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THE DIVERSITY, EXTRACELLULAR ENZYMATIC ACTIVITIES AND PHOTOPROTECTIVE COMPOUNDS OF YEASTS ISOLATED IN ANTARCTICA

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ABSTRACT

The diversity of yeasts collected from different sites in Antarctica (Admiralty Bay, King George Island and Port Foster Bay and Deception Island) and their ability to produce extracellular enzymes and mycosporines were studied. Samples were collected during the austral summer season, between November 2006 and January 2007, from the rhizosphere of *Deschampsia antarctica*, ornithogenic (penguin guano) soil, soil, marine and lake sediments, marine water and freshwater from lakes. A total of 89 isolates belonging to the following genera were recovered: *Bensingtonia, Candida*, *Cryptococcus, Debaryomyces*, *Dioszegia, Exophiala, Filobasidium, Issatchenkia* (*Pichia*), *Kodamaea, Leucosporidium*, *Leucosporidiella, Metschnikowia, Nadsonia, Pichia*, *Rhodotorula*, and *Sporidiobolus*, and the yeast-like fungi *Aureobasidium, Leuconeurospora* and *Microglossum. Cryptococcus victoriae* was the most frequently identified species. Several species isolated in our study have been previously reported to be Antarctic psychophilic yeasts, including *Cr. antarcticus*, *Cr. victoriae*, *Dioszegia hungarica* and *Leucosporidium scottii*. The cosmopolitan yeast species *A. pullulans*, *C. zeylanoides*, *D. hansenii, I. orientalis, K. ohmeri, P. guilliermondii, Rh. mucilaginosa*, and *S. salmonicolor* were also isolated. Five possible new species were identified. Sixty percent of the yeasts had at least one detectable extracellular enzymatic activity. *Cryptococcus antarcticus, D. aurantiaca, D. crocea, D. hungarica, Dioszegia* sp., *E. xenobiotica, Rh. glaciales*, *Rh. laryngis*, *Microglossum* sp. 1 and *Microglossum* sp. 2 produced mycosporines. Of the yeast isolates, 41.7% produced pigments and/or mycosporines and could be considered adapted to survive in Antarctica. Most of the yeasts had extracellular enzymatic activities at 4°C and 20°C, indicating that they could be metabolically active in the sampled substrates.

Key words: yeasts; Antarctica; diversity; extracellular enzymes; mycosporines.

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INTRODUCTION

The continent of Antarctica has a range of extreme climatic conditions and is considered to be one of the harshest ecosystems in the world. The low temperature, low water availability, low annual precipitation, frequent freeze–thaw cycles, strong winds, high sublimation and evaporation, and the high incidence of solar and especially ultraviolet radiation are significant limiting factors for plant and animal life in Antarctica (8). In addition, the geographic isolation and environmental stress make interesting the study of its endemic organisms (34). Recently, the occurrence and diversity of fungal species in this region has been investigated (2, 13, 32, 33, 34). According to Ruisi *et al*. (34), most of the fungi recorded in this area are anamorphic, have short life cycles, and limit metabolic costs associated with sexual reproduction. More than 1,000 fungal species have been reported from the maritime, continental, and sub-Antarctic regions (6, 29).

Yeasts are able to grow at the low temperatures of Antarctica, suggesting that they are capable of degrading organic compounds at these temperatures and being an active part of the nutrient cycle (1, 36). Most yeast species of Antarctica are basidiomycetous anamorphs, but ascomycetous species may also be endemic to Antarctic habitats (23, 37, 40). The adaptation of these yeasts to cold habitats is also reflected in their structural and biochemical characteristics (37). The production of cold-adapted enzymes, cryoprotectants, and high amounts of polyunsaturated fatty acids in cytoplasmic membranes are examples of these adaptive strategies (40). In addition, the production of photoprotective compounds (carotenoids and mycosporines) by yeasts could be a strategy to survive in extreme environments. Mycosporines are water-soluble, UV-absorbing (310-320 nm) compounds with an aminocyclohexenone unit bound to an amino acid or amino alcohol group (21). The production of these compounds in yeasts from high-altitude lakes exposed to increased UV radiation has been correlated with the survival of some species in these environments (22). The photoprotective role of mycosporines, particularly mycosporine-glutaminol-glucoside, in yeasts as UV protectants and antioxidants has recently been reported (28). Carotenoids function primarily as antioxidants, and their UV-protective role has been also demonstrated in yeasts (26, 27).

