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ISOLATION AND IDENTIFICATION OF FILAMENTOUS FUNGI FROM ISLAND LIVINGSTON, ANTARCTICA

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ABSTRACT

Over the last decades, the Antarctic regions have been investigated mainly for the presence and exploitation of psychrophilic bacteria and archaea, occasionally for algae and more rarely for fungi. The present study reports results concerning the isolation and identification of filamentous fungi from samples of soil taken from Livingston Island, South Shetland Archipelago, West Antarctica. Using conventional media and techniques, all collection sites yielded populations of filamentous fungi, belonging to the phylum Ascomycota (7 genera), Deuteromycota (2), Zygomycota (2) and Basidiomycota (1). Mucor, Cladosporium, Alternaria, Aspergillus and Penicillium were predominant genera. Lecanicillium, Botrytis, Geomyces, Monodictys and Rhizopus were the most frequently isolated genera. Most of the fungal isolates proved to be cold-tolerant

Keywords: Antarctica, cold-tolerance, filamentous fungi, identification, isolation

Introduction

The Antarctic continent offers a range of extreme climatic conditions and constitutes one of the harshest environments on Earth. It is one of the most suitable areas for the search of organisms adapted to low temperatures. The organisms living there (psychrophiles and psychrotolerants) present adaptations in their enzymatic systems, in their membranes and therefore in their genes that represent a great biotechnological potential. Over the last decades the Antarctic regions have been investigated mainly for the presence and exploitation of psychrophilic bacteria and archaea, occasionally for algae and more rarely for fungi (11, 4, 8). Only few species of fungi have been described from the region in the recent past, most of them being from the marine environment (10).

Livingston Island is part of the South Shetlands archipelago situated in Western Maritime Antarctica, 850 km south-southeast of Cape Horn across the Drake Passage. The local climate is typical of the peri-Antarctic islands: humid and windy, cool with an average temperature of 1-3 °C in the warmest month and -7 °C in the coldest month, and very few sunny days indeed. The mycological investigations on the Livingston Island started relatively late. Even now they are

very scarce. Different yeast strains from soil and moss samples were isolated and identified (6).

The aim of this study was to isolate and identify filamentous fungi from samples of soil taken from permanent Bulgarian Antarctic base "St. Kliment Ohridski" on Livingston Island, as well as to determine their temperature growth characteristics and some morphological peculiarities.

Materials and methods

Samples collection. During the Bulgarian Antarctic expedition 2006/07 4 soil samples were collected in the surrounding of the permanent permanent Bulgarian Antarctic base "St. Kliment Ohridski" on Livingston Island in the archipelago South Shetland Islands, West Antarctica (62° 38 '29 "S, 60° 21' 53" W).

Isolation of fungi. Cooke Rose Bengal agar was chosen as growth medium. Soil dilution and soil sprinkle plates were used as isolation techniques. Soil dilutions were made by suspending 1 g of each soil sample in 50 ml of sterile distilled water. These suspensions were stirred for 20 min before making 7-fold falling dilutions and distributing 0.1-ml aliquots onto the medium in the plates. Sprinkle plates were prepared by uniformly distributing the soil directly on the surface of the medium. The plates were incubated at 4, 10 and 25°C for up to 3 weeks. Fungi growing on the agar plates were transferred by subculturing from hyphal tips, colonies or

spores to fresh beer agar.

Identification of fungi. The isolated fungi were identified to the genus level and to the species when possible on the basis of macromorphological and micromorphological characteristics using suitable media, slide cultures (obtained by inoculating microfungi directly on a small square of agar medium) and the most updated keys for identifications.

Temperature preferences. Temperature tolerance by cultivation of the isolated fungi was determined on Sabouraud agar (10 cm diameter Petri dishes) at 0, 4, 10, 12, 15, 20, 25, 30 and 35°C. The diameter of the growing colony was measured crosswise in two directions in 7-day old cultures. The average of these two readings was taken as diameter of the colony. The diameter of each fungal colony was the average of 5 replicates.

Macroscopic study. Colonies of Antarctic fungi were cultivated on Sabouraud agar at corresponding isolated temperature for 7 days. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc.

Microscopic study. Fungal spores were cultivated on Sabouraud medium in glass microchamber (3) at isolated temperature for 6 days. The germination and growth of mycelium was observed daily under a light microscope Docuval (Carl Zeiss, Germany).

