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Review

Soil Yeasts in the Vicinity of Syowa Station, East Antarctica: Their Diversity and Extracellular Enzymes, Cold Adaptation Strategies, and Secondary Metabolites

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Abstract: Antarctica is known as one of the harshest environments on Earth, with a frigid and dry climate. Soil yeasts living in such extreme environments can grow by decomposing organic compounds at sub-zero temperatures. Thus far, a list of lichen and non-lichen fungi isolated from the area near Syowa Station, the base of the Japanese Antarctic research expedition, has been compiled and a total of 76 species of fungi have been reported. Yeast, especially basidiomycete yeast, is the dominant fungus in Antarctica. This mini-review summarizes a survey of the yeast diversity in the soil of Eastern Ongul Island and the ability of these yeasts to secrete extracellular enzymes. We also describe the yeast diversity in the soil of the Skarvesnes ice-free region and how these yeasts have adapted to the sub-zero environment. Further, we describe the secondary metabolites of these yeasts, whose production is induced by cold stress.

Keywords: Antarctica; cold adaptation; extracellular enzymes; soil yeast diversity

1. Introduction

Most of the Earth's biosphere is a low-temperature region exposed to temperatures below 5 °C; this includes the polar regions of Antarctica and the Arctic, the deep sea, and high mountains such as the Himalayas and the Alps. [1]. Antarctica is the southernmost continent on Earth, covering an area of about 14 million km^2, making it the fifth-largest continent in the entire world. About 98% of Antarctica is covered in ice and snow, and coastal temperatures are typically between 5 and −35 °C [2]. Ice- and snow-free areas during the austral summer are distributed along the coast of Antarctica in areas called ice-free regions, where snow and ice melt during the summer months to expose the ground. In Antarctica, most life forms live in these ice-and snow-free areas [3]. Despite being exposed to conditions such as sub-zero temperatures and low availability of nutrients and water, which are detrimental to their survival, fungi living in cold environments can grow at near-zero temperatures. Secretion of extracellular enzymes allows them to utilize complex substances as a source of energy [4]. Therefore, psychrophilic and psychrotolerant fungi play an essential role in the nutrient cycle of polar ecosystems [5,6]. Changes in fungal diversity at polar regions can have a profound effect on local primary production; therefore, understanding the diversity of fungi in polar regions is essential to know the local environment.

To our knowledge, more than 1000 fungal species from about 400 genera have been identified and documented in Antarctica [7]. This list of species identified by culture and collection included 68% ascomycetes, 23% basidiomycetes, and 5% zygomycetes fungi, with the last 4% consisting of various other lineages. A list of lichenized and non-lichenized fungi isolated from the area around Syowa Station, the base of the Japan Antarctic Research Expedition (JARE), has been generated and a total of 76 fungal species have been documented [8,9]. We have investigated the diversity of fungi in East Ongul Island, where the Syowa Station is located, and the Skarvesnes ice-free area that is about 60 km from this island. In Antarctica, yeast, especially basidiomycete yeast, is the dominant fungus. Therefore, in this mini-review, we summarize the diversity of yeasts living in the soil near the Syowa Station in Antarctica, their mechanisms of adaptation to low temperatures, and their ability to secrete extracellular enzymes at low temperatures. We also summarize the secondary metabolites that these yeasts produce, specifically under the cold stress of sub-zero temperatures.

2. Soil Yeast Diversity and Their Ecological Role in East Ongul Island

The Island of East Ongul (69° 1 S, 39° 35 E) is located to the east of Lützow Holm Bay in East Antarctica (Figure 1). In 1957, Syowa Station was established here as a base for the JARE. During the austral summer, East Ongul Island is a snow-and ice-free area. A total of 95 yeast strains were cultured from soil samples from East Ongul Island [10]. These strains were classified as basidiomycetes belonging to 10 genera and 16 species in six families, based on the internal transcribed spacer (ITS) region sequence and the D1/D2 domain sequences of the 26S rDNA gene (Table 1). The dominant yeast genera in East Ongul island soils were *Vishniacozyma* (29.4%), *Naganishia* (23.2%), and *Cystobasidium* (14.7%). At the species level, *Vishniacozyma victoriae* (23.2%), *Naganishia friedmannii* (17.9%), *Cystobasidium ongulense* (10.5%), and *Glaciozyma martinii* (10.5%) were the most frequently isolated yeasts. Our fungal survey did not reveal any ascomycetous yeast from the soil of East Ongul Island [10].