Here, we describe the isolation and identification of yeast species present in the rhizosphere of the grass *Deschampsia antarctica* Desv. (*Poaceae*), ornithogenic (penguin guano) soil, soil, water from lakes, seawater and sediments collected from different locations in Admiralty Bay at King George Island and Port Foster Bay at Deception island, Antarctica. In addition, we tested the ability of these yeasts to produce extracellular enzymes and mycosporines.

MATERIALS AND METHODS

Sampling sites and collections

The samples were collected at different sites in the Antarctic Specially Managed Area (ASMA) in Admiralty Bay, King George Island, South Shetland Islands (62°09’S, 58°28’W) and Port Foster Bay, Deception island (62º55.5'S, 60º37'W), Antarctica. Fourteen collection sites were selected along Admiralty Bay and Port Foster Bay (Table 1), and all were sampled during the austral summer season between November 2006 and January 2007. Samples were collected from the rhizosphere of *Deschampsia antarctica* Desv. (*Poaceae*) (21 samples), ornithogenic (penguin guano) soil (8 samples), soil (11 samples), marine (4 samples) and lake (3 samples) sediments, marine water (5 samples) and lake freshwater (16 samples). The samples were collected with sterile bags (roots, sediments, and soils) or sterile bottles (seawater and freshwater) and transported to the laboratory on ice within 24 h for processing. Marine sediment samples were collected using a Van Veen grab at depths not exceeding 60 m and stored at 4°C until use, not exceeding 24 h.

Yeast isolation

Water from each site (10 mL) was filtered through 0.45-

µm membranes with a 47-mm diameter (Millipore, USA). The membranes were placed on YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2% glucose, 2% agar) containing 100 mg mL^{-1} chloramphenicol (Sigma, USA) and incubated at 15°C for 15 days. To obtain yeasts from soil, sediment, and the rhizosphere of *D. antarctica*, 1 g of each sample was added to 9 mL of Hanks buffer (Sigma, USA), and 100-µL serial dilutions (10^{-1} and 10^{-2}) were inoculated onto YM agar plates and incubated as above. The different yeast morphotypes were counted, purified and maintained on YM agar slants or liquid nitrogen for later identification. All yeast isolates were deposited in the Collection of Microorganisms and Cells of the Universidade Federal of Minas Gerais.

Yeast identification

The yeasts were characterized by standard methods (41), and identifications followed the keys of Kurtzman and Fell (17). Isolates with identical morphological and physiological characteristics were grouped together and subjected to PCR fingerprinting using the microsatellite-primed PCR technique (MSP-PCR) with the core sequences of the phage M13 (5'-GAGGGTGGCGGTTCT-3'). DNA extraction was performed according to the protocols described by Loque *et al.* (23). PCR was performed in a 25-µL reaction containing 2.5 µL of 10X Mg-free PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 1 µL of 2 mM deoxyribonucleotide triphosphates (dNTPs), 1 µL of each 10 *p*mol^{-1} of M13 primer, 5 µL of DNA template, and 0.2 µL of 1 U/µL Taq DNA polymerase (Fermentans, USA). The PCR was performed according to Libkind *et al.* (20). Isolates with identical DNA banding patterns were grouped and considered to belong to the same species (14, 35).

