Identification and enzymatic activities of psychrophilic yeasts isolated from permafrost soil in Mongolia

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Abstract. Psychrophilic yeasts with unique properties have recently become a new source of biologically active products. However, psychrophilic microorganisms, including yeasts that inhabit Mongolian permafrost, are practically unknown. Therefore, this study was the first attempt to reveal living organisms hidden in Mongolian permafrost, in particular yeasts, by isolating and identifying yeast strains, investigating their physiological characteristics and the ability to produce extracellular enzymes. Fifteen strains were isolated at 0°C from soil samples collected from three different permafrost sites in Mongolia. The strains were identified by the D1 / D2 domain sequences of the LSU rRNA gene, and as a result, 6 strains belong to the genus Cystofilobasidium, 7 and 2 strains belong to the genera Naganishia and Vishniacozyma, respectively. Testing the effect of temperature on the growth of the 15 strains showed that all of them were able to grow at 0, 4 and 15°C, 6 strains were able to grow at 28°C, and none of them were able to grow at 37°C. In addition, the extracellular enzyme activity of all strains was determined, 10 strains exhibited lipolytic activity and 11 strains cellulolytic activity, respectively. These yeasts exhibited extracellular enzymatic activity at 0, 4, 15, and 28°C, indicating that they maintain active metabolism under permafrost conditions, which is of great importance both for studying cold-adapted enzymes and microbial activity in the degradation of the permafrost habitat.

Keywords: Cystofilobasidium ssp., enzyme activity, Naganishia ssp., psychrophilic-psychrotolerant yeasts, Vishniacozyma ssp.

1 Introduction

Psychrophilic or cold-loving microorganisms, having an optimal growth temperature of ≤15 °C, effectively colonize all permanently cold environments, from the ocean depths to high mountains and polar regions [1]. Some microorganisms are capable of growth at low temperature but grow optimally above 15°C and are referred to as facultative psychrophiles or psychrotolerants [2]. Almost 71% of the Earth’s surface is covered by oceans and 90% of this volume is below 5°C. The deep sea makes up the largest part of this low-temperature
environment, followed by snow (35%) and permafrost (24%) of the land surface, sea ice (13% of the Earth's surface), and glaciers (10% of the earth's surface). According to [3], permafrost is defined as lithosphere material (soil, sediment, or rock) that is permanently exposed to 0 °C temperature, is frozen for at least 2 years in a row, and can reach depths of more than 1000 m. Both high latitudes and high elevations have permafrost sections; mountains make up a sizable portion of the world's permafrost. Approximately 20 to 70% of the soil under permafrost is made up of ice and only 1% to 7% is not frozen. These salt solutions have low water activity (Aw = 0.8–0.85) [4].

Both prokaryotic and eukaryotic psychrophilic microorganisms thrive well in cold environments, and among these, yeasts can adapt to low temperature better than bacteria [2]. In the Siberian permafrost soil with an estimated age of 3 million years, viable basidiomycetous yeasts were discovered in significant amounts (up to 9,000 cfu / g dry mass) and belonged to the genera Cryptococcus, Rhodotorula and Sporobolomyces [5, 6].

By creating extracellular hydrolytic enzymes, psychrophiles and psychrotolerant organisms have the ability to break down a variety of polymeric substances. These "cold-adapted" or "cold-active" enzymes are appealing for industrial processes requiring high enzymatic activity at low temperatures because they have higher catalytic efficiencies than their mesophilic counterparts at temperatures below 20 °C and exhibit unusual substrate specificities [7].

This study aimed to isolate cold-adapted yeast species from permafrost in Mongolia, the southern boundary of Siberian permafrost, identify them at the molecular level by analysing the D1/D2 sequences, and search for yeast isolates capable of secreting cold-active extracellular enzymes.

2 Materials and Methods

2.1 Sampling Sites and Sample Collection

Soil samples were collected from permafrost at depths of 5, 7, 9, and 10 metres in the permafrost regions of Mongolia, Uuyanga sum of Uvurkhangai province, Otgon sum of Zavkhan province and Galuut sum of Bayankhongor province, in 2017 (Table 1). The sampling was carried out by permafrost researchers from the Institute of Geography and Geoecology (IGG) of the Mongolian Academy of Sciences (MAS) and the samples were stored frozen until use.