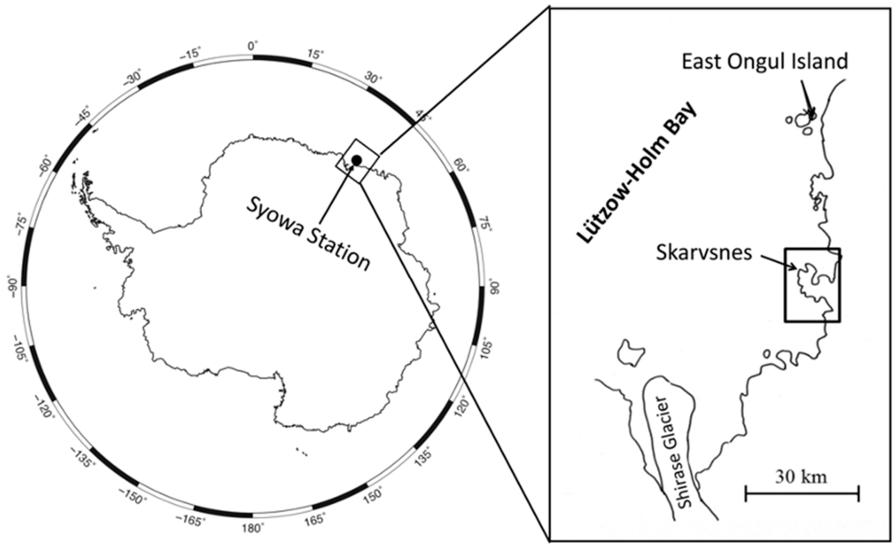

Figure 1. Map of Antarctica and the locations of Syowa Station and the Skarvsnes ice-free area.

The left side of the map shows the Antarctic continent, and the right side shows the area near Syowa Station.

Of these 16 yeast species, it was initially reported that *Cystobasidium lysinophilum*, *Goffeauzyma gilvescens*, *Holtermanniella wattica*, *Mrakia gelida*, *Naganishia albidosimilis*, *Udeniomyces puniceus*, and *Vishniacozyma carnescens* could not be grown in vitamin-free medium [11–14]. However, in our previous study, all species grew in vitamin-free medium and at sub-zero temperatures. The measured total carbon and nitrogen concentrations in the soil samples from 226 points in East Ongul Island were 0.063% ± 0.087% and 0.0025% ± 0.0023%, respectively. Based on this result, the soil on the island is an extreme oligotrophic environment [15]. Therefore, it is thought that the yeasts living on this island have acquired unusual vitamin-free growth characteristics to survive the cold and extremely oligotrophic environment of East Ongul Island.

In general, yeast species are classified into two types according to their ability to grow at low temperatures: psychrophilic and psychrotolerant yeasts. Psychrophilic yeasts show an optimum growth temperature of 15 °C or less, whereas psychrotolerant yeasts have a maximum growth temperature of 20 °C or less [16]. The optimum and maximum growth temperatures were determined for 16 yeast species isolated from the soil of East Ongul Island. Of these yeast species, *Glaciozyma antarctica*, *Gl. martinii*, *M. gelida*, and *Phenoliferia glacialis* were classified as psychrophilic yeasts, whereas the other 12 species were classified as psychrotolerant yeasts (Table 1). All yeast species, irrespective of being psychrophilic or psychrotolerant, were capable of growing at −3 °C [10].

Table 1. List of yeast species isolated from the soil of East Ongul Island.

Species	Number of Strains	Psychrophile or Psychrotolerant
Cystobasidium lysinophilum	2	Psychrotolerant
Cystobasidium ongulense	10	Psychrotolerant
Cystobasidium tubakii	2	Psychrotolerant
Glaciozyma antarctica	1	Psychrophile
Glaciozyma martinii	10	Psychrophile
Goffeauzyma gilvescens	2	Psychrotolerant
Holtermanniella wattica	3	Psychrotolerant
Mrakia gelida	5	Psychrophile
Naganishia adeliensis	1	Psychrotolerant
Naganishia albidosimilis	4	Psychrotolerant
Naganishia friedmannii	17	Psychrotolerant
Phenoliferia glacialis	8	Psychrophile
Tausonia pullulans	1	Psychrotolerant
Udeniomyces puniceus	1	Psychrotolerant
Vishniacozyma carnescens	6	Psychrotolerant
Vishniacozyma victoriae	22	Psychrotolerant

This table presents reconstructed data from a previous study [10].