The internal transcribed spacer (ITS) and D1/D2 domains of the large subunit rRNA gene were sequenced in a representative strain of each MSP-PCR group. The D1/D2 domains were PCR-amplified with primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') according to Lachance *et al.* (19). The ITS domains of the rRNA gene were amplified using the universal primers ITS1 (5'-TCCGTAGGTGAACC TGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described by White *et al.* (42). ITS-amplification and sequencing protocols were performed as described by Vaz *et al.* (39). The amplified DNA was concentrated and cleaned using Wizard Plus SV columns (Promega, USA), and sequenced in a MegaBACE™ 1000 automated sequencing system (GE Healthcare, USA). The consensus sequence data were aligned with all sequences of related species in the GenBank database using Fasta 2.0 (3). The obtained nucleotide sequences and related sequences were aligned using ClustalW (http://www.ebi.ac.uk/clustalw/), and phylogenetic relationships were estimated using MEGA version 2.1 (16).

Extracellular enzymatic activity

All yeasts were tested for their ability to degrade starch, protein (casein), lipids (trybutirin and Tween-80), pectin and cellulose according to procedures described by Brizzio *et al.* (5). Calibrated suspensions of 10^6 cells mL^{-1} grown for 24-48 h were inoculated (10 µL) on the surface of agar plates (9). Plates containing each substrate were incubated at 4°C or 20°C. Enzymatic activity was analyzed after 5 days in samples incubated at 20ºC and after 21 days in those incubated at 4ºC. The halo/colony (h/c) ratio was used as a semi-quantitative assessment of extracellular enzymatic activity. *Sporobolomyces ruberrimus* CRUB1141 (amylase), *Leucosporidiella fragaria* CRUB1211 (protease), *Rhodotorula mucilaginosa* CRUB138 (lipase and pectinase) and *Trichosporon mycotoxinivorans* UFMG-HB20 were used as positive controls for enzymatic activities. Data for both temperatures were statistically compared using Student's *t*-test (9).

Production of photoprotective compounds

Mycosporine detection, extraction, and induction were analyzed as described by Libkind *et al.* (21). Briefly, the isolates were incubated for 4 days at 18ºC in an environmental

test chamber (Sanyo MLR 350) with a 12:12 light:dark photoperiod. The chamber was illuminated with 10 white light fluorescent tubes (Sanyo, 40W) and 5 Q-Panel 340 fluorescent tubes, resulting in photosynthetically active radiation (PAR), UVA and UVB irradiances of 66, 15, and 0.7 Wm^{-2}, respectively. For the screening analysis, isolated colonies were shielded with Ultraphan-395 film (UV Opak, Digefra, Munich, Germany, cutoff: 395 nm) and exposed to PAR only. After exposure, colonies were transferred to distilled water, centrifuged and stored at -20ºC until mycosporine extraction. The pigment production was evaluated by the direct observation of yeast colonies grown on YMA at 5ºC for 2 weeks.

RESULTS AND DISCUSSION

Yeast diversity and identification

The lowest yeast counts were from freshwater and seawater samples, ranging from 2 log CFU L^{-1} (meltwater of the Copacabana Refuge Lake) to 2.54 log CFU L^{-1} (meltwater of the Machu Picchu Station Lake) and 2 log CFU L^{-1} (seawater of Ullmann Point) to 2.24 log CFU L^{-1} (seawater of Botany Point). The highest counts were in soil and sediment samples (Table 1). In this work, counts in soil containing penguin guano ranged from 2 to 3 log CFU g^{-1}; ornithogenic soils may provide rich sources of organic matter. di Menna *et al*. (10) reported variable counts ranging from 0.69 to over 5 log CFU g^{-1} from Antarctic soil samples. Yeasts can grow in many soil types from diverse geographical areas ranging from the arctic to the tropics (4, 18, 30). The role of soil yeasts in terrestrial ecosystems, in particular Antarctic soils, has not been adequately explored.

