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Article

Fungal Diversity in the Inhovde Area, East Antarctica

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Abstract: Antarctica is one of the harshest environments on the planet. Despite this, fungi have been reported in the area for over 150 years. However, no fungi had been reported from the Inhovde area in East Antarctica until now. In this study, we investigated the fungal diversity in the Inhovde area and examined the growth of these fungi at -3°C. We isolated 148 fungal strains from ice and snow samples from the Inhovde area, of which 132 were successfully extracted and analyzed by DNA sequencing. Based on the internal transcribed spacer (ITS) region and 26S rDNA D1/D2 domain sequence similarity, these strains were classified into 6 genera and 10 species of basidiomycetes and 4 genera and 4 species of ascomycetes. A representative strain from each species was selected and evaluated for its ability to grow at -3°C in amino acid-free and vitamin-free media. All strains investigated in this study were able to grow at -3°C in both amino acid-free and vitamin-free media. These results indicate that the fungi in the Inhovde area are well adapted to the oligotrophic environment of Antarctica and play an important role in nutrient cycling at near-freezing temperatures in this area.

Keywords: East Antarctica; fungal diversity; inhovde area

1. Introduction

Antarctica, the southernmost landmass on Earth, covers an area of approximately 14 million km², making it the fifth largest continent in the world. It is subjected to extremely cold and dry conditions, with the lowest recorded temperature being -90°C. Approximately 98% of Antarctica is covered by ice and snow, with coastal temperatures typically ranging from 5°C to -35°C [1]. Ice- and snow-free areas, present during the austral summer, are located around the continent's coast. Most life forms in continental Antarctica are known to inhabit these ice-free and snow-free areas [2].

The first record of fungi from this region was published around 1897-1899 by a Belgian expedition that collected *Sclerotium antarcticum* from Danco Island near the Antarctic Peninsula [3]. This expedition included the late Roald Amundsen (1872–1928), who was the first to reach the South Pole.

Syowa Station, the base for the Japanese Antarctic Research Expedition (JARE), is located on East Ongul Island (69° 1' S, 39° 35' E). The first report on fungi around Syowa Station was published in 1961 [4], followed by three articles reporting 12 ascomycetes and four basidiomycetes [5–7]. From the 1960s to 2013, no additional fungal species were reported by JARE near Syowa Station. Since 2013, fungal diversity has been reported from two sites: East Ongul Island, where Syowa Station is located, and the Skarvsnes ice-free area, about 60 km away from the station [8,9]. To date, these fungal surveys have reported a total of 77 fungal species, including 61 ascomycetes and 16 basidiomycetes [10].

The Inhovde area (69°51'S, 37°06'W) is located between East Ongul Island and the Sør Rondane mountains. Despite previous fungal surveys near Syowa Station, the fungal diversity of the Inhovde area has been completely unexplored.

Fungi serve as decomposers of organic matter in ecosystems, supporting nutrient cycling. Therefore, understanding the diversity of fungi inhabiting Antarctica, which is experiencing rapid environmental changes, is crucial for research aimed at preserving the Antarctic environment [11].

In this study, the diversity of fungi in the Inhovde area is reported for the first time, and the growth of these fungi at low temperatures is also investigated.

2. Materials and Methods

2.1. Sampling Site and Sample Collection

The Inhovde area (69°51'S, 37°06'W) is located in the Lützow Holm Bay, East Antarctica. This area is about 130 km from Syowa Station, beyond the Shirase Glacier (Figure 1).



Figure 1. Map of the location of the Inhovde area.

Ice and snow samples used in this study were collected from Inhovde area during the 56th JARE. The samples were stored at -20 °C immediately after collection until use.

2.2. Isolation of Fungal Strains

Each untreated sample (0.1 g) was plated directly on 5 × potato dextrose agar (PDA, Difco, Becton Dickinson, Tokyo, Japan) at pH8.0 containing 50 µg/mL chloramphenicol and incubated at 10 °C for up to 1 month. Fungal cultures were selected for further isolation and identification based on colony morphology and color, and fungi from colonies of different morphology and color were purified by repeated cultivation on fresh PDA at 10 °C.

2.3. Sequencing and Species Identification

DNA was extracted from fungal colonies using the NucleoSpin Microbial DNA kit (Takara Bio Inc., Shiga, Japan) following the manufacturer's protocols. The extracted DNA was then amplified by polymerase chain reaction (PCR) using KOD-plus DNA polymerase (Toyobo, Osaka, Japan) with the primers ITS1F (5'-GTAACAAGGTTTCCGT) and NL4 (5'-GGTCCGTGTTTCAAGACGG). These primers target two DNA sequences commonly utilized in molecular fungal taxonomy: the ITS region and the D1/D2 domain of the LSU rDNA gene.

