Summary of Doctoral Thesis

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Title	Study on the cold adaptation strategies and biotechnological potential of cryophilic basidiomycetous yeast *Mrakia blollopis*

Despite previous research on the Antarctic mycobiota, knowledge of fungal biodiversity there remains incomplete. Fungal diversity from the Skarvsnes ice-free area, located near the Syowa station, has not yet been studied. Moreover, the only applied study of fungi isolated from Antarctica is Xiao et al.'s patent (特許第5397848 号) from the Japan Antarctica Research Expedition (JARE).

In the present study, I first collected a total 71 fungal isolates from lake sediments and soil surrounding lakes in the Skarvsnes ice-free area, East Antarctica. Based on the sequence similarity of the rDNA ITS region, these isolates were classified into 10 genera. Twenty-three isolates were categorized as Ascomycetous fungi from five genera (*Embellisia*, *Phoma*, *Geomyces*, *Tetracladium*, and *Thelebolus*) and 48 isolates were categorized as basidiomycetous fungi from five genera (*Mrakia*, *Cryptococcus*, *Dioszegia*, *Rhodotorula*, and *Leucosporidium*). Thirty-five percent of culturable fungi were from the genus *Mrakia*, suggesting that they are the predominant species in this area. However, fungal biodiversity in the Skarvsnes ice-free area has not yet been fully researched, because there are still many lakes in the region whose soils and lake sediments remain untouched. Therefore, it would be interesting to conduct further investigations on fungal biodiversity in the Skarvsnes ice-free area using new high-throughput technologies such as next generation sequencing.
Eighteen isolates from the eight genera were selected and tested for both antifreeze activity and growth potential at cold temperatures ranging from − 1 to 25°C. *Rhodotorula* sp. NHT-2 exhibited a high degree of sequence homology (99.8%) to *R. gracilis*, while *Leucosporidium* sp. BSS-1 exhibited a high degree of sequence homology (100%) to *L. antarcticum* (*Glaciozyma antarctica*), and both isolates demonstrated antifreeze activity. All of the 18 isolates examined could grow at − 1°C; however, no antifreeze activity was observed in *Mrakia* spp. and its ability to secrete extracellular polysaccharides was limited. Species of the genus *Mrakia* possessed high amounts of unsaturated fatty acids, suggesting that they have adapted to cold environments by increasing their membrane fluidity.

I then examined the potential of the Antarctic basidiomycetous yeast *M. blollopis*

as a bio-remediation agent under low-temperature conditions. Milk fat curdle in sewage is one of the refractory materials for active sludge treatment under low-temperature conditions. To address this, an Antarctic yeast, strain SK-4, isolated from algal mats on the sediments of Naga-ike Skarvsnes, was used as a bio-remediation agent. The yeast strain exhibited high nucleotide sequence homologies (>99.6%) to *M. blollopi*s CBS8921^{T} in rDNA ITS and D1/D2 sequences and had two unique characteristics when applied to an active sludge, i.e., it used various carbon sources and grew under vitamin-free conditions. Indeed, its BOD removal rate was 1.25-fold higher than that of the control. It may be that the improved BOD removal rate when applying the strain SK-4 can be attributed to the unique activity and characteristics of lipase the strain possesses. Hence, the lipase was purified from strain SK-4 and characterized. As a result, the enzyme was found to be stable under a wide range of temperatures and pHs, even in the presence of various metal ions and organic solvents. Strain SK-4 is therefore a promising bio-remediation agent for cleaning up unwanted milk fat curdles from dairy milk wastewater under low-temperature conditions.

Furthermore, the influence of initial pH on *M. blollopis* strain SK-4 ethanol production at 10°C was investigated. To the best of my knowledge, little is known about basidiomycetous yeast fermentation. Species in the basidiomycetous yeast genus *Mrakia* are known for their ability to ferment sugars. In fact, all of the *Mrakia* species examined in this study could convert glucose and sucrose to ethanol by fermentation. *M. blollopis* strain SK-4 used raffinose, galactose, lactose, and maltose for fermentation at low temperatures. However, little is known about strain SK-4's fermentation ability, and hence SK-4 ethanol productivity was examined under various pH conditions. SK-4 produced ethanol at pHs ranging from 5.0-10.0, with the optimum being 8.0-10.0. SK-4 lipase is stable against metal ions and organic solvents, and its optimum pH was 8.5–9.0. Since Naga-ike is an oligotrophic lake and the pH is 8.5, it was assumed that the unique characters of SK-4 for fermentation under alkaline conditions might have been acquired for survival in the lake's extreme environment.

Strain SK-4 has the unique ability to ferment various sugars under low-temperature conditions. Hence, this yeast is a good ethanol-producing microbial agent candidate. For this purpose, strain SK-4's ability to process carbohydrates into ethanol was examined using glucose, hydrolysates, and lignocellulosic biomass. SK-4's ability for DEF from cellulosic biomass in the presence/absence of Tween 80 at 10°C was also examined. SK-4 produced as much as 48.2 g/L of ethanol from 12% (w/v) glucose solution, and 17.4 g/L ethanol was converted from Japanese cedar hydrolysate.

However, the strain could not use glucose from Eucalyptus hydrolysate for DEF. During the DEF process, SK-4 converted filter paper, Japanese cedar, and Eucalyptus into 12.2 g/L, 12.5 g/L, and 7.2 g/L ethanol, respectively. In the presence of 1% (v/v) Tween 80, the final ethanol concentration increased by approximately 1.1-1.6-fold compared with that in the absence of Tween 80. This is the first report on DEF using cryophilic fungi under low-temperature conditions. *M. blollopis* strain SK-4 has good potential for ethanol fermentation in DEF technology in cold environments. However, when SK-4 was used to ferment mechanochemically treated Eucalyptus and Japanese cedar wood meals (i.e., lignocellulosic biomass) by DEF in the presence of non-ionic surfactant Tween 80, theoretical ethanol yields were 51.2% and 65.1%, respectively. Since these fermentation rates did not exceed 70%, it was necessary to improve fermentation efficiency. By using lipase in addition to Tween 80, DEF with SK-4 was successfully improved to produce 17.3 g/L (fermentation rate = 76.4%) and 17.5 g/L (fermentation rate = 81.0%) ethanol at 10° C from Eucalyptus and Japanese cedar wood meals, respectively. In the presence of 1% (v/v) Tween 80 and 5 U/g dry substrate lipase, the ethanol concentration increased approximately 1.4‐2.4-fold compared with that without Tween 80 and lipase. Consequently, DEF of lignocellulosic biomass with SK-4 in cold environments can be improved by adding Tween 80 and lipase. In this study, I performed the DEF with Acremonium cellulase.