Results and Discussion

Isolation and identification of fungi

A total of 31 isolates were obtained from the analyses of 4 soil samples taken from the area of the Livingston Island, Antarctica by the Bulgarian expedition (January and February 2007) through soil dilution and soil sprinkle plates techniques. Eight strains were isolated from agar plates incubated at 4°C, 8 from plates incubated at 10°C and 15 from plates incubated at 25°. All fungal isolates were obtained in pure cultures by single conidial transfer onto beer agar plates.

The isolates from Antarctic samples were identified as filamentous fungi belonging to the phyla *Deuteromycota* (7 genera), *Ascomycota* (2), *Zygomycota* (2) (only *Rhizopus* is listed in the table) and *Basidiomycota* (1). Most of the total number of isolates were identified to the genus level. The most frequently isolated species belong to the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Geomyces*, and *Lecanicillium*. All of these species have been reported as commonly isolated from Antarctic soil by other authors (1, 5). A total of 14 different strains were identified to specific level and are reported in Table 1.

TABLE 1.

Identified fungal strains and isolation temperature

№	Isolation T (°C)	Species	№	Isolation T (°C)	Species
1.	4	<i>Cladosporium oxysporum</i> Berk. & M.A. Curtis	8.	10	<i>Alternaria alternata</i> group
2.	4	<i>Monodictys austrina</i> Tubaki	9.	10	<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries
3.	4	<i>Cladosporium herbarum</i> (Pers.) Link	10.	10	<i>Cladosporium cladosporioides</i>
4.	4	<i>Lecanicillium muscarium</i> (Petch) Zare & W. Gams	11.	10	<i>Rhizopus stolonifer</i> (Ehrenb.:Fr.) Vuill.
5.	10	<i>Cladosporium oxysporum</i>	12.	25	<i>Epicoccum nigrum</i> Link
6.	10	<i>Geomyces pannorum</i> (Link) Sigler & J.W. Carmich.	13.	25	<i>Aspergillus glaucus</i> (L.) Link
7.	10	<i>Penicillium italicum</i> Wehmer	14.	25	<i>Cadophora fastigiata</i> Lagerb. & Melin

Temperature effects on fungal growth

Variation in growth among isolates was observed when they

were cultivated at temperatures between 4 and 35°C on agar medium and under submerged conditions.

According to the temperature range for growth, the fungal isolates can be classified as extremely psychrophilic, psychrotrophic or mesophilic types (9)(Table 2). Tested psychrophilic strains grew at temperatures between 8 - 25°C, with an optimum growth temperature of 15°C. Only the strain *Cladosporium herbarum* B14-2 grew and formed spores at 4°C. The psychrotrophic (psychrotolerant) strains were able to grow both at low (8°C) and at middle temperatures with a optimum temperature of 25°C. The appropriate temperature range for the mesophiles was 15 - 35°C, while the optimum growth temperature was 28-30°C.

The temperature-growth curves of a psychrophilic, a psychrotolerant, and a mesophilic strain were plotted in Fig. 1 and compared. While the psychrophilic strain *C. oxysporum* started to grow at 4°C, the psychrotolerant *Penicillium* sp. and the mesophilic *A. glaucus* began to form mycelium at 10 and 18°C, respectively.

TABLE 2.

Arrangement of isolated strains according to their range and optimal temperature for growth

Sample №	Isolated strains (number)		
	psychrophiles	psychrotrophs	mesophiles
5	3	2	6
6	2	3	3
7	-	1	-
8	3	2	6
Total	8	8	15

Moreover, the psychrophilic fungus demonstrated maximum radial growth in wide temperature range between 12 and 20°C. At the same time, optimal temperature regimes for colony formation of the psychrotrophic and mesophilic strains were found at 25°C and 30°C, respectively. The results from growth under submerged cultivation in bioreactors confirmed temperature characteristics of the model strains (data not shown).

