RESEARCH/REVIEW ARTICLE

Phosphate solubilizing ability of two Arctic *Aspergillus niger* strains

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Abstract

Many filamentous fungi were isolated from the soils of Ny-Ålesund, Spitsbergen, Svalbard, and were screened in vitro for their phosphate solubilizing ability. Two strains of *Aspergillus niger* showed good tricalcium phosphate (TCP) solubilizing ability in Pikovskaya's medium. The TCP solubilization index was calculated at varying levels of pH and temperatures. The ability of *Aspergillus niger* strain-1 to solubilize and release inorganic-P was 285 μg ml^{-1}, while *Aspergillus niger* strain-2 solubilized 262 μg ml^{-1} from 0.5% TCP after seven days. This is the first report of TCP solubilization by Arctic strains that may serve as very good phosphate solubilizers in the form of biofertilizer.

Phosphorus is an important plant nutrient, playing a key role in the development and yield of crop plants. Phosphorus exists in nature in a variety of organic and inorganic forms. The majority of soils contain insoluble inorganic phosphates, which are of no use to plants unless they are solubilized. Soil contains organic phosphorus that can be used by plants only if it is mineralized. Phosphate solubilizing micro-organisms convert these insoluble phosphates into soluble form through the processes of acidification, chelation and exchange reaction (Earl et al. 1979; Starkanova et al. 1999; Narsian & Patel 2000; Reyes et al. 2002). A number of phosphate solubilizing bacteria, fungi and actinomycetes have been reported (Vassileva et al. 1998; Gaur 1990; Chabot et al. 1993; Khalil 1995; Subba Rao 1999). Filamentous fungi—particularly *Aspergillus niger* gr. and some species of *Penicillium*—from non-polar habitats have been tested for solubilization of inorganic phosphorus (Asea & Kucey 1988; Gaur 1990; Vassilev et al. 1996; Goenadi et al. 2000; Narisan & Patel 2000; Pandey et al. 2008).

The solubilization of inorganic phosphorus at low temperatures by cold adaptive bacteria has been tested (Trivedi & Pandey 2007). However, to our knowledge, the study reported here is the first investigation of phosphate solubilizing fungi from Arctic soils. Fungi obtained from different soil samples of tundra in the Arctic archipelago of Svalbard were investigated for their phosphate solubilization properties using tricalcium phosphate (TCP) in solid and liquid media. The purpose of this work was to document the fungal diversity in the sampled soils and to obtain phosphate solubilizing fungal strains from an Arctic region that have the potential to be used as biofertilizers for agricultural crops in colder climates across the world such as mountainous areas.

Materials and methods

Study sites and sampling

Ny-Ålesund (78° 55′ N, 11° 56′ E) is on the west coast of Spitsbergen, the largest island of the Svalbard Archipelago. Topographical features of Ny-Ålesund include nearby glaciers, terminal moraines and glacial streams flowing northward to Kongsfjorden. Within the marine terraces, gravelly and stony plains predominate. The sampling sites were situated in different habitats, such as near a glacier, in a wetland and on plains (Fig. 1). The mean temperature in the coldest month (February) is –14 °C, while the warmest month (July) has a mean