A total of 89 yeast isolates were obtained and identified in following genera: *Bensingtonia, Candida*, *Cryptococcus, Debaryomyces*, *Dioszegia, Exophiala, Filobasidium, Issatchenkia* (*Pichia*), *Kodamaea, Leucosporidium*, *Leucosporidiella, Metschnikowia, Nadsonia, Pichia*, *Rhodotorula*, and *Sporidiobolus*, and the yeast-like fungi *Aureobasidium, Leuconeurospora* and *Microglossum* (Tables 2 and 3). Most were basidiomycetous anamorphs, and *Cryptococcus* species were the most frequently isolated yeasts in all samples. *Cryptococcus* species are frequently found in a variety of Antarctic sites and substrates, and many are psychrophilic (40). *Cryptococcus* species may be able to utilize available nutrients in oligotrophic systems, whereas most ascomycetous species cannot (7).

Table 1. Location of sampling sites and viable yeast counts isolated in different sources in Antarctica.

Substrate	Sites and location	Number of samples	Log CFU/g or log CFU/L[a]	Mean Log CFU/g or CFU/L	Standard deviation (Log)
Rhizosphere of *Deschampsia antarctica* Desv. (*Poaceae*)	Machu Picchu Station (62°07'S, 58°23'W); Plaza point (62°03'S, 58°24W); Brazilian Refuge II (62°04'S, 58°25'W) Demay point (62°12'S, 58°19'W); Hennequin point (62°05'S, 58°24'W); Botany point (62°05'S, 8°19'W); Ulmann point (62°05'S, 58°20'W); Henry Arctowisky Station (62°09'S, 58°27'W)	21	2 – 3.2[b]	2.4	0.4
Ornithogenic (penguin guano) soil	Demay point (62°12'S, 58°19'W); Chabrier Rock (62°11'S, 58°18'W)	8	2 - 3	2.1	0.3
Soil	Ulmann point (62°05'S, 58°20'W); Henry Arctowisky Station (62°09'S, 58°27'W); Botany point (62°05'S, 8°19'W); Comandante Ferraz Brazilian Station (62º05'S, 58º24'W)	11	2 – 2.9	2.3	0.3
Sediment	Port Foster, Deception Island (62º55'S, 60º37'W); Macchu Picchu station (62°07'S, 58°23'W); Copacabana United States Refuge (62°10'S, 58°26'W)	7	2 – 3.2	2.4	0.4
Seawater	Botany point (62°05'S, 58°19'W); Ulmann point (62°05'S, 58°20'W)	5	2 – 2.9	2.1	0.2
Freshwater from lakes	Macchu Picchu Station (62°07'S, 58°23'W); Copacabana United States Refuge (62°10'S, 58°26'W)	16	2 – 3.7	2.3	0.5

[a]CFU(colony forming units)/g or /L; [b] Minimal and maximum counts.

Table 2. Identification of yeasts isolated from different substrates in Antarctica.