One of the principal challenges faced by cold-adapted microorganisms including yeasts is the adverse effect of low temperatures on the rate of enzymatic reactions. Reduced temperature can cause protein denaturation [17], and even enzymes that remain appropriately folded can slow or arrest the release of enzymatic reaction products [18,19]. In other words, enzymes secreted by microbes show low activity and difficulty in decomposing organic compounds under low-temperature conditions. Carrasco et al. (2012) examined the enzyme secretion ability of 24 strains comprising 12 genera isolated from the soil on King George Island, Antarctica, at the optimal growth temperature of these strains [20]. Further, Vaz et al. (2011) tested the secretion ability of five enzymes (amylase, lipase, esterase, protease, cellulase) at 4 °C and 20 °C for 89 strains of 16 genera isolated from the soil of King George Island and Deception Island [21]. *Vishniacozyma victoriae* secreted lipase at 4 °C, and *Leucosporidium scottii* secreted lipase and cellulase at 4 °C, but these two species could not secrete any extracellular enzymes at 20 °C. Few studies have investigated the secretion of extracellular enzymes by yeasts isolated from Antarctic soil at different temperatures. An extracellular enzyme secretion experiment for yeasts inhabiting Antarctic soil at different temperatures was thus planned and carried out.

Based on the growth characteristics of psychrophilic and psychrotolerant yeast at temperatures below 0 °C, we predicted that psychrophilic yeast strongly secreted extracellular enzymes even at temperatures below 0 °C, whereas psychrotolerant yeast did not strongly secrete extracellular enzymes at temperatures below 0° C. Indeed, when investigating the secretion temperature of the extracellular enzymes of yeasts isolated from the soil of East Ongul Island from −3 to 25 °C, psychrophilic yeasts, such as *Gl. antarctica*, *Gl. martinii*, *M. gelida*, and *P. glacialis*, did not clearly secrete extracellular enzymes at −3 °C and 4 °C. On the contrary, psychrotolerant yeasts such as *Go. gilvescens*, *H. wattica*, *T. pullulans*, and *U. puniceus* secreted significant amounts of enzymes even at temperatures below freezing [10].

This result suggests that psychrotolerant yeasts play a more important role in the nutrient cycle compared to psychrophilic yeasts under sub-zero temperature conditions in soils at East Ongul Island.

3. Soil Yeast Diversity and Their Cold Adaptation Strategies in the Skarvsnes Ice-Free Area

The Skarvesnes ice-free area is located in the central Soya coast of East Antarctica, approximately 60 km from Syowa Station (Figure 1). This area is a known ice-free area where snow melts and the soil is exposed during the austral summer. We isolated a total of 71 fungal strains from lake sediments and soil collected by the 48th JARE in the Skarvsnes ice-free area and reported the diversity of fungi inhabiting this area [22]. Of these fungal strains, 51 strains were isolated from soil; 35 of 51 fungal strains were yeast species, all of which were classified as basidiomycetes. For these 35 yeast strains, a list of species names and accession numbers of the ITS region sequence is shown in Table 2. Notably, most of these strains have not been previously identified to the level of species in the original article. We attempted to reclassify these strains using DNA sequence data deposited in the DNA databank. If a strain could not be reclassified at the species level based on the DNA sequence data, it was shown at the genus level. The major yeast species inhabiting the soil in this area are as follows: *Vishniacozyma victriae* (32%), *Mrakia gelida* (26%), *M.robertii* (12%), and *Goffeauzyma gastrica* (9%). Although only about 60 km away from the Skarvsness ice-free area and East Ongul Island, the fungal diversity in the soil was quite different in both areas.

Table 2. List of yeast species isolated from the soils of the Skarvesnes ice-free area by the 48th JARE and their ITS region accession numbers.

