01$, resp.). Among the modified media, maximum solubilization (4.36%) was recorded in the case of the medium supplemented with starch on day 28 (Figure 4(e)). The minimum decline in pH (4.23 on day 35)

was also recorded in the same medium. In this case, biomass production was observed to be persisting even after day 42 of incubation. The persistence of solubilization was found to be correlated to the biomass production (significant at $P \leq 0.01$). The solubilization of iron phosphate by *A. sydowii*, in both B2 and B5, was significantly ($P \leq 0.0003$, and $P \leq 0.05$, resp.) higher, than the iron phosphate in the normal Pikovskaya's broth containing iron phosphate.

3.3. Effect of Fungal Inoculation on Plant Growth of Maize and Wheat. Positive response of inoculation with three species of *Aspergillus* in two test crops, maize and wheat, were recorded under greenhouse conditions. In case of maize, in *A. glaucus* inoculated plants, the increase in root and shoot length, root, shoot, and seed weight, and harvest index ranged between 1.20 to 1.35 times, while, in the case of *A. niger*-inoculated treatments, the increases ranged between 1.18 to 3.0 times. Except root weight, all the parameters tested were significantly higher due to inoculation with *A. glaucus* and *A. niger*. *A. sydowii* inoculation resulted in the increases in various growth parameters, ranging between 1.21 and 2.50 times, with all the parameters being significantly higher, except shoot length (Table 1).

In wheat-based experiments, inoculation with *A. niger* and *A. sydowii* resulted in increases in root and shoot length, root, shoot, and seed weight, and harvest index ranging between 1.22 to 2.11 times, and 1.14 to 2.07 times, respectively, all parameters being statistically increased over the controls. These increases ranged between 1.11 to 1.82 times

FIGURE 3: Effect of carbon ((a), (c), and (e)) and nitrogen ((b), (d), and (f)) sources on the aluminum phosphate solubilization efficiency and biomass production of *A. glaucus* ((a) and (b)), *A. niger* ((c) and (d)), and *A. sydowii* ((e) and (f)). *y*-axis shows both phosphate solubilized and biomass produced (separately mentioned on *x*-axis).

FIGURE 4: Effect of carbon ((a), (c), and (e)) and nitrogen ((b), (d), and (f)) sources on the iron phosphate solubilization efficiency and biomass production of *A. glaucus* ((a) and (b)), *A. niger* ((c) and (d)), and *A. sydowii* ((e) and (f)). y-axis shows both phosphate solubilized and biomass produced (separately mentioned on x-axis).

TABLE 1: Effect of phosphate-solubilizing *Aspergillus* species on the growth of maize and wheat under greenhouse conditions.