UFMGCB code	Closest related species/GenBank accession numbers	Similarity (%)	Identification
ANT 92	*Aureobasidium pullulans* DBVPG 5028 / GQ911487	100	*A. pullulans*
ANT 84, 85	*Bensingtonia yamatoana* CBS7243 / AF189896	99	*B. yamatoana*
ANT 28, 22, 33, 37, 44, 108, 109	*Candida glaebosa* CBS 5691 / CGU45757	99	*C. glaebosa*
ANT 60, 98	*Candida sake* CBS 5740 / AY536216	100	*C. sake*
ANT 168	*Candida spencermartinsiae* CBS 10894 / FJ008044	100	*C. spencermartinsiae*
ANT 130	*Candida zeylanoides* CBS 619 / U45832	99	*C. zeylanoides*
ANT 21	*Cryptococcus antarcticus* CBS7687 / A.F075488	99	*Cr. antarcticus*
ANT 29, 35, 53, 56, 77, 97, 100, 102, 103, 104, 106, 120, 128, MFA 17	*Cryptococcus victoriae* CBS 8884 / AF444741	99	*Cr. victoriae*
ANT 20, 105, 111, 112	*Debaryomyces hansenii* NRRL Y-17914 / DHU94927	99	*D. hansenii*
ANT 76	*Dioszegia aurantiaca* PYCC 5856 / AY562141	99	*D. aurantiaca*
ANT 68	*Dioszegia crocea* CBS 6714 / AF075508	99	*D. crocea*
ANT 73, EACF 103	*Dioszegia hungarica* CBS 5124 / AF314231	99	*D. hungarica*
ANT 99	*Dioszegia* sp. CRUB 1147 / EF595753	98	*Dioszegia* sp.
ANT 126, 141, 145, 149	*Exophialla xenobiotica* CBS 115831 / FJ358246	99	*E. xenobiotica*
ANT 39	*Filobasidium capsuligenum* CBS 4381 / AF444695	94	*Filobasidium* sp.
MF 50	*Issatchenkia (Pichia) orientalis* CBS 573 / AY497684	100	*I. orientalis*
MFA 14	*Kodamaea ohmeri* ATCC 46053 / AF335976	100	*K. ohmeri*
ANT 24, 62, 67, 80	*Leuconeurospora pulcherrima* CBS 343.7 / AF096193	97	*Leuconeurospora* sp.
ANT 61, 81, 91, 139, 170	*Leucosporidiella creatinivora* CBS8620 / AF189925	99	*L. creatinivora*
ANT 30, 69, 70, 71, 75, 78	*Leucosporidiella fragaria* CBS 6254 / AF070428	99	*L. fragaria*
ANT 101, 118	*Leucosporidiella muscorum* CBS 6921 / AF070433	99	*L. muscorum*
ANT 63, 133, 158, 160, 166, EACF 149	*Leucosporidium scottii* CBS 5930 / AF070419	99	*L. scottii*
ANT 116, 169	*Metschnikowia australis* NRRL Y-7014 / U76526	100	*M. australis*
ANT 96b, 142, 150, 152	*Microglossum rufum* AFTOL 1292 / DQ470981	92	*Microglossum* sp.1
ANT 146	*Microglossum rufum* AFTOL 1292 / DQ470981	89	*Microglossum* sp. 2
ANT 50, 54	*Nadsonia commutata* CBS 6640 / U73598	100	*N. commutata*
MF 36	*Pichia guilliermondii* CBS 2030 / U45709	99	*P. guilliermondii*
ANT 117, 121, 122, 123	*Rhodotorula glacialis* DBVPG 4797 / EF643740	99	*Rh. glacialis*
ANT 11, 131, 136	*Rhodotorula laryngis* CBS 2221 / AF189937	99	*Rh. laryngis*
ANT 153, MFC14	*Rhodotorula mucilaginosa* Y-17501 / AF189951	99	*Rh. mucilaginosa*
ANT 86	*Sporodiobolus salmonicolor* CBS 490 / AF070439	99	*S. salmonicolor*

Table 3. Yeast species isolated from different sources in Antarctica.