2.2 Isolation of yeast from soil

To isolate yeast, 1 g of soil sample was suspended in 9 ml of distilled water (1/10), stirred for 30 min and 0.1 ml of the resulting suspension was further serially diluted. Then 0.1 mL of 1/100 and 1/1000 dilutions were spread on Petri plates on the surface containing potato dextrose agar (PDA, Difco, Becton–Dickinson, Tokyo, Japan) and yeast malt extract agar (YM) (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, 20 g/L agar) supplemented with chloramphenicol. The plates were incubated at 0°C, 4°C, and 15°C for 3 weeks. The yeast colonies obtained from these
cultures were subsequently cultured onto YM agar plates for purification. The yeast isolates obtained from all dilutions were examined microscopically and preserved in 20% glycerol at -80°C.

2.3 Identification of Yeast Isolates

Yeast isolates were identified based on the sequences of the D1/D2 domain of the large subunit (LSU) ribosomal RNA (rRNA) gene.

Genomic DNA extraction was carried out using the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific Inc., USA) according to the manufacturer’s instructions. Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region, 5.8S gene and the D1/D2 domain of the LSU rRNA gene was performed using the GoTaq® Green Master Mix (Promega Corp., Madison, WI, USA) and ITS5 (5'-GGAAGTAAAGTCGTAACAAGG-3') and NL4 (5'-GTCCGTGTTCAGACGG-3'), primers described by [8]. The thermal profile was 94 °C 2 min, followed by 30 cycles of 98 °C 10 sec, 56 °C 30 sec, 68 °C 1:30 min, and a final extension step at 4 °C for 5 min. PCR products were verified on 1% agarose gels and purified using the AccuPrep® PCR/Gel Purification Kit (Bioneer, Korea), and sent to Macrogen, Korea for commercial sequencing with primers, NL1 (5’ GCATATCAATAAGCGGAGGAAAAG 3’) and NL4, for the D1/D2 domain of the LSU rRNA gene.

For phylogenetic analysis, the D1/D2 domain sequences were aligned with closest reference sequences, obtained from a BLAST homology search on the NCBI website, using the CLUSTAL_X software and phylogenetic trees were constructed using the neighbor-joining method [9].

2.4 Determination of the Effect of Temperature on the Growth of Yeasts

The yeasts were tested for the ability to grow at different temperatures (0, 4, 15, 28, and 37 °C) on YM agar plates. The plates were inoculated with yeast cells grown for 24-48 h, and incubated at different temperatures. Growth was monitored visually on a daily basis for a week, and growth of plates incubated at 0 °C and 4 °C was monitored for 2 weeks [10].

2.5 Production of Hydrolytic Enzymes

Sorbitan monooleate (Tween 80) was used to detect lipase activity. Tween 80 medium agar consisted of 10 g/L peptone, 5 g/L NaCl, 0.1 g/L CaCl2.2H2O, 20 g/L agar, 10 mL (v/v) tween 20 or tween 80 [11].

Screening for cellulose activity was done on YM agar supplemented with 0.5% sodium carboxymethylcellulase and the detection was done by Congo red solution (0.2%) and destained with NaCl 1M [12, 13].

The diameter of each clearance zone was measured, and the enzyme activity was calculated according to the following formula:

\[ \text{Extracellular enzyme secretion ability} = \frac{\text{clear opaque zone diameter} - \text{colony diameter}}{\text{colony diameter}} \]
3 Results

3.1 Isolation of Yeast from Permafrost Soil

Culturable yeasts in permafrost soils in Mongolia ranged from $2 \times 10^2$ to $2 \times 10^3$ colony forming unit per gram (cfu/g) (Table 1). After incubation at 0, 4, and 15 °C, number of yeast colonies grown from a sample taken at a depth of 5 m from permafrost in the Uyanga sum of Uvurkhangai aimag was $2 \times 10^3$, $4 \times 10^2$, and $3 \times 10^2$, respectively.

Table 1. Permafrost sampling information and yeast abundance in each sampling sites.

