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Psychrophilic fungi from Schirmacher Oasis, East Antarctica

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This communication presents results of a preliminary study on the fungal biodiversity of soils of Schirmacher Oasis, East Antarctica. Using 2% malt extract agar medium, serial dilution method was followed to recover the fungi in culture from the soil samples. Fungal colonies were visible in culture plates only when maintained at 2–5°C for up to 45 days. Several taxa of fungi were recovered.

Keywords: Psychrophilic fungi, Schirmacher Oasis, soils, serial dilution method.

PSYCHROPHILES thrive at very low temperatures. These include organisms living in deep sea (–1 to 4°C), Arctic and Antarctic (–1 to –35°C during wintertime), and glacial ice habitats (–5°C). Little is known on the biodiversity of such habitats, especially microfungal diversity. A few reported organisms from these habitats are gaining popularity in recent years with the advent of genomics and proteonomics[1–3]. Further, some of these, especially fungi and bacteria, are now known to produce unique enzymes and secondary metabolites of immense biotechnological potential.

The physiological and ecological mechanisms that help fungi to overcome and survive cold environmental conditions are well explained by Robinson[4]. He indicated that there is a predominance of sterile mycelia in the Antarctic soils and this could be a physiological adaptation to overcome the harshness of sub-zero temperatures. He also attributed the production of melanin by these fungi as a protective mechanism for survival under extreme temperatures.

Antarctica is a continent located at the South Pole. Barring 2% of the area, thick sheets of ice cover the remaining parts. Only a few species of fungi and bacteria have been described from the region in the recent past, most of them being from the marine environment, i.e. sea water and sea ice. Little investigation has been carried out on soil microorganisms of ice-free areas. Studies of Nichols *et al.*[5] resulted in the recovery of 769 strains of Actinobacteria from the Antarctica. They suggested that the terrestrial environments of the region are a rich source of novel and rare genera. They also studied at molecular level, the total microbial diversity of the polar and deep-sea environment.

India has established a permanent research station, Maitri at Schirmacher Oasis, East Antarctica and launched a series of scientific expeditions since 1981. Earlier studies suggested that life at Schirmacher Oasis is dominated by lichens[6], mosses[7] and algae[8,9]. Studies on bacteria and yeast were conducted by Shivaji[10]. Effect of temperature on bacterial populations was observed by Matondkar[11]. Microfaunal studies of the region were carried out by Ingole and Parulekar[12].

Sharma[13] reported nine species of fungi from the Antarctica region. These include *Arthrobotrys ferox* on moss, *Torulopsis psychrophila* and *Phoma herbarum* on bird excreta, *P. herbarum* on skeletal remains, *Acremonium antarcticum* and *A. psychrophilum* on lichens and species of *Torulopsis*, *Psychrophila* and *Cryptococcus* on ornithogenic soils. Besides, a few alien species of fungi, viz. *Hormoconis resinae* on oil spills and species of *Dacrymyces* and *Exidia* on wooden debris have also been reported by Sharma[13]. Some of the tropical saprophytic fungi, viz. *Chaetomium globosum*, *Stemphylium* sp., *Curvularia lunata*, *Memnoniella echinata*, *Aureobasidium pullulans*, *Aspergillus niger*, *Paecilomyces varioti*, *Penicillium funiculosum* and *Cladosporium* sp., were exposed to Antarctic environment for a period of 14 months by Dayal *et al.*[14], in order to study their viability, growth rate and virulence, but no major variation in activity was observed. In subsequent years, steady increase in summer temperatures and concomitant glaciological changes resulted in further exposure of soils in Antarctica and warranted continued studies on the life of the region.

The authors had an opportunity to examine the fungal diversity of soils of Schirmacher Oasis based on samples collected during a recent expedition by one of the authors.