Identification	Origin of isolates													
	A(n=21)[a]	B(n=1)	C(n=7	D(n=1)	E(n=3	F(n=6	G(n=1)	H(n=1)	I(n=4)	J(n=2)	K(n=2)	L(n=3)	M(n=11)	N(n=5)
Aureobasidium pullulans	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Bensingtonia yamatoana	-	-	1	-	1	-	-	-	-	-	-	-	-	-
Candida glaebosa	-	1	3	-	-	2	-	-	-	1	-	-	-	-
C. sake	2	-	-	-	-	-	-	-	-	-	-	-	-	-
C. spencermartinsiae	-	-	-	-	-	-	-	-	-	-	-	1	-	-
C. zeylanoides	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Cryptococcus antarcticus	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Cr. victoriae	7	1	2	-	-	1	-	-	1	-	-	-	1	1
Debaryomyces hansenii	1	-	-	-	-	3	-	-	-	-	-	-	-	-
Dioszegia aurantiaca	1	-	-	-	-	-	-	-	-	-	-	-	-	-
D. crocea	1	-	-	-	-	-	-	-	-	-	-	-	-	-
D. hungarica	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Dioszegia sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Exophiala xenobiotica	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Filobasidium sp.	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Issatchenkia (Pichia) orientalis	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Kodamaea ohmeri	-	-	-	-	-	-	-	-	-	-	-	-	3	-
Leuconeurospora sp.	2	-	1	-	1	-	-	-	-	-	-	-	-	-
Leucosporidiella creatinivora	1	-	-	-	1	-	-	-	-	-	-	2	-	1
L. fragaria	5	-	1	-	-	-	-	-	-	-	-	-	-	-
L. muscorum	1	-	-	-	-	-	-	-	1	-	-	-	-	-
Leucosporidium scottii	-	-	-	-	-	-	1	-	-	-	2	3	-	-
Metschnikowia australis	-	-	-	-	-	-	-	-	1	-	-	1	-	-
Microglossum sp.1	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Microglossum sp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Nadsonia commutate	-	-	1	-	-	-	-	-	-	1	-	-	-	-
Pichia guilliermondii	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Rhodotorula glacialis	-	-	-	-	-	-	-	-	4	-	-	-	-	-
Rh. laryngis	-	-	-	-	-	-	-	1	-	-	2	-	-	-
Rh. mucilaginosa	-	-	-	-	-	-	-	-	-	-	-	-	3	1
Sporidiobolus salmonicolor	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total of isolates	26	2	9	1	3	6	1	1	7	2	4	7	9	11

[a]A. Rhizosphere of *Deschampsia antarc*tica Desv. (*Poaceae*); B. Ornithogenic soil (Demay point); C. Ornithogenic soil (Chabrie Rock); D. Soil (Cmte Ferraz Brazilian Station); E. Soil (Ulmann point); F. Soil (Henry Arctowisky Station); G. Soil (Botany point); H. Lake sediment (Port Foster, Deception Island); I. Marine sediment (Machu Picchu Station); J. Lake sediment (Copacabana United States Refuge); K. Seawater (Botany point); L. Seawater (Ulmann point); M. Freshwater (Copacabana United States Refuge); N. Freshwater (Machu Picchu Station); n= number of samples

We recovered *Cryptococcus antarcticus*, *Cr. victoriae*, *Dioszegia hungarica*, and *Leucosporidium scottii*, which are reported psychrophilic yeasts from Antarctica (40). According to Ruisi *et al*. (34) the majority of yeasts present in Antarctic habitats are psychrotolerants, and others are psychrophiles. The preponderance of psychrotolerance is probably a response to the wide temperature fluctuations in Antarctica that allows microorganisms to survive in unstable environments (29).

Cryptococcus victoriae was the most frequently identified species in our study. It was isolated from seven substrates, and most isolates were obtained from the rhizosphere of *D. antarctica*. It was also isolated from penguin guano, soil, sediment and freshwater samples. *Cryptococcus victoriae* was originally described from Antarctica, but some occurrences were recorded in Portuguese seawater as well as in the roots of *Puccinela distans* and *Atriplex sagittata* and spores of arbuscular mycorrhizal fungi collected in Germany (31, 40). The occurrence of *Cr. victoriae* in the rhizosphere of *D. antarctica* and roots of temperate plant species suggests that this yeast may prefer the rhizosphere of plants in cold and temperate ecosystems.

Leucosporidium scottii was isolated only from seawater (Table 3). This yeast inhabits low-temperature environments, mostly polar or temperate regions during cold seasons (12). Although the isolates were obtained from seawater in this study, this species is considered to have a widespread distribution and occupy different substrates.

The ascomycetous yeast *Metschnikowia australis* is indigenous to Antarctic seawater (11), and we isolated it from marine sediment and seawater (Table 3). *Metschnikowia australis* was the predominant yeast species associated with the thalli of the algal species *Adenocystis utricularis*, *Desmarestia anceps*, and *Palmaria decipiens* in Antarctica (23). These algal thalli were reported as a new habitat for *M. australis.* Therefore, its isolation from seawater substrates in our study suggests a widespread distribution in Antarctica. The rare yeast *Nadsonia commutata* was isolated from penguin guano soil and seawater sediment. This species was discovered in soil from East Falkland Island in the Atlantic Ocean and from soil in the Carpathian Mountains of Eastern Europe (15). *Nadsonia commutata* has a low maximum growth temperature (22-27^{o}C) and is considered to be a psychrophilic yeast (25). This is the first report of its occurrence in Antarctica.