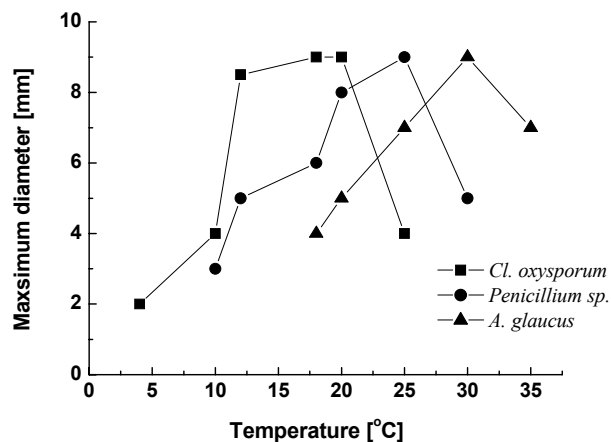


Fig. 1. Effect of temperature on the radial growth of three strains Antarctic (psychrophilic, psychrotolerant and mesophilic) on beer agar

Colony morphology of Antarctic isolates

Figure 2 shows some morphological peculiarities of the isolated Antarctic fungi. Macroscopic studies demonstrated that melanized fungi are the dominating species in this extreme environment (Fig. 2 A, B), suggesting the beneficial role of the melanin in their survival under extreme conditions.

Melanin has been referred to as “fungal armor” due to the ability of the polymer to protect microorganisms against a broad range of stress factors including low temperatures and UV-radiation (7). Many of the tested strains showed colonies with concentric rings of thinner and denser growth (Fig. 2 A, B, C) or rings with conidia and rings of sterile mycelium. In some cases the surface of the colony was uneven with many irregular convolute wrinkles and deep radial furrows (Fig. 2 D, E F). Cultivation under stress conditions could lead to ring extension of the colony as multicellular meristematic clusters. This type of growth ensures an optimal surface to volume ratio for the colonies, a character that was supported to improve the survival ability under stressful conditions (8).

Microscopic studies showed unique mycelial characters of Antarctic strains isolated at 4°C. As it can be seen in Fig. 3, the mycelium of *Penicillium* sp. 1-6-4 contains abundant intercalary, swollen, thick-walled cells (Fig. 3A) that formed chlamydospores (Fig. 3B). Chlamydospores in the fungi are generally considered as dormant resting spores, a makeshift adaptation to tide over unfavorable growth conditions (10). 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 (2).

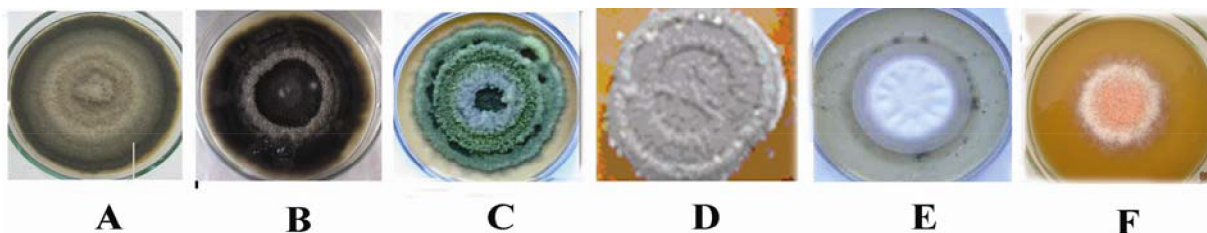


Fig. 2. Morphological characteristic of Antarctic fungi

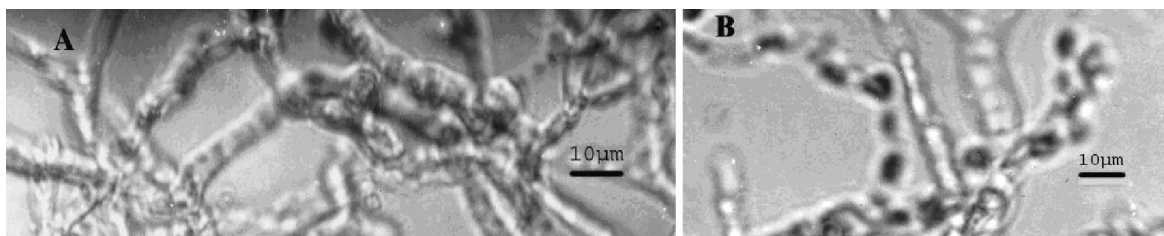


Fig. 3. Modification of mycelia to overcome cold temperature in psychrophilic isolate 1-6-8 (A) Intercalary cells; (B) Chlamydospores.

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