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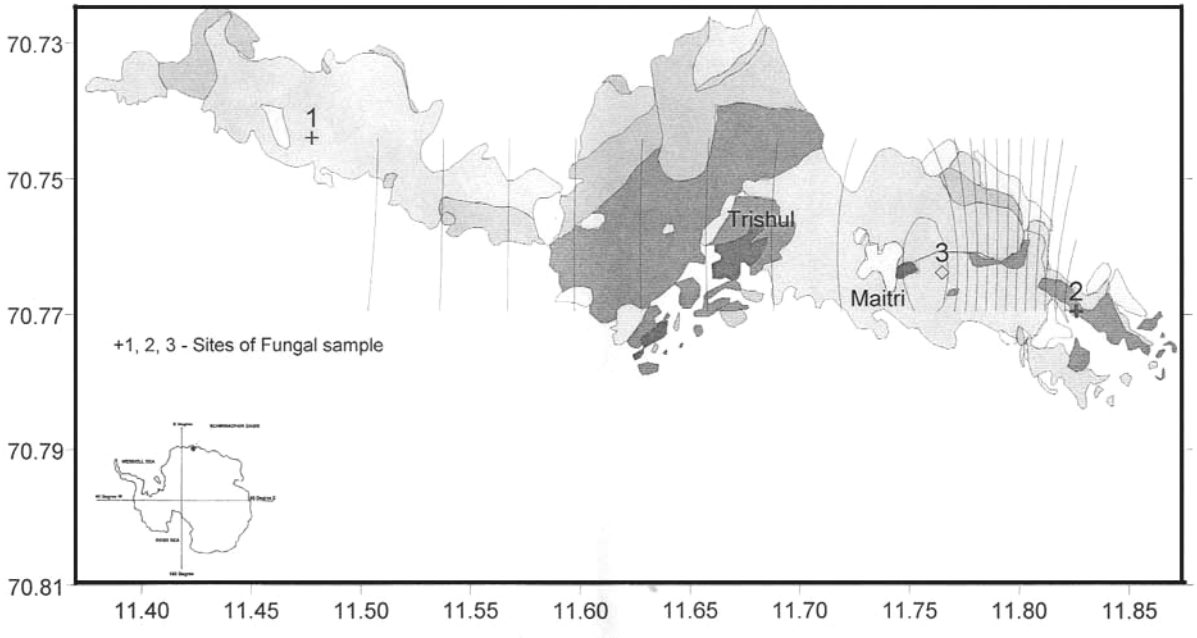

Figure 1. Soil sample collection sites in Schirmacher Oasis[24].

Table 1. GPS locations and characteristics of soil sample used for assessment of fungal diversity in Schirmacher Oasis

Sample no.	Date of collection	GPS location	Altitude (m)	Depth of collection (cm)	Soil temperature (°C)	pH
1	17.01.2004	70°44′38.3″ 11°28′41.7″	72	5	0	5.75
2	30.01.2004	70°45′49.8″ 11°45′55.2″	92	5	+1	5.27
3	03.02.2004	70°46′10.5″ 11°49′34.7″	112	5	+1	5.96

Schirmacher Oasis (70°46′04″–70°44′21″S; 11°49′54″–11°26′03″E), located approximately 70 km south of Princes Astrid coast, consists of a number of rocky hills and valleys that extend from Dronning Moud land (Figure 1). Its elevation varies from 0 to 236 m asl. The oasis, with width of 3 km and a length of 20 km, is oriented in an east-west direction. The northeastern and northwestern corners of the area are on ice-shelf, while the southwestern extremity is on polar ice-sheet. The southeastern end lies on a rocky outcrop. The area has three types of lakes, viz. proglacial lake, land-locked lake and epi-shelf lake.

The average annual temperature is –10°C and mean wind velocity is about 10 ms^{-1}. The average precipitation (snow) ranges between 250 and 300 mm and relative humidity is 15–20%. Air temperature ranges between –5°C and +9.2°C during summer months and between –12 and –33°C during winter. According to India Meteorological Department (IMD), the highest temperature of the year was 9.2°C during the polar summer on 30–31 December 2003 and the lowest temperature of the year was –33°C during polar winter on 24 July 2003. During summer, the polar ice melts and water often flows into the lakes.

The valleys are ice-free because the mountains block the flow of ice from the polar plateau and low precipitation and strong winds lead to little accumulation of snow in the area. The lakes occupy closed basins and vary in surface area, depth and ice-cover thickness. The observations presented here were derived from 3 GPS locations (Table 1) of Schirmacher Oasis, Dronning Moud land, East Antarctica.

