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Taxonomic Description

Vishniacozyma ellesmerensis sp. nov., a new psychrophilic yeast isolated from a retreating glacier in the Canadian High Arctic

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The GenBank/EMBL/DDBJ accession number for the ITS region and 26S rRNA D1/D2 domain sequences of JCM 32573 (UAMH 11973) is LC335796, and that of JCM 32574 (UAMH 11974) is LC335797.
The MycoBank number for *Vishniacozyma ellesmerensis* sp. nov. is MB825501.

Abbreviations: ITS, internal transcribed spacer

Abstract

Two strains of a psychrophilic basidiomycetous yeast species belonging to the genus *Vishniacozyma* were isolated from sediments and soil at the front of a retreating glacier on northern Ellesmere Island in the Canadian Arctic. Analysis of the large-subunit D1/D2 region and the internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) indicated that these strains represented a novel species. The closest relatives of the novel species were *Vishniacozyma globispora* and *V. dimennae*, which exhibited sequence identities of 82.2% and 81.6%, respectively, to the ITS region of the new species, and contained 61 and 57 nucleotide substitutions , respectively, in the D1/D2 domain, in comparison to the novel species. Strains of the new species were able to grow at sub-zero temperatures and in vitamin-free medium. These characteristics likely allow the strains to survive and grow in perennially cold, oligotrophic habitats in the Canadian High Arctic. The name *V. ellesmerensis* sp. nov. is proposed. The type strain is JCM 32573 (=UAMH 11973=G3-4-8) and the MycoBank number is MB825501.

INTRODUCTION

Polar regions occupy about 14% of Earth's landmass [1] and provide vast habitats for microbial colonization and growth. Fungi in cold environments can grow and decompose diverse types of organic compounds at sub-zero temperatures and they therefore play an important role in the nutrient cycles of polar microbial ecosystems [2–4].

Basidiomycetous yeasts have been widely reported to represent the dominant fungi in polar habitats [5-10]. Many of these yeasts have been found only in their asexual stage and were classified into the anamorphic genera *Cryptococcus* or *Rhodotorula* [11-13]. However, species classified as *Cryptococcus* have been found to be distributed across four orders: *Tremellales*, *Trichosporonales*, *Filobasidiales*, and *Cystofilobasidiales* [12]. Thus, many species previously classified as *Cryptococcus* have now been incorporated into newly described genera [14]. Recently, the genus *Vishniacozyma* was newly established from the Victroae and Dimennae clades of the genus *Cryptococcus* [15]. Currently, the genus *Vishniacozyma* consists of a total of 11 species: *Vishniacozyma carnescens*, *V. dimennae*, *V. foliicola*, *V. globispora*, *V. heimaeyensis*, *V. nebularis*, *V. penaeus*, *V. psychrotolerans*, *V. taibaiensis*, *V. tephrensis*, and *V. victoriae* [16].

In this study, two yeast colonies were isolated from sediments and soil at the front of a retreating glacier in the Canadian High Arctic. Based on physiological testing and molecular analysis of the internal transcribed spacer (ITS) sequences and the 26S large-subunit (D1/D2 domain), these strains were classified as a new basidiomycetous yeast species in the genus *Vishniacozyma*, for which the name *V. ellesmerensis* sp. nov. is proposed.

METHODS

Study site and sampling

Sampling was at the Walker Glacier (unofficial name) on the northern coast of Ellesmere Island in the Canadian High Arctic. This region is at the northern limit of Quttinirpaaq National Park, Nunavut, where climate-related effects on the cryosphere have been observed over the last 20 years [17]. GPS measurements on 20 July 2013 from a datum pole that had been installed at this site by Paul T. Walker on 10 July 1959 (lat. 83 00.601'N; long. 72 12.387'W) showed that the glacier had retreated by 71 m, at an average rate of 1.3 m/y over this 54-year period. Repeat GPS measurements at this site during the present study (21 July 2016) showed a further retreat of 10 m, giving an average rate of 3.3 m/y. This 2.5-times faster rate of glacial melting and retreat would indicate a recent acceleration of climate warming at this far northern site.

