

Diversity of Soil Yeasts Isolated from South Victoria Land, Antarctica

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Abstract Unicellular fungi, commonly referred to as yeasts, were found to be components of the culturable soil fungal population in Taylor Valley, Mt. Discovery, Wright Valley, and two mountain peaks of South Victoria Land, Antarctica. Samples were taken from sites spanning a diversity of soil habitats that were not directly associated with vertebrate activity. A large proportion of yeasts isolated in this study were basidiomycetous species (89%), of which 43% may represent undescribed species, demonstrating that culturable yeasts remain incompletely described in these polar desert soils. *Cryptococcus* species represented the most often isolated genus (33%) followed by *Leucosporidium* (22%). Principle component analysis and multiple linear regression using stepwise selection was used to model the relation between abiotic variables (principle component 1 and principle component 2 scores) and yeast biodiversity (the number of species present at a

given site). These analyses identified soil pH and electrical conductivity as significant predictors of yeast biodiversity. Species-specific PCR primers were designed to rapidly discriminate among the *Dioszegia* and *Leucosporidium* species collected in this study.

Introduction

Large portions of the earth's terrestrial surface are covered by deserts or cold biospheres. Additionally, global climate change may increase the rate of desertification [23] and exposed land from glacier retreat in certain parts of the globe [33], yet surprisingly little is known about the community structure of soil eukaryotic microorganisms and their adaptation to these habitats. A recent study has shown that the first organisms to colonize newly exposed surfaces are not photosynthetic organisms, but are heterotrophic microbial communities [42] that primarily utilize ancient carbon sources [3]. The complexity of temperate soil communities makes the study of food-webs more difficult. The polar deserts of Antarctica are unique in that they have no vascular plants, therefore no rhizosphere, and the food-webs are presumably less complex than those found in lower latitudes. Unicellular fungi, broadly described as yeasts, may be the predominant soil fungi in interior continental regions of Antarctic [43, 45] and therefore integral to the function of these microbial soil communities.

The investigation of yeast biodiversity in extreme environments such as deep-sea hydrothermal vents, waters draining acidic mine tailings, and polar regions is an active field of research [13, 21, 22, 32, 40]. Organisms from these habitats may hold keys to nutrient cycling, metal detoxification, and food-webs in extreme environments, as well as

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potentially provide unique biomolecules for industry and medicine. Investigation of microbial activity in polar desert soils may give clues to the development of nascent soils and to early terrestrial ecosystems formed after much of the globe was covered by glaciers during “Snow-ball Earth” [26, 34]. Identification of microfungi from continental Antarctica, especially in the dry sections of South Victoria Land, has been the focus of our recent work [13].

The exposed land area in Antarctica comprises less than 2% of the land mass of the continent [39], including both continental and maritime regions. The soil habitats span a wide range of moisture and organic carbon contents. South Victoria Land soils are typically polar deserts [7], although there are some areas near the Ross Sea that have higher nutrient input associated with vertebrate animal activity (e.g., bird or seal colonies) and higher soil moisture. The interior polar desert region has some of the oldest exposed surfaces on earth [7] with sharp gradients of soil surface salinity ranging from coastal to interior sections [11]. Apart from human research activities, there are no vertebrates in the interior desert sections and, unlike the sub-Antarctic and Antarctic peninsula, there are no vascular plants. Most of the biological activity is restricted to below ground and is possibly driven more by abiotic than biotic factors [24]. These soils have some of the lowest organic carbon levels on earth [4, 9, 25]. Additionally, organic carbon sources remaining from the shores of paleolakes may provide energy for soil communities dominated by heterotrophs [30, 31].

The variety of habitats across the Antarctic can support a range of cosmopolitan fungi, but the Ross Desert of South Victoria Land imposes more restrictive growth conditions of high UV, low moisture, multiple freeze-thaw cycles, and high winds, thereby limiting the active fungal communities to those most adapted to extreme environments [36]. Thus, the biodiversity of this soil system is less complex than those found in areas with vascular plants, and presumably more tractable for defining soil food-webs. These food-webs consist of bacteria, fungi, primary producers, and a small number of invertebrates, dominated by nematodes [17, 20]. Additionally, it is estimated that about 30% of the soils are completely devoid of soil invertebrates [20]. Although meta-genomic-based studies offer the opportunity to find an array of species that are not culturable, they may miss those that are not highly abundant in these low biomass soils and may identify some that are not active in the soil community. Furthermore, with culture-based methods, isolates can be assessed for individual physiological capabilities such as nutrient utilization, maximum growth temperature, and freeze-thaw survivability.