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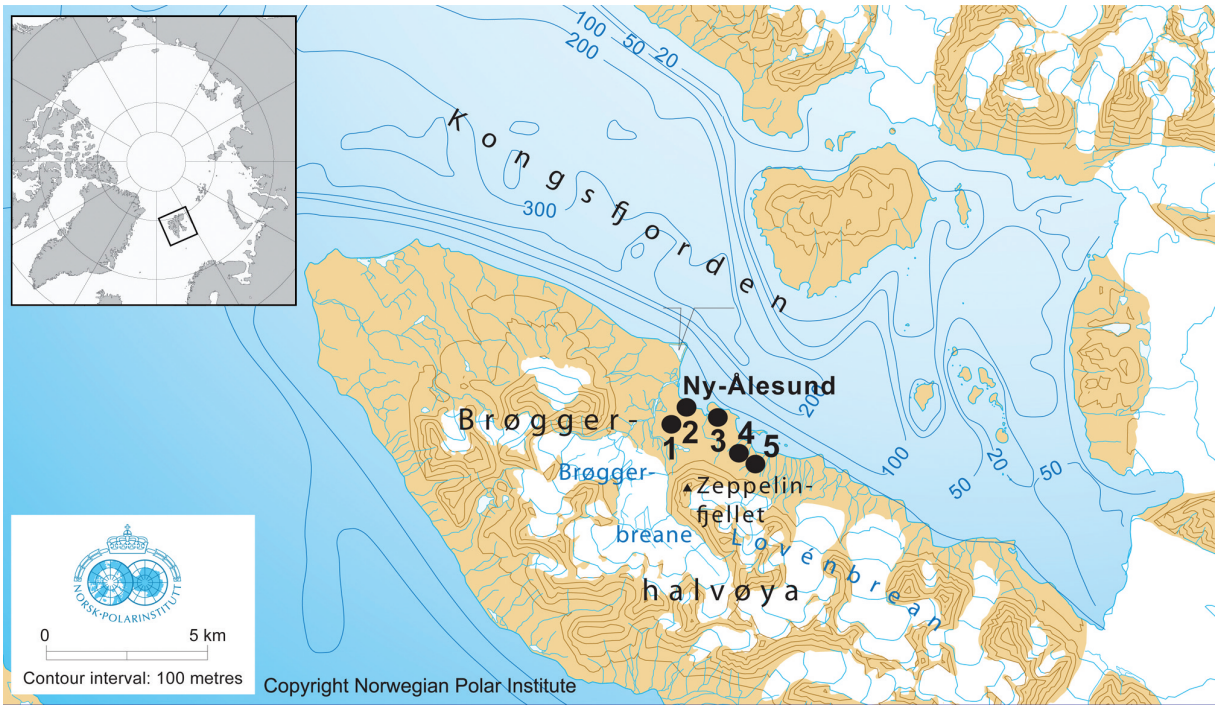

Fig. 1 Sampling sites in Ny-Ålesund, Spitsbergen, Svalbard. The location of the archipelago of Svalbard is shown in the inset.

temperature of +5 °C (Nygaard 2009). The soils of the area are loose and poorly developed and support tundra vegetation (Klimowicz & Uziak 1988).

In the present study, soil samples were collected from the surface to 10 cm depth from vegetated and barren lands in the area of Ny-Ålesund during the 2007 Indian Arctic Expedition. The samples were placed in sterile ampules (Himedia, Mumbai, India) and stored at 4 °C until studied.

Collected from five different sites, nine soil samples were designated as F1 Veg, F1 Barr, F2 Veg, F3 Veg, F3 Barr, F4 Veg, F4 Barr, F5 Veg and F5 Barr (Table 1). "Veg" refers to a vegetated area and "Barr" refers to a barren area at the collection sites (F1–F5). Located in the near vicinity of the glacier Austre Brøggerbreen, F1 had fragmentary moss vegetation. F2 was at a comparatively higher altitude with high plant diversity. The moss *Sanionia uncinata* was dominant in the area along with healthy flowering plants such as *Deschampsia alpina* and *Dryas octopetala*. Sites F3 and F4 were low-lying plains with scanty moss and lichen vegetation. F5 was a coastal area with moss, lichen and *Dryas* sp.

For the isolation of fungi, 1 g of soil sample was taken and serially diluted up to 10^{-7} (Waksman 1916). Soil suspension (0.2 ml) was used as inoculum for the isolation of fungi on three different culture media: malt extract agar, corn meal agar and potato dextrose agar. The plates were initially incubated at 4 °C for 20 days and later at 15 °C for 7 days. The growing fungal colonies having different morphological features were purified and transferred onto potato dextrose agar slants for detailed study. Pure and well-sporulating cultures were identified on the basis of morphotaxonomy with the help of standard literature (Barnett 1960; Rapper & Fennell 1965; Von Arx 1974; Ellis 1971, 1976; Barron 1977; Pitt 1979; Carmichael et al. 1980; Gams et al. 1980; Kirk et al. 2008).

Diversity indices for the fungi at different localities were calculated using PAST software (Hammer et al. 2001) and statistical calculators available online (http:/biome.sdsu.edu/fastgroup/cal_tools.htm).