Treatment	Shoot length (cm)	Root length (cm)	Biomass production and yield (g dry weight)			Harvest index
			Shoot	Root	Seed	
Maize						
Control	56.70 ± 8.37	11.50 ± 1.90	0.28 ± 0.16	0.04 ± 0.22	0.84 ± 0.02	0.32
<i>A. glaucus</i>	68.20 ± 3.20*	15.30 ± 2.51*	0.38 ± 0.06*	0.05 ± 0.03	0.93 ± 0.06*	0.39
ANOVA	$F_{1,18} = 15.53$ $P = 0.001$	$F_{1,18} = 25.73$ $P = 0.05$	$F_{1,18} = 5.39$ $P = 0.03$	$F_{1,18} = 2.14$ $P = 0.16$	$F_{1,18} = 3.16$ $P = 0.01$	NA
<i>A. niger</i>	67.31 ± 2.16*	15.23 ± 1.25*	0.61 ± 0.14*	0.12 ± 0.02	1.12 ± 0.06*	0.69
ANOVA	$F_{1,18} = 11.93$ $P = 0.002$	$F_{1,18} = 22.83$ $P = 0.0002$	$F_{1,18} = 5.39$ $P = 0.03$	$F_{1,18} = 2.14$ $P = 0.16$	$F_{1,18} = 6.23$ $P = 0.006$	NA
<i>A. sydowii</i>	74.81 ± 5.32	17.21 ± 3.26*	0.58 ± 0.16*	0.10 ± 0.03*	1.02 ± 0.04*	0.68
ANOVA	$F_{1,18} = 25.21$ $P = 0.87$	$F_{1,18} = 9.46$ $P = 0.006$	$F_{1,18} = 12.83$ $P = 0.002$	$F_{1,18} = 11.36$ $P = 0.003$	$F_{1,18} = 4.56$ $P = 0.004$	NA
Wheat						
Control	29.80 ± 4.86	16.85 ± 2.68	0.09 ± 0.03	0.13 ± 0.02	0.27 ± 0.06	68.08
<i>A. glaucus</i>	33.20 ± 4.10	20.40 ± 3.63*	0.13 ± 0.05*	0.16 ± 0.05	0.36 ± 0.06*	124.14
ANOVA	$F_{1,18} = 2.86$ $P = 0.10$	$F_{1,18} = 6.18$ $P = 0.02$	$F_{1,18} = 4.62$ $P = 0.04$	$F_{1,18} = 2.41$ $P = 0.13$	$F_{1,18} = 11.58$ $P = 0.003$	NA
<i>A. niger</i>	39.15 ± 6.03*	20.60 ± 3.84*	0.19 ± 0.03*	0.17 ± 0.05*	0.37 ± 0.10*	102.78
ANOVA	$F_{1,18} = 14.58$ $P = 0.001$	$F_{1,18} = 6.41$ $P = 0.02$	$F_{1,18} = 43.74$ $P = 0.0001$	$F_{1,18} = 6.40$ $P = 0.02$	$F_{1,18} = 7.11$ $P = 0.015$	NA
<i>A. sydowii</i>	35.40 ± 5.32*	19.20 ± 2.15*	0.14 ± 0.04*	0.27 ± 0.17*	0.34 ± 0.05*	82.92
ANOVA	$F_{1,18} = 6.04$ $P = 0.02$	$F_{1,18} = 4.66$ $P = 0.04$	$F_{1,18} = 4.97$ $P = 0.04$	$F_{1,18} = 5.94$ $P = 0.02$	$F_{1,18} = 6.42$ $P = 0.02$	NA

ANOVA: analysis of variance; NA: not detected; *significant increment as compared to control.

higher than the controls and were statistically significant in case of *A. glaucus*.

4. Discussion

The phosphate solubilization efficiency by microorganisms depends on the form of insoluble phosphate. The three fungal cultures used in the present study exhibited differential responses towards solubilization of aluminium and iron phosphate. The Al and Fe phosphate solubilization efficiency observed, in the present study, are lower than previously reported tricalcium phosphate solubilization efficiency [15]. The cultures are likely to have better applications due to their potential towards solubilization of Al and Fe phosphates, which are known for lesser solubility through microbial activity. Lopez et al. [18] and Puente et al. [19] have demonstrated the solubilization efficiency of more insoluble forms of phosphates in rock weathering through microbial activities, with reference to desert-plant-based studies. Positive correlation between phosphate solubilization and pH reduction developed in most cases is attributed to the production of organic acids and proton extrusion. Production of organic acids by microbial cultures has been emphasized as one of the main mechanisms involved in phosphate solubilization [2, 20].

The effect of nutritional aspects, such as carbon and nitrogen sources, on microbial phosphate solubilization efficiency has been studied by various workers [8, 21]. In the present

study, the nitrogen-source-based modifications in the media brought significant changes in the solubilization efficiency of aluminium phosphate, production of biomass, and changes in pH, as compared to carbon sources. Nitrogen in ammonium form is necessary for P solubilization by production of organic acids through NH_4^+/H^+ exchange mechanisms [5, 22]. Solubilization of iron phosphate was found to be the best in starch-containing medium as compared to other carbon sources. Starch is the only polysaccharide used in the present study as a carbon source and is likely to create nutritional stress conditions that, in turn, might support phosphate solubilization by virtue of producing secondary metabolites.