As part of a microbial survey in the region, sediments were scraped from the surface of the melting glacier face and additional samples were taken of surface sediments (mineral soil) that had been deposited and exposed by the receding glacier. The sediments were transferred aseptically to sterile 5-mL sample tubes. Within 1 h of sampling, the tubes were transferred to a –20°C freezer and then stored at that temperature until subsequent analysis.

Yeast isolation

Subsamples (0.1 g) of the glacial sediment or soil were directly placed on potato dextrose agar (PDA; Difco, Becton Dickinson Japan, Tokyo, Japan) containing 50 μg/mL chloramphenicol and incubated at 10°C for a period of up to 3 weeks. Yeast samples were chosen for isolation based on colony morphology. Two cream-colored yeast colonies were isolated from glacial sediment and from soil located 40 m from the glacier terminus. They were purified by repeated streaking on fresh PDA. The resulting pure cultures of *V. ellesmerensis* were deposited at the Japan Collection of Microorganisms (JCM), Riken, Japan and at the UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada.

DNA sequencing and phylogenetic analysis

DNA was extracted from yeast colonies using an ISOPLANT II kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's protocol. The fragment covering the ITS region and D1/D2 domain was amplified from extracted DNA by polymerase chain reaction (PCR), using KOD-plus DNA polymerase (Toyobo, Osaka, Japan) and the fragment covering the ITS region and D1/D2 domain was amplified using the following primers: ITS1F (5′-GTAACAAGGTTTCCGT) and NL4 (5′-GGTCCGTGTTTCAAGACGG). The PCR conditions were as follows: primary template melting, 5 min at 94°C; 35 cycles of 10 s at 98°C (melting), 30 s at 54°C (primer annealing), and 90 s at 68°C (elongation); and 10 min at 68°C (final elongation). The PCR was performed using an Eppendorf Mastercycler Nexus (Eppendorf Japan, Tokyo, Japan). The amplified DNA fragments were purified using Sephacryl S-400HR (Sigma-Aldrich Japan, Tokyo, Japan). Sequences were determined using an ABI Prism 3130xl Sequencer (Applied Biosystems, Life Technologies Japan, Tokyo) [17].

The contiguous ITS and D1/D2 region sequences were aligned with the MAFFT program ver. 7.273 [18] using the L-INS-I algorithm. Maximum likelihood with an HKY+G+I model was performed using MEGA7 [19]. A bootstrap analysis with 1000 replicates was performed to estimate the confidence of the tree nodes and a bootstrap percentage of at least 50% was considered supportive in all constructed trees in this study. *Saitozyma podzolica* CBS

6819^{T} (AF444321/AF075481) was used as an outgroup in this analysis.

We also determined the sequence identities and nucleotide variation in the ITS region and D1/D2 domain among the species most closely related to *V. ellesmerensis* using the EMBOSS water alignment tool (http://www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html).

Physiological and morphological characterizations

The effects of temperature on the growth of fungi on PDA plates were determined for the temperature range of –3 to 25°C (at –3°C, 4°C, 10°C, 13°C, 15°C, 17°C, 20°C, 22°C and 25°C) for up to 8 weeks. The assessment of carbon assimilation was performed in glass vials with yeast nitrogen base liquid medium for carbon assimilation tests according to standard methods [20], with incubation for 2 weeks at 15°C. Assimilation of nitrogen and other physiological tests were also carried out in glass vials according to previously described protocols [20]. Strains were examined for their sexual state after growth on the following media, which were incubated at 15°C for up to 8 weeks: yeast mold agar (YMA, 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, and 20 g/L agar), 5% malt extract agar (5% MA, 50 g/L malt extract, and 30 g agar/L), and corn meal agar (CMA; Difco). The temperatures in the incubator was regularly verified with an alcohol thermometer. All experiments were carried out independently in three vials or on three plates and the results were recorded at the end of each week of incubation.