The soil samples were collected by Corer, during 17 January to 3 February 2004 from different GPS locations. The temperature of the samples measured at the site was 0 to +1°C. However, it may range on the day of observation between –2 and +2°C. The samples were packed in sterile plastic bags and maintained at –40°C using dry ice during transportation. They were later stored in the cold laboratory of National Centre for Antarctic and Ocean Research, Goa until study.

Isolation of soil fungi was carried out following the method proposed by Waksman[15]. One gram of soil sample was suspended in 100 ml of sterile distilled water and serially diluted to 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions. About 0.5 ml of each aliquot was spread evenly on surface of 2% MEA

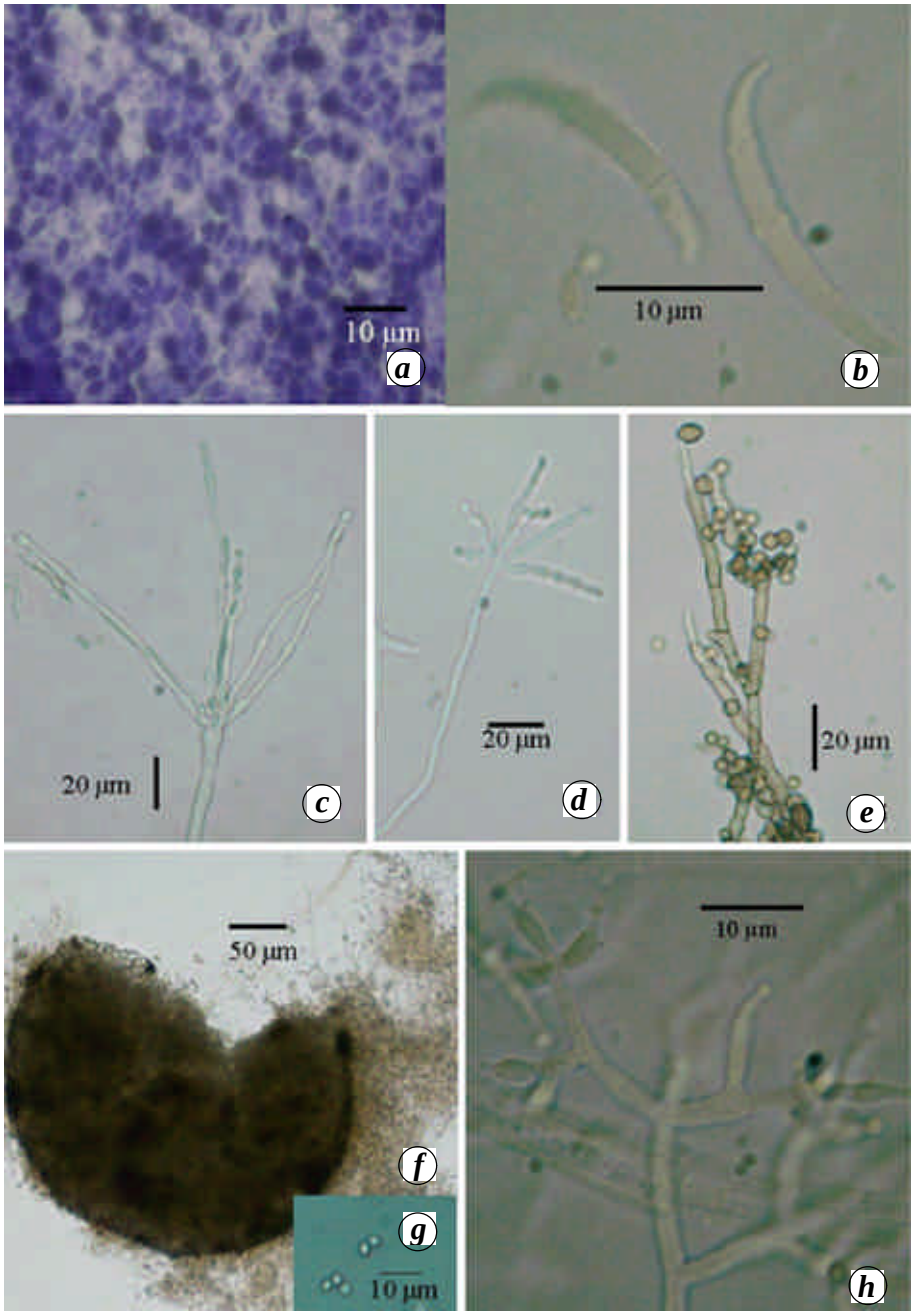

Figure 2. ***a***, *Torulopsis psychrophila*; ***b***, *Fusarium* sp., ***c***, ***d***, *Aspergillus* sp.; ***e***, *Cladosporium* sp.; ***f***, Undetermined pycnidal taxon; ***g***, Spores of ***f***; ***h***, *Trichoderma* sp.

(malt extract agar) medium with 5.5 pH (soil pH recorded at the collecting site) embedded with a cocktail of antibiotics. Each dilution was plated in triplicates. The plates were sealed and incubated at 2–5°C, a temperature range recorded at study sites for about 45 days before isolation. The method was followed for all the samples studied. The recovered fungi were identified down to generic/species level based on morphological characters and following the standard literature[16–19]. Growth rate of the isolates was calculated in terms of mm/day, after centrally point-inoculating each of the cultures on 2% MEA plates and incubating at 2–5°C for 45 days[20].

In all, 16 fungal and one yeast isolate were recovered from the samples analysed. The results obtained are depicted in Table 2. A pictorial representation is given in Figure 2 *a–h*. While the plates were examined twice every week at regular intervals of 3–5 days from the first day of incubation, the first sign of fungal growth was visible only after 20 days. In general, all the fungi recovered were slow-growers. They appeared on culture plates almost simultaneously after three weeks of incubation.

Fungi belonging to genera *Acremonium*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Trichoderma* were represented in Antarctic soils. These are commonly known soil