Four isolates of *Rh. glacialis* were obtained from marine sediments of the Machu Picchu Station. This yeast is a true psychrophilic species and was originally isolated from alpine glacier cryoconite and mud in a thawing zone on the Stubaier Glacier near Innsbruck in Tyrol, Austria (24). This species was also isolated from subglacial and meltwater glacier sediments in Italy (38). The maximum growth temperature of the type strain of this species is 20^{o}C. However, two isolates in our study were able to grow at 25^{o}C.

Several cosmopolitan yeast species including *A. pullulans*, *C. zeylanoides, D. hansenii, I. orientalis, K. ohmeri, P. guilliermondii, Rh. mucilaginosa*, and *S. salmonicolor* were isolated (Table 3). Most of these mesophilic yeasts were isolated at low frequency in water samples and could represent immigrants carried by water or result from human presence in Antarctica.

In this work, some yeast isolates showed more than 1% nucleotide differences in the D1/D2 domains of the rRNA gene compared with the most closely related known species and thus could represent novel taxa. The isolate identified as *Filobasidium* sp. (UFMGCB-ANT39, GenBank access number HQ184184) had 20 substitutions compared to its closest related species, *F. capsuligenum*. The isolate identified as *Dioszegia* sp. had almost identical sequences to strain *Dioszegia* sp. CRUB 1147, which was isolated from altitudinal lakes in Argentina. The Antarctic and Argentinean isolates probably represent a new species closely related to *Dioszegia hungarica*. Four yeast-like isolates had 97% similarity (10 nucleotide differences and 5 gaps) compared with the type strain *Leuconeurospora pulcherrima* CBS 343.7 (GenBank accession number AF096193), and they could represent new species.

These strains were identified as Leuconeurospora sp. (UFMGCB-ANT62, GenBank access number HQ184181). The yeast-like fungal species identified as *Microglossum* sp. 1 (UFMGCB-ANT96b, GenBank access number HQ184182) and *Microglossum* sp. 2 (UFMGCB-ANT96b, GenBank access number HQ184183) had 92% (36 nucleotide differences and 10 gaps) and 89% (43 nucleotide differences and 20 gaps) similarity, respectively, compared with *Microglossum rufum* AFTOL 1292 (DQ470981). *Microglossum* sp. 1 differed from *Microglossum* sp. 2 by nine nucleotides in the D1/D2 domains of the rRNA gene, and both were considered to be two new, distinct species.

Extracellular enzymatic activity

Activity of at least a single extracellular enzyme was detected in 58 isolates (60% of total yeast isolates) at either 4 or 20ºC (Table 4). Cellulolytic and esterase activities were most frequent and were present in 76% of isolates. Significantly higher levels of Tween 80 hydrolysis (t=3.1461; p=0.00192) were observed at 4ºC compared to 20ºC. No significant differences between temperatures were observed for the other tested enzymatic activities. Most *Cr. victoriae* isolates were able to hydrolyze Tween 80 and cellulose at 4 and 20ºC, most of which were isolated from the rhizosphere of *D. antarctica*. Most *L. scottii* isolates could hydrolyze protease and pectinase at pH 7. Furthermore, most of the isolated yeasts were able to hydrolyze the compounds tested at low temperatures, providing further evidence of their metabolic adaptation to cold environments.