RESULTS AND DISCUSSION

DNA sequencing and phylogenetic analysis

In total, 325 fungal strains were isolated from the nine glacial sediment samples collected from the Walker Glacier site in the extreme High Arctic. Strain JCM 32573^{T} was isolated from surface sediments scraped from the glacier, and strain JCM 32574 was isolated from surface mineral soil deposits located 40 m from the glacier terminus, within the zone of retreat since 1959 (see above). Among these fungal strains, both were classified as belonging to the genus *Vishniacozyma* based on sequence similarity of the ITS region and the D1/D2 domain. Phylogenetic analysis of the ITS region and D1/D2 domain indicated that the two isolated strains represented a novel species that was closely related to *V. globispora* and *V. dimennae* in the *Vishniacozyma* clade (Fig. 1). The ITS region and D1/D2 domain sequences of the new *Vishniacozyma* species were therefore compared with the corresponding sequences from these closely related species. The ITS region of *V. ellesmerensis* exhibited 87 and 84 nucleotide

substitutions relative to those of *V. dimennae* and *V. globispora*, respectively, giving sequence identities of 81.6% and 82.2% (Table 1). In the D1/D2 domain, there were 57 and 61 nucleotide substitutions between *V. dimennae* and *V. globispora*, respectively (Table 1). Strains JCM 32573 and JCM 32574 exhibited 100% sequence identities with each other in their ITS regions and D1/D2 domains and were considered to represent a novel species.

Physiological characterization

The genus *Vishniacozyma* produces starch-like compounds and can utilize galactose, trehalose, and cellobiose, but cannot assimilate nitrate, methanol, and ethanol [12]. Our isolates from the High Arctic glacier site exhibited all of these characteristics, confirming that they belonged to the genus *Vishniacozyma*. Cultures of *V. ellesmerensis* could assimilate inulin, melezitose, and lactose and grew in vitamin-free conditions, but they were not able to utilize citrate and could not grow at 22°C on PDA for up to 8 weeks. In contrast, *V. dimennae* and *V. globispora* showed the opposite physiological characteristics (Table 2). The optimum growth temperature of *V. ellesmerensis* was 15-17°C, and the maximum growth temperature tolerated by this new species was 20°C.

Based on their phylogenetic and physiological characteristics, strains JCM 32573 and JCM 32574 represent a novel species in the genus *Vishniacozyma*, for which the name *V. ellesmerensis* sp. nov. is proposed.

Description of *V. ellesmerensis* M. Tsuji sp. nov.

The name *V. ellesmerensis* (el.les.mer.en'sis. N.L. fem. adj. ellesmerensis refers to the collection site for this species on Ellesmere Island, Nunavut, near the northern terrestrial limit of Canada). Yeast cells after 1 week at 15°C on CMA were ellipsoidal to cylindrical in shape and measured 6–8 μm × 3–4 μm, with proliferation occurring by polar budding (Fig. 2). Sexual activity was not observed. Pseudohyphae and true hyphae were not formed. Streak culture after 1 week on 5% MA at 15°C yielded yellowish cream- or pinkish-colored colonies, with a smooth and glossy surface and an entire margin. Glucose was not fermented. Glucose, D-galactose, sucrose, D-arabinose, L-arabinose, cellobiose, lactose, maltose, melezitose, raffinose, D-ribose, L-rhamnose, L-sorbose (weak or slow), trehalose D-xylose, galactitol, D-glucitol, inulin, *myo*-inositol, D-mannitol, ribitol (positive or weak), D-xylitol, starch, salicin, DL-lactate, succinate, D-gluconate, D-glucuronate, and methyl-α-D-glucoside were assimilated. Melibiose, *N*-acetyl-D-glucosamine, ethanol, methanol, erythritol, glycerol, citrate, potassium nitrate, and sodium nitrate were not assimilated. No growth occurred on 50% (w/v) glucose and 5% glucose

medium with 10% NaCl (w/v) and 0.01% cycloheximide. DBB and urease reactions were positive. Amino acids and vitamins were not required for growth. The maximum temperature for growth was 20°C, and optimal growth was observed at 15–17°C. Growth occurred at –3°C on PDA.

The holotype JCM 32573^{T} was isolated from glacial sediments at Walker Glacier, Ellesmere Island (lat. 83°00'N, long. 72°12'W), Nunavut, Canada, and has been preserved in a metabolically inactive state at the Japan Collection of Microorganisms, Riken, Japan, and the UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Toronto, Canada as UAMH 11973^{T}. The paratype JCM 32574 was isolated from soil deposits located 40 m from the glacier terminus, and has also been deposited in the JCM, and in the UAMH as UAMH 11974. The MycoBank deposit number is MB825501.

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Conflicts of interest

The authors declare no conflicts of interest.