The PCR conditions were as follows: initial denaturation at 94 °C for 5 minutes; 35 cycles of 98 °C for 10 seconds (denaturation), 50-65 °C for 30 seconds (primer annealing), and 68 °C for 90 seconds (extension); followed by a final extension at 68 °C for 10 minutes. PCR was performed using an Eppendorf Master Cycler Nexus (Eppendorf Japan, Tokyo, Japan). The amplified DNA fragments were purified using Sephadryl S-400HR (Sigma-Aldrich, Tokyo, Japan). DNA sequencing was

performed on an ABI Prism 3130xl sequencer (Applied Biosystems, Life Technologies, Tokyo, Japan), and the sequences were deposited in GenBank. The GenBank accession numbers for all sequences analyzed in this study are listed in Supplementary Table S1. Fungal species identifications were conducted through BLAST analysis based on sequence homology.

The ITS region and D1/D2 domain sequences of *Cystobasidium* sp. were aligned using CLUSTAL W (<http://clustalw.ddbj.nig.ac.jp/>) and manually adjusted. The aligned sequences were used for phylogenetic reconstruction analysis by the neighbor-joining method in MEGA X [12]. Bootstrap analysis with 1000 replicates was performed to determine the confidence of the tree nodes.

2.4. Determination of Growth Abilities of Fungal Strains in the Inhovde Area

Fungal growth tests at -3°C were conducted on PDA plates. Vitamin dependence of growth was tested in liquid medium in glass vials according to standard methods [13] at 10°C. The amino acid requirement test was performed in liquid medium containing yeast nitrogen base without amino acids (YNB, Difco, Becton Dickinson, Tokyo, Japan) and 2% glucose at 10°C.

3. Results

3.1. Fungal Diversity of the Inhovde Area

In the current study, 148 fungal strains were isolated from ice and snow samples from the Inhovde area, of which 132 were successfully extracted and analyzed by DNA sequencing. Note that some strains were not identified to the species level in this study. When classification to the species level was not possible, the strains are presented at the genus or family level.

Based on the sequences of the ITS region and D1/D2 domain of the LSU rDNA gene, the strains were classified as basidiomycetes, belonging to six genera and 10 species: *Cystobasidium ongulense*, *Cystobasidium* sp., *Glaciozyma watsonii*, *Leucosporidium yakuticum*, *Mrakia gelida*, *Mrakia robertii*, *Naganishia adeliensis*, *Naganishia albidosimilis*, *Naganishia friedmannii*, and *Vishniacozyma victoriae*; and as ascomycetes, belonging to four genera and four species: *Aotearoamycetes* sp., *Leuconeurospora* sp. (yeast-like), *Pseudeurotium hygrophilum*, and *Symbiotaphrina microtheca*(yeast-like). At the species level, the most frequently isolated fungi were *V. victoriae* (35.6%), *G. watsonii* (28.0%), and *C. ongulense* (15.2%). All species classified as basidiomycetes were in yeast form. Two of the four ascomycetous fungal species (*Leuconeurospora* sp. and *Symbiotaphrina microtheca*) exhibited yeast-like morphology.

Table 1. Number of strains isolated from Inhovde area.

Speices	Number of strains
Ascomycota	
<i>Aotearoamycetes</i> sp.	3
<i>Leuconeurospora</i> sp.	1
<i>Pseudeurotium hygrophilum</i>	1
<i>Symbiotaphrina microtheca</i>	1
Basidiomycota	
<i>Cystobasidium ongulense</i>	20
<i>Cystobasidium</i> sp.	2
<i>Glaciozyma watsonii</i>	37
<i>Leucosporidium yakuticum</i>	1
<i>Mrakia gelida</i>	5
<i>Mrakia robertii</i>	9
<i>Naganishia adeliensis</i>	1
<i>Naganishia albidosimilis</i>	1
<i>Naganishia friedmannii</i>	4
<i>Vishniacozyma. victoriae</i>	46
Total	132

3.2. Growth Characteristics of Fungi in the Inhovde Area

In this study, one strain from each species was randomly selected. These selected strains were used as representative strains for each species in subsequent experiments.

To evaluate the growth characteristics of the selected fungal species at sub-zero temperatures, their ability to grow on PDA plates at -3 °C was examined. Additionally, their growth in vitamin-free and amino acid-free media at 10 °C was tested to assess their ability to thrive in oligotrophic environments. All 14 species were able to grow at -3 °C, as well as in vitamin-free and amino acid-free media (Table 2).