<table>
<thead>
<tr>
<th>№</th>
<th>Sampling sites</th>
<th>Location</th>
<th>Soil depth (m)</th>
<th>Total yeast counts (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uvurkhangai province Uyanga sum</td>
<td>46°27'34.21&quot;N 102°16'36.39&quot;E</td>
<td>5</td>
<td>$2 \times 10^3$ 4 $10^2$ 3 $10^2$</td>
</tr>
<tr>
<td>2</td>
<td>Zavkhan province Otgon sum</td>
<td>47°12'36.20&quot;N 97°36'29.67&quot;E</td>
<td>7</td>
<td>$7 \times 10^2$ 2 $10^2$ 2 $10^2$</td>
</tr>
<tr>
<td>3</td>
<td>Zavkhan province Otgon sum</td>
<td>47°12'36.20&quot;N 97°36'29.67&quot;E</td>
<td>9</td>
<td>$2 \times 10^2$ 2 $10^2$ -</td>
</tr>
<tr>
<td>4</td>
<td>Bayankhongor province Galuut sum</td>
<td>46°42'1.43&quot;N 100° 8'34.07&quot;E</td>
<td>7</td>
<td>- - - -</td>
</tr>
<tr>
<td>5</td>
<td>Bayankhongor province Galuut sum</td>
<td>46°42'1.43&quot;N 100° 8'34.07&quot;E</td>
<td>10</td>
<td>- - - -</td>
</tr>
</tbody>
</table>

A sample taken at a depth of 7 m from permafrost in the Otgon sum of Zavkhan aimag yielded $7 \times 10^2$, $2 \times 10^2$, and $2 \times 10^2$ colonies at these temperatures, while a sample from a depth of 9 m yielded $2 \times 10^2$ colonies at 0 and 4 °C, and no growth was observed at 15 °C. Also, no yeast growth was observed in the samples collected in the Galuut sum of Bayankhongor aimag.

Fifteen were selected from yeast colonies grown at 0 °C on two different culture media based on colony characteristics such as texture, elevation, size and pigmentation for further study.

3.2 Identification of Yeast

A total of 15 yeast strains isolated at 0 °C were identified based on the D1/D2 domain of the 26S rRNA gene. The strains were basidiomycetous yeasts belonging to 3 genera and 6 species (Table 2). Using the CLUSTAL_X program, the D1/D2 domain sequences of the strains were compared with the most similar sequences obtained as a result of the BLAST search, as well as with the sequences of type species, and phylogenetic trees were constructed using the neighbor-joining method. The sequences of six strains U5-23, U5-32, U5-36, O7-34, O7-31, and O9-28 were 99.67-100% similar to the sequences of representatives of *Cystofilobasidium macerans* (Table 2) and grouped into clusters on the phylogenetic tree (Fig. 1A), which indicates that these strains belong to *Cystofilobasidium macerans*. 
Table 2. Yeast strains isolated from permafrost soils in Mongolia identified by LSU D1/D2 domain sequence comparison with the BLAST match with the NCBI GenBank database.

<table>
<thead>
<tr>
<th>№</th>
<th>Strain</th>
<th>Obtained GenBank accession No.</th>
<th>Top BLAST search results</th>
<th>Similarity %</th>
<th>Reference accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>U5-23</td>
<td>LC772161</td>
<td>Cystofilobasidium macerans</td>
<td>99.83</td>
<td>NG_059011</td>
</tr>
<tr>
<td>2</td>
<td>U5-24</td>
<td>LC772162</td>
<td>Naganishia adeliensis</td>
<td>100</td>
<td>KF891469</td>
</tr>
<tr>
<td>3</td>
<td>U5-25</td>
<td>LC772163</td>
<td>Vishniacozyma sp.</td>
<td>99.66</td>
<td>OP941491</td>
</tr>
<tr>
<td>4</td>
<td>U5-30</td>
<td>LC772164</td>
<td>Naganishia albidus</td>
<td>100</td>
<td>MW990007</td>
</tr>
<tr>
<td>5</td>
<td>U5-32</td>
<td>LC772165</td>
<td>Cystofilobasidium macerans</td>
<td>100</td>
<td>MF448255</td>
</tr>
<tr>
<td>6</td>
<td>U5-33</td>
<td>LC772166</td>
<td>Naganishia albidus</td>
<td>100</td>
<td>MW990007</td>
</tr>
<tr>
<td>7</td>
<td>U5-36</td>
<td>LC772167</td>
<td>Cystofilobasidium macerans</td>
<td>99.67</td>
<td>NG_059011</td>
</tr>
<tr>
<td>8</td>
<td>O7-26</td>
<td>LC772168</td>
<td>Naganishia adeliensis</td>
<td>100</td>
<td>MF462742</td>
</tr>
<tr>
<td>9</td>
<td>O7-27</td>
<td>LC772169</td>
<td>Naganishia albidosimilis</td>
<td>99.83</td>
<td>MF448297</td>
</tr>
<tr>
<td>10</td>
<td>O7-29</td>
<td>LC772170</td>
<td>Naganishia adeliensis</td>
<td>99.83</td>
<td>MF891469</td>
</tr>
<tr>
<td>11</td>
<td>O7-31</td>
<td>LC772171</td>
<td>Cystofilobasidium macerans</td>
<td>100</td>
<td>MF448255</td>
</tr>
<tr>
<td>12</td>
<td>O7-34</td>
<td>LC772172</td>
<td>Cystofilobasidium macerans</td>
<td>100</td>
<td>MF448281</td>
</tr>
<tr>
<td>13</td>
<td>O7-35</td>
<td>LC772173</td>
<td>Vishniacozyma victoriae</td>
<td>100</td>
<td>MN848499</td>
</tr>
<tr>
<td>14</td>
<td>O9-28</td>
<td>LC772174</td>
<td>Cystofilobasidium macerans</td>
<td>100</td>
<td>MF448255</td>
</tr>
<tr>
<td>15</td>
<td>O9-37</td>
<td>LC772175</td>
<td>Naganishia adeliensis</td>
<td>100</td>
<td>MF891469</td>
</tr>
</tbody>
</table>