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Figure Legends

Fig. 1. Phylogenetic tree based on the ITS region and D1/D2 domain sequences. Maximum likelihood analysis of the ITS region and D1/D2 domain sequences of *Vishniacozyma ellesmerensis* and closely related species. The *V. ellesmerensis* strains investigated in this study are highlighted in bold font. *Saitozyma podzolica* CBS 6819 was designated as the outgroup. The tree was constructed by maximum likelihood analysis with MEGA7. Bootstrap values higher than 50% are shown. The scale bar represents 0.05 substitutions per nucleotide position.

Fig. 2. Morphology of *Vishniacozyma ellesmerensis*

Vegetative cells of *Vishniacozyma ellesmerensis* after growth at 15°C for 1 week on CMA. *Bar*: 10 μm.

Table 1 – Number of nucleotide substitutions in the D1/D2 domain and ITS region sequences among the type strains of species in the genus *Vishniacozyma*.

	1	2	3	4	5	6	7	8	9	10	11	12
1		50	54	11	57	18	56	18	17	25	13	10
2	125 (76.0)		57	51	16	52	35	52	46	52	47	50
3	93 (78.4)	87 (81.6)		51	61	54	55	49	43	50	59	55
4	46 (91.2)	125 (77.1)	95 (78.1)		58	7	51	12	8	18	15	11
5	129 (76.1)	21 (95.8)	84 (82.2)	122 (77.6)		54	43	59	53	59	56	56
6	50 (90.2)	125 (76.1)	96 (77.7)	11 (97.8)	123 (76.4)		51	13	9	14	17	13
7	N	N	N	N	N	N		49	42	47	48	50
8	35 (91.4)	58 (83.9)	70 (80.7)	29 (92.6)	75 (81.2)	29 (92.6)	N		13	13	19	18
9	43 (90.5)	93 (77.9)	63 (81.7)	6 (98.7)	96 (78.1)	11 (97.5)	N	24 (93.5)		17	8	11
10	71 (86.4)	132 (76.0)	105 (76.0)	49 (90.8)	134 (75.6)	43 (91.6)	N	47 (88.4)	46 (89.8)		22	19
11	20 (96.2)	136 (75.0)	94 (78.2)	50 (90.2)	135 (74.1)	53 (89.6)	N	40 (90.1)	44 (90.3)	73 (85.8)		10
12	29 (94.4)	133 (75.6)	93 (78.4)	54 (89.8)	129 (76.2)	56 (89.0)	N	18 (95.1)	51 (88.7)	73 (86.4)	43 (91.8)	

Species: 1. *Vishniacozyma carnescens*; 2. *V. dimennae*; 3. *V. ellesmerensis* sp. nov.; 4. *V. foliicola*; 5. *V. globispora*; 6. *V. heimaeyensis*; 7. *V. nebularis*; 8. *V. penaeus*; 9. *V. psychrotolerans*; 10. *V. taibaiensis*; 11. *V. tephrensis*; 12. *V. victoriae*.

The upper right triangle shows the number of nucleotide (nt) substitutions in the D1/D2 domain sequence. The lower left triangle indicates the number of nt substitutions and the sequence identity (%, in parentheses) between each pairs of species in the ITS region sequences.
N: data not available for comparison.
The ITS region sequence of *V. nebularis* has not been deposited in a DNA database.

Table 2. Comparison of selected phenotypic properties of *Vishniacozyma ellesmerensis* and phylogenetically closely related species

Characteristics	1	2	3
Assimilation of			
Inulin	+	–	–
Melezitose	+	–	–
Lactose	+	–	+
Maltose	+	+	–
D-Ribose	+	–	+
Soluble starch	+	V	–
Ethanol	–	–	+
myo-Inositol	+	–	+
DL-Lactate	+	–	w
Citrate	–	s	w
Growth on/at			
Vitamin-free	+	–	–
20°C	W	+	+
22°C	–	+	+
25°C	–	+	+

Species: 1. *Vishniacozyma ellesmerensis* sp. nov.; 2. *V. globispora*; 3. *V. dimennae* +, positive; w, weak; s, slow; -, negative; v, variable. Data for *V. globispora* and *V. dimennae* are taken from Boekhout et al. [9], Fonseca et al. [10], and this study.