Screening for TCP solubilizing ability

All fungal isolates were screened for their phosphate solubilizing activity on Pikovskaya's medium (Himedia) by spot inoculation and incubated at 10, 15, 20, 25 and 30 °C for seven days (Pikovskaya 1948). A clear zone around a growing colony indicated phosphate solubilization. After primary screening the positive isolates were

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Table 1 Screening of isolates for tricalcium phosphate solubilization (TCP) properties and the presence of each fungus in different soil samples from vegetated (Veg) and barren (Barr) collection sites in the vicinity of Ny-Ålesund, Spitsbergen, Svalbard.

		Sampling site								
		F1		F2	F3		F4		F5	
		78 55.082 N 11 51.527 E 36 m a.s.l.		78 55.165 N 11 52.660 E 60 m a.s.l.	78 55.254 N 11 54.256 E 30 m a.s.l.		78 54.978 N 11 57.330 E 31 m a.s.l.		78 54.817 N 11 58.378 E 17 m a.s.l.	
Isolated fungi	TCP solubilization	Veg	Barr	Veg	Veg	Barr	Veg	Barr	Veg	Barr
Aspergillus aculeatus	–		+							
Aspergillus nidulans	–		+	+						
Aspergillus flavus gr.	–		+							
Aspergillus niger strain-1	+[a]			+						
Aspergillus niger strain-2	+[a]			+						
Acremonium sp.	–			+						
Arthrinium sp.	–	+								
Cladosporium cladosporioides	–	+		+						
Cladosporium sp.	–			+						
Corynespora sp.	–									+
Chrysosporium panorum	–			+						
Epicoccum sp.	–			+						
Hypoxylon sp.	–		+							
Mortierella sp.	–		+							
Mucor sp.	–				+					
Myrothecium sp.	–				+					
Paecilomyces roseolus	–	+								
Penicillium sp.	–		+			+				
Phialophora sp. 1	–			+						
Phialophora sp. 2	–								+	
Non-sporulating	–		+	+	+	+	+	+		+

[a]Clearing zone formed around colony.

taken for detailed study at different pH and temperature regimes. Phosphate solubilization index was calculated using the following formula (Edi-Premono et al. 1996):

$$\text{Solubilizing index} = \frac{\text{Colony diameter} + \text{clearing zone}}{\text{Colony diameter}}.$$

Quantitative estimation of phosphate solubilization in broth culture

Pikovskaya's broth medium (pH 7.2) was prepared and 100 ml of the medium was dispensed in 250 ml conical flasks. Insoluble phosphate in the form of TCP (500 mg) was added to each flask and was then sterilized at 15 lb pressure for 20 min. Ten-day-old culture grown on PDA medium was used as an inoculum source and 1.0 ml of spore suspension of the isolates was inoculated in triplicate. A set that was not inoculated was maintained as a control. Flasks were kept on an incubator shaker for seven days at 20 °C and 140 rpm. After incubation, the contents of each flask were filtered through grade 42 filter paper (Whatman, Maidstone, Kent, UK). Water-soluble phosphorus was measured using the chlorostanous-reduced molybdophosphoric acid blue method (Jackson 1973).

Result and discussion

During the course of isolation of fungi from the soil samples collected in Ny-Ålesund, 21 fungal taxa belonging to 14 different genera were isolated (Table 1). Screening of these isolates for their TCP solubilizing ability revealed that among different groups of fungi only two strains of *A. niger* group (Fig. 2) were found to be TCP solubilizers in Pikovskaya's medium. Both *A. niger* strains were grown at different temperatures and pH for TCP solubilization. Both the isolates showed maximum clearing zones at pH 7.2 and 20 °C (Figs. 3–5). The maximum solubilization index was observed in strain-1 (2.2) followed by strain-2 (1.12).