Although yeasts are thought to be primarily degraders and utilize simple sugars, the role of many, especially basidiomycetes, may be more complex, and their function in soil ecosystems remains unclear. Identification of the

yeast diversity in soil communities, especially those with a few members as the polar deserts, is an important step in the development of a model for food-web processes. To investigate the diversity of yeasts found in polar desert soil, we collected samples from a range of habitats not associated with high nutrients or with vertebrate activity. Sites associated with seabirds and marine mammals have higher nutrient inputs and human activity can disturb the soil and inadvertently disperse or deposit non-indigenous microorganisms. This study utilized one culture medium and two culture temperatures to isolate yeasts from soil samples. Data from this study show that, despite the limited scope of culture media, 43% of the basidiomycetous species isolated represent potentially novel species.

Materials and Methods

Sampling Sites

Twenty sites spanning a variety of soil types were surveyed in Southern Victoria Land (Fig. 1, Table 1). Seven sites in upper Wright Valley [the Labyrinths; 03Lab1, 03Lab2 (later named Dauphin pond), 03Lab3, 03Lab4, 03Lab6, 03Lab8] were each within a meter of the edge of a pond, and an additional site was within 5 m of the edge of a hyper-saline Don Juan Pond (03DJ1). One site on Sponsors Peak (03SP24) and one on a peak above Niebelungen Valley, in the Asgard Range, (03NB35) were sampled. The sample from the most northern site, 03SP24, was composed of Ferrar dolerite soil, and the Asgard Range site (03NB35) was from mafic dike and sandstone-derived soil.

Two short transects and five individual sites were established in Taylor Valley. One site was in the Lake Bonney basin valley bottom (03T14), at the edge of a seasonal stream flood-plain (Priscu Stream). Two more sites were located above the north shore of Lake Fryxell (03T21 and 03T23). Site 03T21 was near the stream run-off from a rock glacier. The fourth site (03T30) was on the marine side of Commonwealth Glacier along the north side of Taylor Valley. The fifth individual sample site, 03G5, was located in a region of older exposed soil on the Nussbaum Riegel. Transect CW was a short transect of two sites (03CW1 and 03CW2) 17 m apart, with 03CW1 being 14 m to the shore of a melt pond near the terminus of Commonwealth Glacier. The YB transect (03YB1, 03YB2, and 03YB3) followed a seasonal melt stream along the south side of Taylor Valley.

Taylor Valley soils are primarily unconsolidated alluvium consisting of sand-sized particles imbedded in cobble and boulders, composed of gneiss, diorite, and schist [7]. Typic Anhyturbels are most abundant in the coastal region of Taylor Valley while the drier interior regions are dominated by Typic Anhyorthels [8]. The most southern site near the