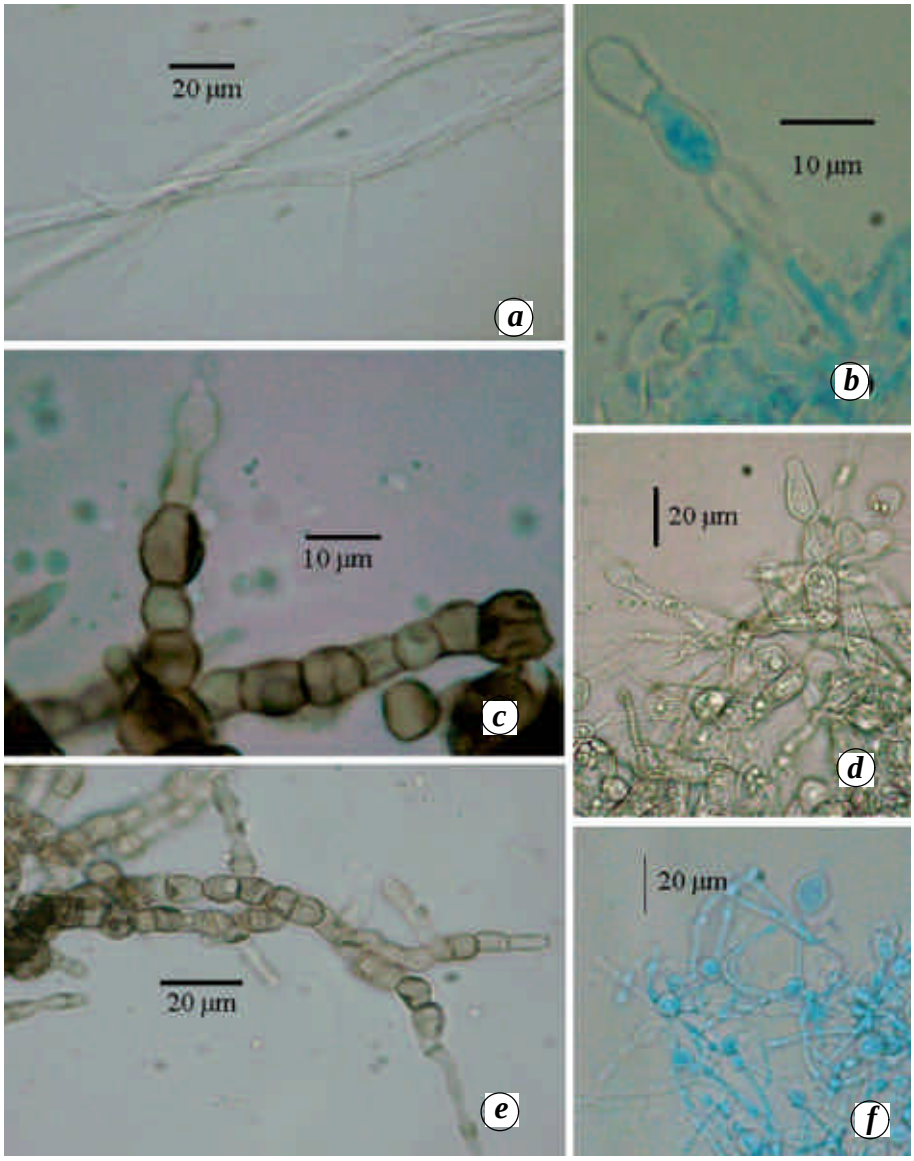

Figure 3. Modification of mycelia to overcome temperature stress in psychrophiles. ***a***, Mycelial cords; ***b***, Bulbous intercalary cells; ***c***, ***d***, Thick-walled swollen cells; ***e***, ***f***, Chlamydospores.

fungi. However, they differ from their mesophilic counterparts in having unique mycelial characters. The mycelia of most fungi recovered from the Antarctic soils were found to be unique in that they consistently had abundant intercalary, swollen, thick-walled cells that sometimes formed conspicuous mycelial cords besides chlamydospores (Figure 3 *a–f*). Chlamydospores in the fungi are generally considered as dormant resting spores, a makeshift adaptation to tide over unfavourable growth conditions. It is possible that Antarctic fungi in general possess these features as an adaptation to survive in extreme low temperatures that prevail in the polar region.

Though modified subsequently by many workers, the dilution plate count technique of Waksman[15] is one of the widely used and accepted methods available to enumerate culturable microorganisms in the soil. The technique is based on the principle that complete detachment and dispersion of cells from the soil would give rise to discrete colonies when incubated on a petri plate containing nutrient medium and further aid in the estimation of microbial biodiversity.