Species	Strain	Accession Number
Cystobasidium laryngis	ABH-3	AB774463
Dioszegia fristingensis	ARJ-3	AB774458
Dioszegia fristingensis	HYT-1	AB774459
Glaciozyma watsonii	KGK-2	AB774460
Goffeauzyma gastrica	TKU1-1	AB773891
Goffeauzyma gastrica	BSS-1	AB773892
Goffeauzyma gastrica	MOA-2	AB774233
Mrakia blollopis	MOA-3	AB775474
Mrakia gelida	AGK-2	AB774465
Mrakia gelida	ABU1-1	AB774468
Mrakia gelida	EBH-3	AB774470
Mrakia gelida	EBH-4	AB774471
Mrakia gelida	NKU-1	AB775661
Mrakia gelida	NGU-1	AB775662
Mrakia gelida	NIN-6	AB775663
Mrakia gelida	BSU2-3	AB775471
Mrakia gelida	EBN-1	AB775203
Mrakia gelida	NRI-1	AB775469
Mrakia robertii	SMI-2	AB775472
Mrakia robertii	MOA-4	AB775660
Mrakia robertii	NRI-1	AB775468
Mrakia robertii	NRI-3	AB775470
Naganishia friedmannii	NHU-1	AB773893
Phenoliferia glacialis	NHT-2	AB774464
Vishniacozyma victoriae	OGA-2	AB774232
Vishniacozyma victoriae	ARI-3	AB773887
Vishniacozyma victoriae	NIK-1	AB774234
Vishniacozyma victoriae	NIK-2	AB774235
Vishniacozyma victoriae	NIK-3	AB774236
Vishniacozyma victoriae	NIN-5	AB774237
Vishniacozyma victoriae	ARJ-4	AB773888
Vishniacozyma victoriae	OGN2-4	AB774230
Vishniacozyma victoriae	ABH-4	AB773886
Vishniacozyma victoriae	JZN-4	AB773890
Vishniacozyma victoriae	OGA-1	AB774231

The contents of Table 2 are reconstructed based on the data from previous studies [8,9,22].

Robinson (2001) stated that microorganisms benefit from the physiological characteristics of cryoprotectants, such as sugars, polyols, fatty acids, antifreeze proteins (also known as AFP, ice-binding proteins), and cold-active enzymes, to survive during polar winters [16]. All 35 strains of yeast isolated from the soils of the Skarvsnes ice-free area were able to grow at −1 °C, but these strains could not grow at 25 °C [22]. Some fungi secrete extracellular AFP to prevent their cells from freezing when exposed to extremely cold temperatures. Fungal AFP has been found in the snow mold fungus, which is a known plant pathogen in both wheat and rice [23–25]. In the soil of the Skarvesen ice-free area, only *Phenoliferia glacialis* NHT-2 exhibited antifreeze activity. The results of this study, consistent with those of previous studies, indicate that few Antarctic fungi exhibit antifreeze activity and that almost all of them employ other strategies to survive such extreme environments [26,27].

Pathan et al. (2010) noted that cold-adapted yeasts generally contain high amounts of unsaturated fatty acids, such as C18:1 and C18:2, and these fatty acids are considered essential for survival at low temperatures [28]. The fatty acid composition results of *Mrakia* spp. and *V. victoriae* suggest that they are adapted to low temperature environments by maintaining their cell membrane fluidity [29,30]. In the Skarvesnes ice-free area, yeasts of the genera *Mrakia* and *Glaciozyma* in particular, had higher concentrations of unsaturated fatty acids in their cells [22]. Based on the combined antifreeze-protein activity tests and fatty acid analysis, yeasts inhabiting Antarctic soil with AFPs are adapted to the cold temperature environment because of their antifreeze activity, although they are in the minority. Most yeasts in Antarctic soil increase the contents of unsaturated fatty acids in their cells to maintain or improve cell membrane fluidity under a cold environment to adapt to the cold temperatures.

Mrakia blollopis SK-4 was isolated from a lake in the Skarvesnes ice-free area of East Antarctica and *M. blollopis* TKG1-2 was isolated from the soil in the same area. When the SK-4 and TKG1-2 strains are cultivated at the optimal growth temperature of 10 °C, the cell numbers of both strains become almost the same [31]. However, when both strains are cultivated at −3 °C, the SK-4 strain grows to a cell number almost the same as that when cultured at 10 °C, though its growth rate is slow. On the contrary, when the TKG1-2 strain is cultivated at −3 °C, it grows only to approximately half the number of cells as that when cultured at 10 °C [31]. Thus, Antarctic basidiomycetous yeasts *Mrakia blollopis* SK-4 and TKG1-2 exhibit distinct growth characteristics under sub-zero conditions. SK-4 isolated from the lake grows efficiently under sub-zero temperature conditions, whereas TKG1-2 isolated from the soil does not grow well under these conditions. However, these strains show over 99.5% identity with strain CBS 8921, a type of strain of *M. blollopis*, in the ITS (internal transcriptional spacer) region and the D1/D2 domain of the 26S rDNA [32]. Under the current taxonomic criteria, these two strains with differing growth ability below sub-zero temperatures belong to the same species. The cold stress-induced metabolite responses of these two *M. blollopis* strains were analyzed using capillary electrophoresis-time of flight mass spectrometry (CE-TOFMS). *M. blollopis* SK-4 that was isolated from a lake, grew well below the freezing temperature and accumulated high concentrations of TCA cycle metabolites, lactic acid, aromatic amino acids, and polyamines in the cells in response to cold shock. Polyamines are known to function in cell growth and development, and aromatic amino acids induce improved cell growth at low temperatures. On the contrary, strain TKG1-2 isolated from soil did not grow efficiently under sub-zero temperatures, and cold stress strongly induced metabolites of the TCA cycle, whereas other metabolites did not accumulate much in these cells. As activation of the TCA cycle and consequent production of ATP is essential for responses to cold stress, Antarctic yeast may require other metabolites that accumulate at sub-zero temperatures for growth [31].