The sequences of strains U5-24, O7-26, O7-29 and O9-37 were 99.83-100% similar to those of members of *Naganishia adeliensis*, and the sequences of strains U5-33 and U5-30 were 100% similar to those of members of *Naganishia albidus*, the sequence of strain O7-27 was 99.83% similar to that of *Naganishia albidosimilis* isolate lhWW59 (Table 2), and they were clustered with members of the corresponding species (Fig. 1B).

The resting 2 strains belonged to the genus *Vishniacozyma*, strain U5-25 belonged to *Vishniacozyma carnescens* and strain O7-35 belonged to *Vishniacozyma victoriae*, respectively (Table 2, Fig 2). The sequence of strain U5-25 was 99.66% and 99.32% similar to that of *Vishniacozyma* sp. isolate KBP_Y-6982 and *Vishniacozyma carnescens* CBS 973\(^1\) fell into one cluster with them (Fig. 1B).

3.3 Growth of Yeast Strains at Different Temperatures

The ability of yeast strains to grow at temperatures from 0 to 37 °C was tested on YM plates to assess their growth characteristics. After incubation for 7 days, all strains were able to grow at 4 and 15 °C. Then after 14 days, strains belonging to the genera *Naganishia* and *Vishniacozyma* showed from visible to strong growth at 0 °C, strong growth at 4, 15, and 20 °C, except *V. carnescens* U5-25, which showed visible growth at 20 °C. Strains belonging to the genus *Cystofilobasidium* showed from visible to strong growth at 4 and 15 °C, weak to strong growth at 0 °C, and less growth at 20 °C.
Identification and enzymatic activities of psychrophilic yeasts

A

Cystofilobasidium macerans lhWW1 (MF448255)
Cystofilobasidium macerans CBS 2206 (AF189848)

Cystofilobasidium macerans CBS 10757^T (NG_059011)
Cystofilobasidium lacus-mascardii CRUB 1046^T (AY158642)
Cystofilobasidium capitatum CBS 6358^T (AF075465)
Cystofilobasidium ferigula CBS 7202^T (KY107454)
Cystofilobasidium bisporidii CBS 6346^T (AF189832)
Cystofilobasidium infirmominlatum CBS323^T (AF075505)
Phaffia rhodozyma CBS590^T (AF189871)

Holtermanniella festucosus VKM Y-2930^T (AY462119)

B

Naganishia adeliensis A1 (KF891469)
Naganishia adeliensis CBS 8351^T (AF137603)

Naganishia albicus PEX-25 (MW990007)
Naganishia albicus CBS 142^T (AF075474)

Naganishia albidosimilis CBS 7711^T (AF137601)
Naganishia albidosimilis lhWW59 (MF448297)

Vishniacozyma carnescens CBS 973^T (AB035054)
Vishniacozyma sp. KBP Y-6982 (OP941491)

Vishniacozyma victoriae NPCC 1263 (MN848499)
Vishniacozyma victoriae CBS 8685^T (AF363647)
Fig. 1. Phylogenetic trees based on the sequences of the D1/D2 domain of the LSU rRNA gene showing the position of representative yeast strains in relation to closely related species. The trees were constructed by the neighbor-joining method, and bootstrap percentages based on 1000 replications are displayed for each node; bootstrap values higher than 50% are shown. Strains isolated in the course of this work and branches supported by 90% or more are highlighted in bold. The trees were constructed for genera: Cystofilobasidium (A), Naganishia and Vishniacozyma (B).