Phosphate solubilization in Pikovskaya's broth medium for both isolates was analysed quantitatively. The culture

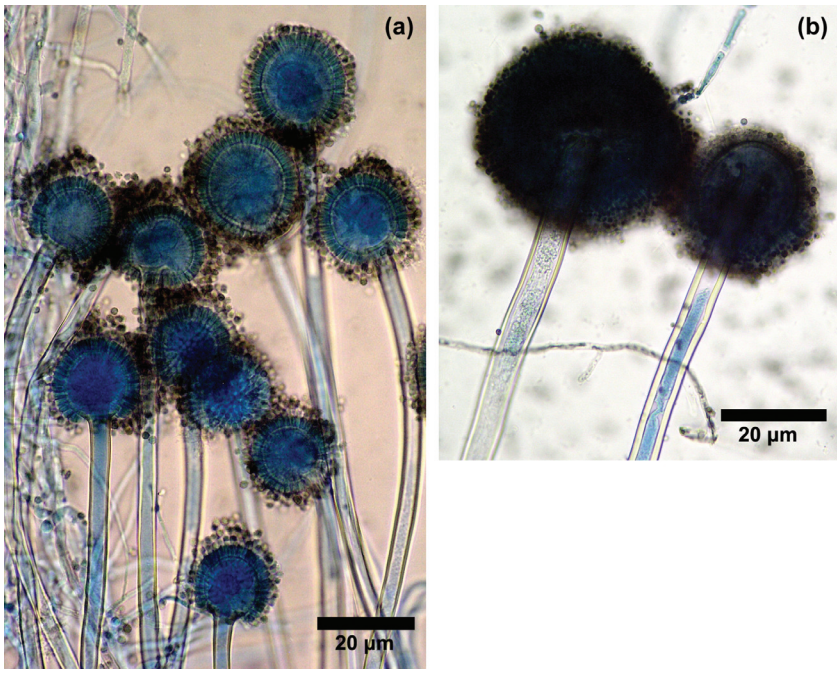

Fig. 2 (a) *Aspergillus niger* strain-1 (NFCCI-2140). (b) *Aspergillus niger* strain-2 (NFCCI-2141).

was sampled daily to determine the change in pH (Fig. 6) of the liquid broth. The pH of the culture broth dropped significantly as compared to the control, where it remained constant at 7.2. *A. niger* strain-1 (NFCCI-2140) decreased the pH from 7.2 to 4, while strain-2 (NFCCI-2141) decreased the pH from 7.2 to 3.4 (Fig. 6). The differences in drop in pH by the two strains indicates the varying diffusion rates of different organic acids secreted by these two tested strains of fungi. The drop in pH during the experiment reported here resembles results from non-polar areas (Alam et al. 2002; El-Katatny 2004; Pradhan & Shukla 2005; Nopparat et al. 2007). After seven days incubation in Pikovskaya liquid medium, it was observed that strain-1 solubilized and released 285 µg P ml^{-1} whereas strain-2 produced 262 µg P ml^{-1}.

The relation between the drop in the pH of the medium and TCP solubilization was examined in detail in this study. However, no significant relationship could be established. Similar observations have been made by those working with fungi from tropical habitats in India (Das 1963; Ahmad & Jha 1968; Sethi & Subba-Rao 1968; Narsian & Patel 2000).

We found that the diversity of fungi varied in the different soils tested. Soil sample F2 showed significant diversity (Shannon diversity index = 2.1) while other localities had less fungal diversity (Table 2). The two strains of *A. niger* that we found to be good phosphate

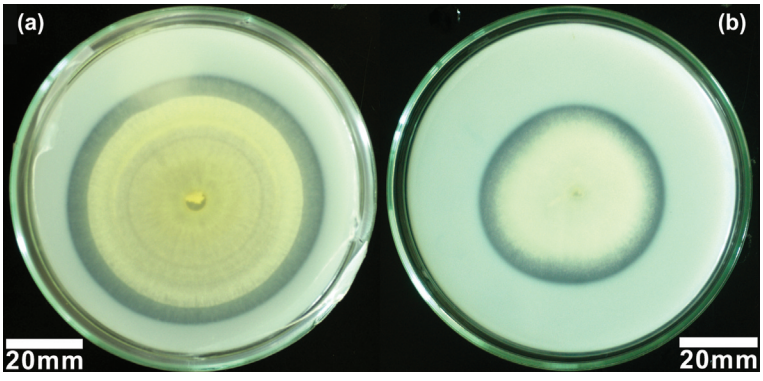

Fig. 3 Clearing zones around colonies of (a) *Aspergillus niger* strain-1 and (b) strain-2.