Table 2. Growth ability of fungal strains isolated from Inhovde area.

species	Growth at -3 °C	Growth in vitamin-free medium	Growth in amino acids- free medium
Ascomycota			
<i>Aotearoamyces</i> sp.	w	+	+
<i>Leuconeurospora</i> sp.	+	+	+
<i>Pseudeurotium hygrophilum</i>	+	+	+
<i>Symbiotaphrina microtheca</i>	w	+	+
Basidiomycota			
<i>Cystobasidium ongulense</i>	+	+	+
<i>Cystobasidium</i> sp.	+	+	+
<i>Glaciozyma watsonii</i>	+	+	+
<i>Leucosporidium yakuticum</i>	+	+	+
<i>Mrakia gelida</i>	+	+	+
<i>Mrakia robertii</i>	+	+	+
<i>Naganishia adeliensis</i>	+	+	+
<i>Naganishia albidosimilis</i>	+	+	+
<i>Naganishia friedmannii</i>	+	+	+
<i>Vishniacozyma. victoriae</i>	+	+	+

4. Discussion

In the present study, 46 strains of *Vishniacozyma victoriae* were isolated. *V. victoriae* is a cosmopolitan yeast species. This yeast has been reported from various cold environments around the world including the Canadian High Arctic [13]. In Antarctica, this yeast had also been identified from Victoria Land, Lichen Valley, Vestfold Hills, Davis Base, the Skarvsnes ice-free area and East Ongul Island [8,9,15–17].

In this study, 37 strains of *Glaciozyma watsonii* were isolated from the samples. *G. watsonii* was identified from meltwater stream sediment, glacial meltwater stream sediment and seawater [18–20]. The genus *Glaciozyma* is a fungus with a remarkable ability to adapt to cold temperatures, being able to grow even on PDA media frozen at -80°C (Tsuiji unpublished data).

Cystobasidium ongulense strains were originally isolated from the soil of East Ongul Island, East Antarctica [21]. This yeast species has also been reported from Italian Alps [22]. In the present study, 19 strains of *Cystobasidium ongulense* were isolated from samples collected from Inhovde area. Three strains, *Cystobasidium* sp. NIPR00539, NIPR00543 and 056-1-20Y-2, were also closely related to *C. ongulense* but had two nucleotide substitutions in the ITS region and four in the D1/D2 region compared to the type strain, JCM 31527.

Phylogenetic analysis using sequences of the ITS and D1/D2 regions indicated that NIPR00539, NIPR00543 and 056-1-20Y-2 branched off from JCM 31527 (Figure 2). This suggests that the three strains are new species closely related to *C. ongulense*.

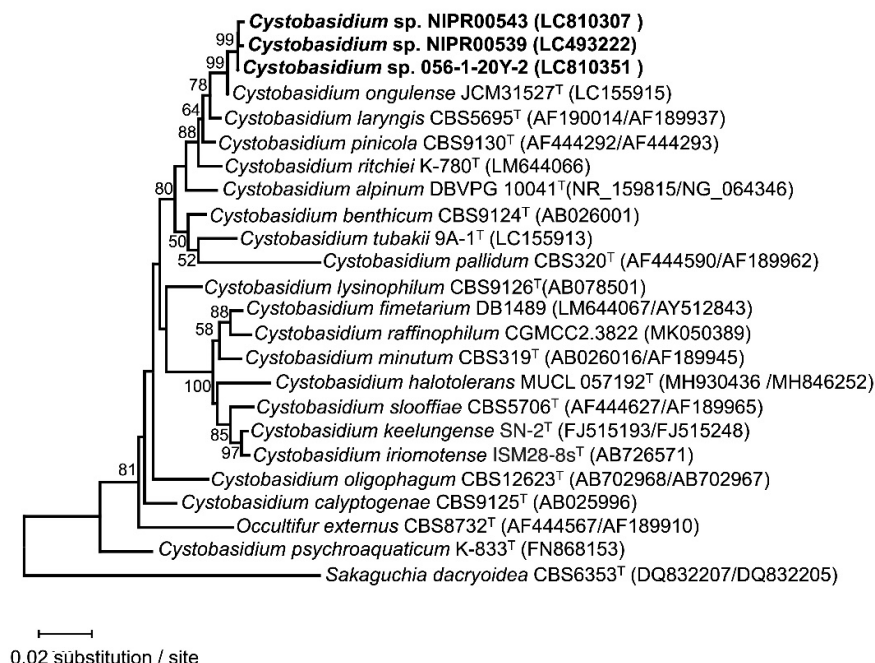


Figure 2. Phylogenetic tree based on the ITS region and the LSU D1/D2 domain.

di Menna (1966) reported that approximately 24% of the culturable yeasts from Antarctic soil were *Mrakia* spp. [23]. In this study, five *Mrakia gelida* and nine *M. robertii* strains were isolated from samples from the Inhovde area. *M. gelida* is one of the most commonly isolated yeasts from cold environments worldwide [24]. In Antarctica, this species has been isolated from soil at Scott Base and from lake sediment in the ice-free area of Skarvsnes, East Antarctica [8,9,22]. *M. robertii* was first reported from Mossell Lake, Antarctica, and the Italian Alps [26]. This yeast has also been reported from the Antarctic Peninsula and East Ongul Island. [9,26].