Six strains (40% of the total strains) could grow at 28 °C, and all of them were belonged to the genus Naganishia. On the other hand, none of the yeast strains could grow at 37°C (Table 3).

Table 3. Growth ability of yeast strains isolated from permafrost soils in Mongolia at different temperatures.

<table>
<thead>
<tr>
<th>№</th>
<th>Strain</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 °C</td>
<td>4 °C</td>
</tr>
<tr>
<td>1</td>
<td>C. macerans U5-23</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>N. adeliensis U5-24</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>V. carnescens U5-25</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>N. albidus U5-30</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>C. macerans U5-32</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>6</td>
<td>N. albidus U5-33</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>7</td>
<td>C. macerans U5-36</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>N. adeliensis O7-26</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>N. albidosimilis O7-27</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>N. adeliensis O7-29</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>C. macerans O7-31</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>C. macerans O7-34</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>V. victoriae O7-35</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>C. macerans O9-28</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>15</td>
<td>N. adeliensis O9-37</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Growth of yeast strains: ++ strong growth, + visible growth, w weak growth, - absence of growth

3.4 Screening of enzymes activities

The ability of yeast strains to produce hydrolytic enzymes was evaluated at 0, 4, 15, and 28°C. All strains except N. adeliensis O7-26 exhibited at least one extracellular enzymatic activity at 0, 4, 15, and 28°C.
Ten strains were positive for lipase, depending on the temperature at which it was tested. Two strains belonging to the genus *Vishniacozyma*, *V. carnescens* U5-25 and *V. victoriae* O7-35, showed lipolytic activity regardless of the temperature at which it was tested, but the highest at 15 °C. *N. albidosimilis* O7-27 also demonstrated lipolytic activity at all temperatures, but the highest at 28 °C. Out of 6 strains belonging to *C. macerans*, 4 had lipolytic activity depending on the temperature (Fig. 2A). Interestingly, the representative strains of this species formed 2 clusters on the phylogenetic tree (Fig. 1A), and 2 lipase-negative strains fell into a cluster with the type strain of the species, *C. macerans* CBS 10757T, while 4 lipase-positive strains clustered with other strains. Moreover, *C. macerans* U5-32 and *C. macerans* O7-31 had highest activity at 4 °C.

Eleven strains were positive for cellulase, depending on the temperature at which it was tested. Two strains belonging to the genus *Vishniacozyma* and 3 strains belonging to *Naganishia* showed cellulolytic activity at low temperatures, at 15°C and below. All 6 strains belonging to *C. macerans* had a pronounced cellulolytic activity in a wide temperature range with maximum activity at 15°C, with the exception of *C. macerans* O9-28, which did not show activity at 28°C (Fig. 2B).

**Fig. 2.** Extracellular enzymatic activity of yeast strains at different temperatures. (A) Lipolytic activity, (B) Cellulolytic activity.

**4 Discussion**

In this study, the abundance of yeast in the permafrost soils in Mongolia ranged from $2 \times 10^2$ to $2 \times 10^3$ cfu/g. Studies of the distribution and diversity of yeasts in glacial habitats of different geographic areas revealed varying numbers of yeasts; from 5 to over $10^5$ cfu/g in soil of Ross Dependency of Antarctica [15], 9 x $10^3$ cfu/g in Siberian permafrost soil [5], over $10^4$ cfu/mL in snow and ice cores of the high-altitude Belukha
glacier of Altay Mountains [16], up to $9.6 \times 10^3$ cfu/g in subglacial sediment in glaciers of the Italian Alps [17], and $10^5$ cfu/g in sediments of Calderone Glacier [18].