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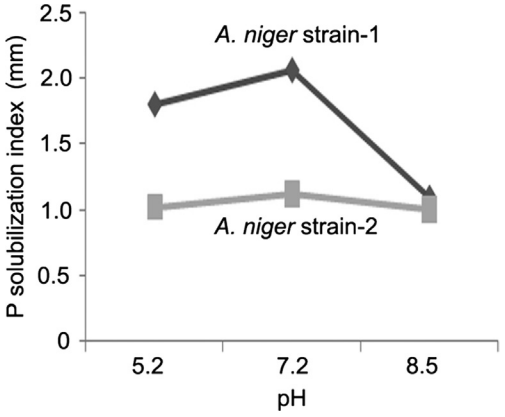

Fig. 4 Effect of pH on the phosphate solubilization index for the two *Aspergillus niger* strains tested.

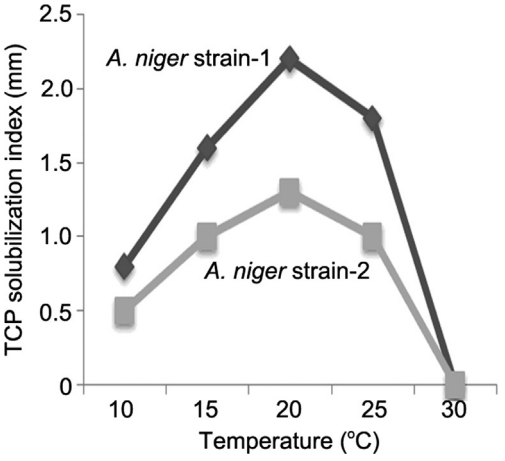

Fig. 5 Effect of temperature on tricalcium phosphate (TCP) solubilization by the two *Aspergillus niger* strains tested.

solubilizers were from site F2, a location with very good plant diversity. It is well known that many plants benefit from their association with soil microbes that promote plant growth (Glick 1995; Illmer et al. 1995; Richardson 2001). Among them, phosphate solubilizing microbes increase the availability of phosphate to plants, resulting in higher plant growth and, in the case of crops, higher yield (Kueey et al. 1989). It is possible that the presence

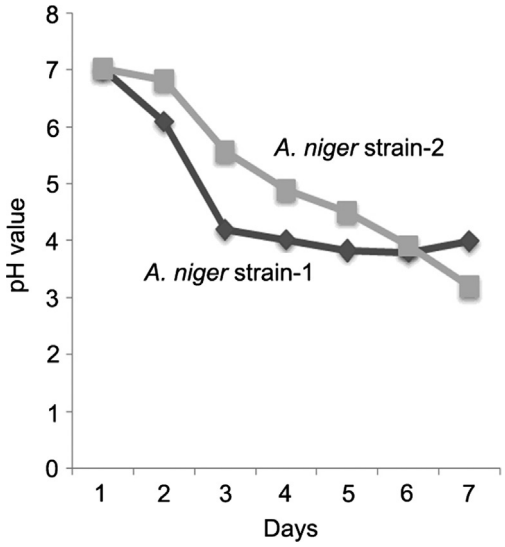

Fig. 6 Decrease in pH of media during incubation of the two tested *Aspergillus niger* strains.

of good phosphate solubilizing fungi may be one of the reasons that the plant community in the F2 area is so well developed.

To the best of our knowledge, this is the first report of phosphate solubilizing fungi from the Arctic tundra. Isolating filamentous fungi from the soils of Ny-Ålesund, we documented 21 fungal taxa including non-sporulating forms. Two strains of *A. niger* were found to be good phosphate solubilizers at 20 °C. In the future, these promising strains may be utilized as biofertilizers to increase agricultural yields in colder regions around the world, such as the Himalayas and other mountainous areas.

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Table 2 Diversity indices of filamentous fungi in soil samples from vegetated (Veg) and barren (Barr) collection sites in the vicinity of Ny-Ålesund, Spitsbergen, Svalbard.

Indices	F1 Veg	F1 Barr	F2 Veg	F3 Veg	F3 Barr	F4 Veg	F4 Barr	F5 Veg	F5 Barr
Distinct fungal taxa	2	7	10	3	2	1	1	1	2
Individuals	14	34	60	22	2	5	7	9	13
Shannon diversity index	0.5983	1.83	2.18	1.09	0.6931	0	0	0	0.6172
Simpson's diversity index	0.4082	0.8235	0.8761	0.6612	0.5	0	0	0	0.426

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