The genus *Naganishia* has been found in a variety of ecosystems, including marine waters, glaciers, soil, and Antarctica [27–30]. In this study, one strain of *Naganishia adeliensis*, one strain of *N. albidosimilis*, and four strains of *N. friedmannii* were isolated. There are also reports that *N. friedmannii* has a high biosurfactant production capacity in Antarctica. [31].

A strain of *Leucosporidium yakuticum* was obtained in this study. The original paper reported that this yeast was found in permafrost in Russia [32]. To the best of our knowledge, this is the second report of the fungus from Antarctica, following a report from the South Shetland Islands [16], and the first from continental Antarctica.

Neighbor-join analysis of the ITS region and the LSU D1/D2 domain sequences of *Cystobasidium* sp. strains and closely related species. *Cystobasidium* sp. strains investigated in this study are highlighted in bold font. *Sakaguchia dacryoidea* CBS 6353 was designated as the outgroup. Bootstrap percentages of neighbor-join analysis over 50% from 1000 bootstrap replicates are shown from left on the branches. The scale bar represents 0.02 substitutions per nucleotide position.

The genus is one genus in a species, *Aotearoamycetes nothofagi*, which is an ascomycetous fungus found in the forests of the Notochaetidae family in New Zealand.[34]. *Aotearoamycetes* sp.cultured in this study showed low homology (94.9% in the ITS region and 97.3% in the D1/D2 region) with the type strain of *A. nothofagi*. This suggests that *Aotearoamycetes* sp. isolated from the Inhovde region is a new species.

The genus *Leuconeurospora* is reported to have been isolated from King George Island, Antarctica [17]. To the best of our knowledge, this is the first report of this fungus from continental Antarctica.

Pseudeurotium hygrophilum has been reported to be isolated from sediment cores from Hope Bay, Antarctic Peninsula and from Lake Baikal, Russia [34,35]. In the present study, a strain of *Pseudeurotium hygrophilum* was isolated from samples from the Inhovde area. *Symbiotaphrina microtheca*

is also known as *Sarea microtheca* and *Tromeropsis microtheca*. Information on this fungus is limited but is probably the first report from continental Antarctica.

In this study, all representative strains indeed showed growth in vitamin-free media, amino acid-free media and at subzero temperatures. In general, continental Antarctica is characterized by an oligotrophic environment [21]. Considering this and the current observations, the fungi inhabiting the area may have acquired their growth characteristics to survive cold and oligotrophic environments such as that of the Inhovde area.

5. Conclusions

Of the 14 fungal species obtained in this study, the following six (two basidiomycetes and four conidiomycetes) were newly reported from the Syowa Station area: *Cystobasidium* sp., *Leucosporidium yakuticum*, *Aotearoamycetes* sp., *Leuconeurospora* sp., *Pseudeurotium hygrophilum*, *Symbiotaphrina microtheca*. So far, 61 species of ascomycetous fungi and 16 species of basidiomycetous fungi have been reported from near the Showa Station area [36]. The results of the present study increased the total number of fungal species isolated from the Showa Station area to 83, of which 65 were ascomycetes and 18 were basidiomycetes. These results indicate that these fungi play an important role in nutrient cycling in the ecosystem of the Inhovde area at near-freezing temperatures.

In recent years, fungi inhabiting Antarctica have garnered attention as new microbial resources, due to their psychrophilic characteristics, including cold-active enzymes and secondary metabolites. The fungi isolated from the Inhovde area in this study also hold potential as new microbial resources that could contribute to achieving carbon neutrality.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Species names, strain names and GenBank accession numbers of the ITS region and D1/Dr2 domain sequences of fungal isolates from Inhovde area.

Author Contributions: Conceived and designed the experiments: MT. Performed the laboratory experiments and analyses: MT. Analyzed the data: all authors. Wrote the manuscript: MT with input from all authors.

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Data Availability Statement: The DNA sequences of Antarctic fungi obtained in this study have been deposited in the DNA Data Bank, and the list of accession numbers of the registered DNA sequences can be found in Table S1.

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Conflicts of Interest: The authors declare no conflicts of interest.

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