To the best of our knowledge, the genus *Mrakia* is the only ethanol fermentable fungal species inhabiting the continental Antarctic region. The SK-4 strain can produce over 5% (v/ v) ethanol, whereas TKG1-2 could produce less than 2% (v/v) ethanol [33]. Therefore, to investigate the relationship between ethanol production ability and ethanol tolerance ability, we tested the ethanol tolerance ability of SK-4 and TKG1-2 isolated from a site near the Syowa Station. These were evaluated for their ability to grow in media containing ethanol. Consequently, the SK-4 strain, which has superior ethanol fermentation ability, was also revealed to have superior ethanol tolerance, but the TKG1-2 strain, which cannot produce much ethanol, has weak ethanol tolerance [34]. In other words, *Mrakia* strains with superior growth ability at sub-zero temperatures have high ethanol fermentation ability and high ethanol tolerance [34].

We have already reported the whole-genome sequence of *Mrakia blollopis* SK-4 isolated from a lake [35]. Comparing the genome sequence of the unpublished data for TKG1-2 strain with that of the SK-4 strain, the genome of the TKG1-2 strain is about 10% larger than the SK-4 strain. Examining differences in the genomic sequences of SK-4 and TKG1-2 strains could allow the determination of the gene sequences and metabolic pathways that are responsible for the differences in sub-zero growth ability and stress tolerance between the two strains.

4. Secondary Metabolites Induced by Cold Stress

Antarctica has one of the harshest environments on Earth. Yeasts inhabiting such environments demonstrate surprising viability in extreme environments and are an attractive source for microbial resources as well as for basic research. We used gas chromatography-tandem mass spectrometry (GC-MS/MS) to investigate the production of useful secondary metabolites from basidiomycetes *Mrakia* spp. and *Cystobasidium* spp. and *Tausonia* spp. isolated from the area around Syowa Station. The results are shown in Table 3.

Table 3. List of secondary metabolites produced at sub-zero temperatures by yeasts isolated from soils near the Syowa Station.

Species	Chemical Compound	Principal Applications of Chemical Compounds
Mrakia blollopis	Peltatol A	anti-HIV activity
Mrakia blollopis	Pinacidil	reduces blood pressure
Mrakia blollopis	Pirbuterol	bronchodilatation
Cystobasidium ongulense	Altretamine	anti-neoplastic agent
Cystobasidium ongulense	Lucyoside M	anti-inflammatory activity
Cystobasidium ongulense	Tegafur	anti-neoplastic agent
Tausonia pullulans	Acebutolol	the treatment of hypertension and arrhythmias
Tausonia pullulans	Epothilone D	anti-neoplastic agent
Tausonia pullulans	Isopentenyl adenosine	promotes cell division

Yeasts living in the soil around the Syowa Station were induced by cold stress to produce useful secondary metabolites including the bronchodilator Pirbuterol and the anti-neoplastic agent, Altrethamine. However, of the cold stress-induced secondary metabolites of these yeasts, only about 10% could be identified by mass spectrometry, and the remaining 90% of the metabolites were unknown [36]. As human health is threatened by the appearance of unknown viruses and pathogens such as the current COVID-19 pandemic, the unknown secondary metabolites produced by yeasts living in Antarctic soils may be good candidates for searching chemical compounds that could be used as raw materials for new drugs. We thus hope that pharmaceutical companies will consider the yeasts from Antarctica as candidates for the discovery of raw materials for designing new drugs.

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