Maximum solubilization of aluminium phosphate, in some of the instances, was recorded with less production of biomass. In case of *A. glaucus*, maximum biomass was produced in presence of starch, while maximum aluminium phosphate was solubilized in the media supplemented with fructose and sucrose. Depending on the nutrient and P sources, the fungus may use alternative metabolic pathways resulting in secretion of different organic acids [3, 23]. Similar results have been reported in previous studies, where species of *Aspergillus* and *Paecilomyces* have been evaluated for solubilization of tricalcium phosphate at different temperatures [15, 24]. Evaluation of the factors responsible for production of lesser fungal biomass in the medium vis-à-vis lethal and sublethal concentration of Al and Fe is an area of important future research. Independent of the excretion of organic acids, the toxic effect of Al^{3+} can affect the release of P from

AlPO_4 [2]. The results in the present study are contradictory to the earlier findings of Barroso et al. [21] that reported a correlation between the production of greater biomass and a reduction in pH in the medium supplemented with AlPO_4 than that containing calcium phosphate. Decreased solubilization of phosphate in the presence of aluminium in comparison to iron has also been reported by Vyas et al. [25]. Inefficiency of *A. sydowii* growth in the presence of Fe during the initial period of incubation was an interesting observation. However, in later phases of growth, the fungus again started acting on the substrate probably because it was in need of the nutrients, thus releasing P from insoluble forms. In addition, after the initial shock, the cells are likely to utilize the available free P for metabolism first, later solubilizing P to acclimatize to the stressed environment [26].

At suboptimal growth conditions, where phosphate solubilization was found to be maximum, a highly significant negative correlation was observed between a reduction in pH and the production of biomass. This was an indication of the direct relationship between cell proliferation and phosphate solubilization with reference to pH and growth conditions. Metabolic alteration, due to extracellular pH and involvement of different signaling pathways, has been reported [27]. The role of these signaling molecules in view of the mechanisms involved in solubilization of phosphate also requires attention in future research.

Use of P-solubilizing fungi in plant nutrition is well reported [28, 29]. In the present study, the three fungal cultures exhibited their ability to enhance the growth of two crops. Inoculation with *A. niger* produced a maximum harvest index in maize, while *A. glaucus* produced a maximum index in wheat. Yield and harvest index are considered the most important parameters, evaluating the performance of PGP microorganisms. Microorganisms are important in agricultural nutrient cycling to reduce the need for chemical fertilizers [30]. Wahid and Mehana [31] reported the impact of phosphate-solubilizing fungi on wheat and faba bean, *Penicillium pinophilum* being the most efficient. Organic acids help in the mobility of nutrients in soil and hence can have a positive impact on plant growth. There is evidence that organic acids are capable of mobilizing phosphorus [32, 33]. Several factors have been known to improve the quality of soil, phosphorus mobilization, in particular [27]. The efficiency of organic acid production by the *Aspergillus* spp., used in the present study, has been evaluated [34]. These cultures were also found to produce phosphatases [15], which are likely one of the factors responsible for P mobilization [35].

The fixation of P depends on the soil acidity. In acidic soils, free oxides and hydroxides of Al and Fe fix P, while in alkaline soils P is fixed with Ca and Mg. Aluminium and iron phosphate-solubilizing microorganisms play a major role in P mobilization in low temperature environments of Himalayan soils, which are mostly acidic. Effectiveness of the *Aspergillus* spp., used in the present study, has already been demonstrated for their tricalcium phosphate solubilization efficiency [15]. The cited report also revealed the tolerance of these fungi to a wide range of pH. The present study documented the ability of the best three tricalcium phosphate-solubilizing fungi for their aluminium and iron phosphate solubilization

in the presence of different carbon and nitrogen sources. Based on the previous [15] and present investigations, it is concluded that these fungi can be used as phosphate solubilizers in different environmental conditions and hence are ecologically important in soil nutrient management in mountain ecosystems. Further, due to their cold-tolerant properties, these fungi also provide an opportunity to develop bioformulations for field application in low-temperature environments.

Documentation of microbial diversity, including mycorrhizae, in various ecological niches of the Indian Himalayan Region (IHR) with particular reference to biotechnological applications, such as development of microbial inoculants for colder regions, has received attention in recent times [36–39]. Cold-tolerant bacterial inoculants, possessing plant growth promoting and biocontrol properties, have already been developed in suitable formulations with particular reference to field applications under mountain ecosystems [40–46]. Phosphate solubilization, biocontrol properties, and tolerance to low temperature have been the preferred characteristics for screening suitable inoculants for biotechnological applications. These investigations are important as the distribution of microorganisms is largely governed by environmental specificities.

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