Forty percent of all yeast strains isolated in the present study belonged to C. macerans. This species is the sexual stage of Cryptococcus macerans, and recognized as Cystofilobasidium macerans sp. nov. with the self-fertile type strain CBS 10757T after observing the entire sexual cycle [19]. Indeed, C. macerans has been extensively isolated from glacial habitat samples; soil of Antarctica (original taxonomic designation Rhodotorula macerans) [2, 15], frozen environmental samples of Iceland [20], high Arctic glacier ice of Norway [21], glacial meltwater of Argentina [10], and sediments of glacier of Italy [18], suggesting that it is a cold-adapted species. Sixty percent of all yeast strains isolated in this study belonged to 3 species of the genus Naganishia, namely N. adeliensis, N. albidus and N. albidosimilis, and 2 species of the genus Vishniacozyma, namely V. carnescens and V. victoriae. These species previously belonged to the genus Cryptococcus [22], the main genus representing glacial habitats; about 25-40% of the total species isolated from Antarctic, European and South American glaciers belong to this genus [2]. All 5 species were found in Antarctica and the Arctic, while C. adeliensis, C. albidus, and C. victoriae were also found in European glaciers, and C. adeliensis and C. victoriae in South American glacier-associated habitats [2]. Further, C. victoriae and C. carnescens were isolated from glacial biomes in Argentina and Norway, where the latter was the dominant species (de García et al., 2012).

Among them, C. carnescens and C. victoriae are known to be psychrophilic or psychrotolerant, and C. adeliensis, C. albidus and C. albidosimilis are mesophilic as their maximum temperatures for growth are $\geq 30$ °C [23]. However, in the present study, strains belonging to N. adeliensis, N. albidus and N. albidosimilis (synonyms of C. adeliensis, C. albidus and C. albidosimilis) showed strong growth at $\leq 20$°C, even some could not grow at 28 °C. Four of them showed strong growth at 28 °C, but the same growth was observed at lower temperatures. According to the results obtained, all strains were classified as facultative psychrophiles or psychrotolerants.

Lipolytic activities (hydrolysis of Tween-80 and/or tributirin) was the most predominantly expressed extracellular enzyme activity and was higher at 4 °C than at 20 °C in most studies [10, 17, 24, 25, 26, 27]. Among the isolates reported in the above studies, one isolate of C. macerans showed the highest lipolytic activity at 4 °C on tributirin agar [10], and C. adeliensis, C. carnescens and C. victoriae expressed lipolytic activity at 5 °C and 20 °C [25]. In the present study, 2 strains of C. macerans exhibited the highest lipolytic activity at 4 °C, which is consistent with the results of the above studies. Representative strains of V. carnescens, V. victoriae and N. albidosimilis had lipolytic activity at wide range of temperatures, from 0 °C to 28 °C, and the similar results were reported on yeasts isolated from East Ongul Island of Antarctica [14]. Also, [26] reported that most C. victoriae isolates hydrolyzed Tween 80 and cellulose at 4 and 20°C.

In some studies, yeast isolates were not able to hydrolyze cellulose at the assessed temperatures [10, 17, 24]. However, [25] found cellulase activity in 53.0% of 148 strains tested, including C. adeliensis, C. carnescens, and C. victoriae, while [27] detected hydrolysis of carboxymethyl-cellulose in 96 out of 212 strains. Interestingly,
yeasts from permafrost in Mongolia showed high cellulolytic activity in terms of both percentage of positive strains (73.3%) and enzyme secretion ability (highest 5.1 or halo zone of 37mm) over a wide temperature range from 0 °C to 28 °C. All C. macerans strains had a pronounced cellulolytic activity. Moreover, strains belonging to V. carnesens, V. victoriae, N. adeliensis, and N. albidus showed cellulolytic activity at ≤15°C, in contrast to results of [14] where no activity was detected for N. adeliensis and higher activity at ≥15°C for V. victoriae. The high cellulase activity of yeasts from Mongolian permafrost compared to the yeasts isolated from aquatic environments such as glacial and subglacial sediments, water and ice [10, 24] can be associated with the vegetation cover of the active soil layer above the permafrost; they may involve in the decomposition cellulose, which constitutes up to 20% of the biomass of land plants.

5 Conclusion

Psychrophilic basidiomycetous yeasts, belonging to the taxa that extensively inhabit the global glacial environments, were isolated for the first time from permafrost in Mongolia. The yeast strains exhibited pronounced extracellular enzymatic activity at 0, 4, 15, and 28 °C, which indicates that they maintain an active metabolism under permafrost conditions. These results are of great importance both for the study of cold-adapted enzymes, which have the potential for industrial application, and for the study of microbial activity in the Mongolian permafrost degrading due to climate warming. Furthermore, bacteria and filamentous fungi were also isolated (data not shown), confirming that Mongolian permafrost is a reservoir of both prokaryotic and eukaryotic microorganisms.

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References