Production of photoprotective compounds

In this study, isolates belonging to the Tremellales group, including the pigmented species *Dioszegia aurantiaca, D. crocea, D. hungarica* and *Dioszegia* sp. and the non-pigmented species *Cr. antarcticus,* were mycosporine-positive (Table 4). All of the isolates were obtained from the rhizosphere of *D. antarctica*. Libkind *et al*. (22) detected mycosporine production in *Dioszegia* species isolated from lakes in the Patagonian Mountains, which can be explained by the high-altitude and exposure to high UV radiation. All *Cr. victoriae* isolates, also belonging to the Tremellales group, were mycosporine-negative. *Exophiala xenobiotica* and *Rh. laryngis* also produced mycosporines. Organisms that can synthesize UV-protective compounds can occupy a great diversity of habitats (34), especially those habitats exposed to high UV radiation (22) such as in this work. The ascomycetous yeast-like fungi *Microglossum* sp. 1 and *Microglossum* sp. 2 also produced mycosporines. This report is the first of mycosporine-positive yeast-like ascomycetous species in Antarctic environments.

Twelve isolated species produced carotenoids or other pigments. Pigment production is a strategy to survive the stressful conditions of Antarctica. Although it does not produce mycosporines, the ubiquitous species *R. mucilaginosa* enhances UV-B survival by producing the carotenoid pigment torularhodin (27). In our study, 41.7% of the isolated yeasts were able to produce pigments and/or mycosporines and thus could be considered adapted to survive in Antarctica. In addition, most of the yeast isolates had extracellular enzymatic activities at both temperatures tested, indicating that they may be metabolically active in the sampled substrates.

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Table 4. Extracellular enzymatic activity, mycosporine and pigment production of yeasts from Antarctica.

Yeast species	Number of positive isolates																
	n[a]	Lip(trybutirin)		AmA		EsA (Tween 80)		PrA		PecA pH7		PecA pH5		CelA		Myc[b]	Pigmented species[b]
		4ºC	20ºC	4ºC	20ºC	4ºC	20ºC	4ºC	20ºC	4ºC	20ºC	4ºC	20ºC	4ºC	20ºC		
Candida glaebosa	7													2		-	-
C. sake	2													1		-	-
C. spencermartinsiae	1													1		-	-
C. zeylanoides	1	1	1													-	-
Cryptococcus antarcticus	1			1	1						1					+	-
Cr. victoriae	14	1				8	8							5	9	-	-
Debaryomyces hansenii	4													2		-	-
Dioszegia aurantiaca	1															+	+
D. crocea	1					1	1									+	+
D. hungarica	2					1	1									+	+
Dioszegia sp.	1					1	1							1		+	+
Exophialla xenobiotica	4			1		2	2	1	1					1	3	-	+
Filobasidium sp.	1													1	1	-	-
Issatchenkia (Pichia) orientalis	1					1	1									-	-
Leuconeurospora sp.	4			1	2	2		2			1		1	3	2	-	+
Leucosporidiella creatinivora	5	2				5	3	3	3	2		1		2	2	-	-
Leucosporidiella fragaria	7					1	1	1	1					2	2	-	-
Leucosporidiella muscorum	2							2	2	1	1	1	1			-	+
Leucosporidium scottii	6	4	1			4	1	4	3	3	1			2		-	-
Metschnikowia australis	2					1										-	-
Microglossum sp. 1	4												1	2	2	+	+
Microglossum sp. 2	1													1	1	+	+
Nadsonia commutata	2					1	1	1						1	1	-	-
Pichia guilliermondii	1													1	1	-	-
Rhodotorula glacialis	4					4	1	2	2	1		1		1		-	+
Rh. laryngis	3					2	1							1		+	+
Rh. mucilaginosa	2													1		-	+
Total of isolates	84	8	2	2	3	34	22	15	12	7	4	3	3	31	24	14	31

[a]n: number of isolates; Lip: Lipase activity on Trybutirin agar; AmA: Amylolytic activity; EsA: Esterasic activity on Tween 80 agar; PrA: Proteolytic activity; PecA: Pectinolytic activity on pH 7 and pH 5; CelA: Cellulolytic activity; Myc:Mycosporine; +: positive; -: negative.

[b] All isolates belonging to a species with + result were positive for the character.

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