Psychrophiles have unique adaptation in polar regions[21]. Due to harsh climatic conditions, many fungal species are represented as resting spores. Since polar fungi show phenotypic plasticity, it is difficult to identify them using light microscopy. Besides, they are sensitive to temperature and pH changes and also to the nature of culture media used[22]. The community structure of Antarctic soil microbes can be assessed completely by new molecular techniques that focus on the genetic material of the organism as a means

Table 2. Fungi isolated from soils of Schirmacher Oasis, East Antarctica

Fungal species	Sample 1	Sample 2	Sample 3	Growth rate (mm/day)
Acremonium sp. 1	+	+	–	1.044
Acremonium sp. 2	–	+	–	0.844
Aspergillus sp.	–	+	–	–
Cladosporium sp. 1	+	+	+	0.711
Cladosporium sp. 2	+	–	–	–
Fusarium sp. 1	–	+	–	0.933
Fusarium sp. 2	+	–	–	0.377
Non-sporulating sp. 1	+	–	–	0.8
Non-sporulating sp. 2	+	–	–	–
Penicillium antarcticum	–	+	+	0.311
Torulopsis psychrophila (yeast)	+	+	+	0.177
Trichoderma sp.	–	+	–	–
Undetermined pycnidial taxon	+	–	–	–
Undetermined hyphomycete sp. 1	–	–	+	–
Undetermined hyphomycete sp. 2	+	–	–	0.488
Undetermined hyphomycete sp. 3	+	–	–	0.888
Undetermined hyphomycete sp. 4	+	–	–	0.644

of identification, so that the entire community diversity, including unculturable organisms can be analysed and described[23].

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