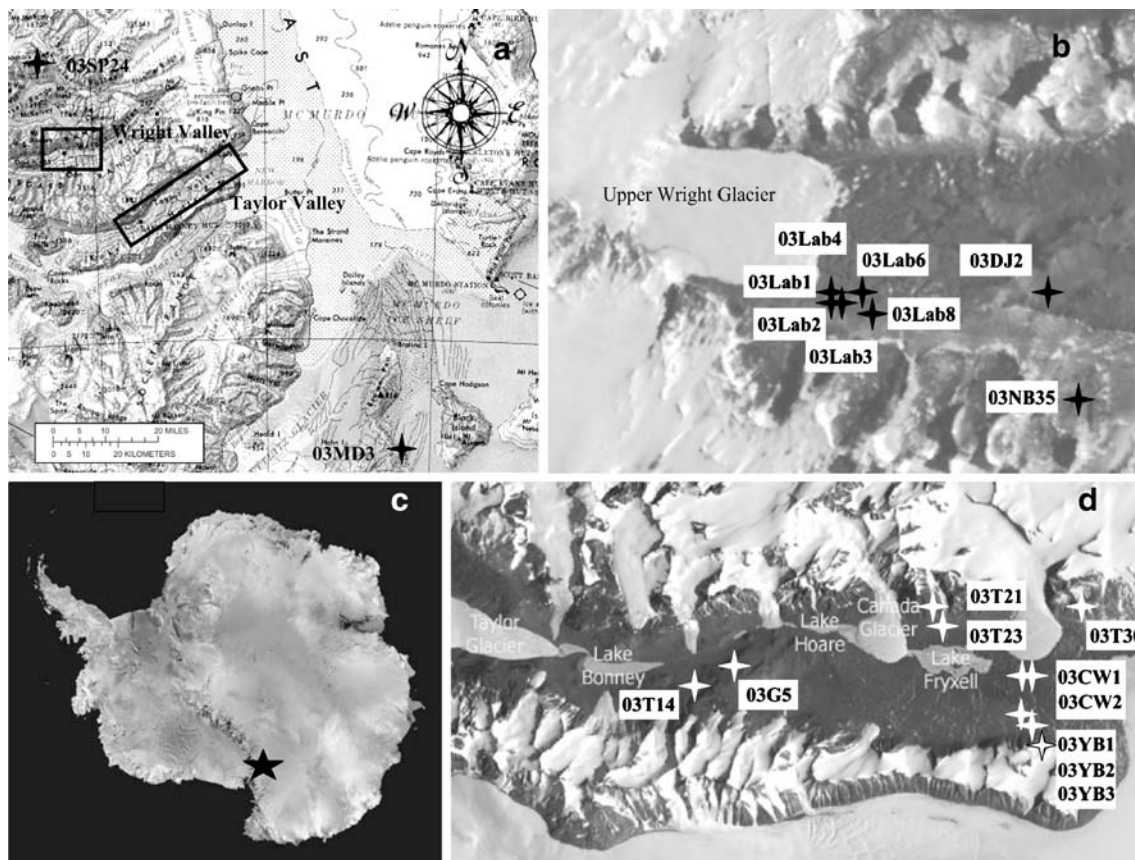


Figure 1 Sample site locations in South Victoria Land, Antarctica (2003–2004). Sites are identified by stars and labels. The entire study area with specific sites 03SP24 and 03MD3 is shown in (a). Sites in Wright

Valley are shown in (b) and Taylor Valley in (d). The location of the study area on the Antarctic continent is indicated with a star in (c)

foot of Mount Discovery (03MD3) was soil that consisted primarily of a thin layer of ground shells and rock (~5 cm) overlaying blue glacial ice.

All sites were sampled during the 2003–2004 austral summer season (November 2003–January 2004). Soil samples (200g) were recovered and handled aseptically as previously described [13]. Latitude and longitude coordinates were taken from each site using a Trimble geodetic real-time kinematic GPS receiver (UNAVCO) unit with submeter accuracy. The samples were transported to McMurdo Station, Crary laboratory, where they were processed within 12 h of arrival.

Soil Chemistry

Soil moisture, pH, electrical conductivity (EC), and salinity: soil moisture was determined using a gravimetric method [5]. A 1:2 soil/distilled deionized water solution was allowed to rehydrate for at least 2 h at 20°C for use in pH and EC determinations. Soil pH values were measured directly at 20°C using a benchtop Orion 720A pH meter [19]. Electrical conductivity was measured using a Corning 311 conductivity meter [35]. Soil salinity (Table 1) was

estimated from soil EC measurements automatically by the conductivity meter [35].

Culturing and Identification of Fungi

A 100-g sample of soil from each site was prepared by sterile distilled water extraction [1, 13]. A total of ten culture plates (100 mm) were inoculated with 100 or 200 μ l of soil–water slurry. A standard culture medium containing antibiotics to suppress bacterial growth [yeast peptone dextrose (YPD) with 0.25 mg ml⁻¹ chloramphenicol and 0.1 mg ml⁻¹ ampicillin] was used. Six plates (three with 100 μ l and three with 200 μ l inoculations) were incubated at 15°C for up to 8 weeks, and representative colony-forming units (CFU) were assessed twice weekly. An additional set of four plates per sample (two each 100 and 200 μ l inoculants) were incubated at 4°C to identify organisms that were restricted to growth below 15°C. Representative colonies from each group were isolated for identification using the ribosomal internal transcribed spacer (ITS) ITS1 and ITS2 as well as the intervening 5.8S gene. Taxonomic position inference was supported with isolate nutrient utilization using standard nutrient utilization proto-

Table 1 Location and soil properties of sites in South Victoria Land. Isolates found at each site are listed by number in Table 2

Site	Longitude (east)	Latitude (south)	Elevation (m)	Distance Marine (km)	Distance glacier (km)	Distance lake/stream (km)	pH	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)	Moisture (%)
03 DJ 1	161.18628	-77.56278	150	56.45	8.72	0.02	7.17	34,590	24.6	1.0
03 G 5	162.6678	-77.68502	490	19.58	1.96	1.62	8.78	461	0.2	3.2
03 Lab 1	160.71832	-77.56657	780	67.51	0.51	0.01	8.35	580	0.3	13.7
03 Lab 2 (Dauphin pond)	160.71352	-77.56498	760	67.57	0.36	0.01	8.06	93.5	0.1	4.9
03 Lab 3	160.70219	-77.56248	810	67.85	0.06	0.01	8.41	195	0.1	9.9
03 Lab 4	160.70834	-77.56035	840	67.69	0.15	0.01	8.69	89.5	0.1	3.0
03 Lab 6	160.75159	-77.56409	800	66.65	1.25	0.01	8.20	23,280	15.9	14.8
03 Lab 8	160.78484	-77.56779	800	65.82	2.10	0.01	8.94	296	0.2	13.3
03 NB35	161.30985	-77.5989	1694	53.32	3.35	1.98	6.76	3,430	2	3.1
03 SP 24	161.41522	-77.30592	1452	50.85	2.56	2.30	8.24	7,150	4.4	3.7
03 CW1	163.33284	-77.58849	25	4.31	0.19	0.04	9.8	915	0.5	4.8
03 CW2	163.33315	-77.58864	26	4.31	0.20	0.05	9.4	503	0.3	5.3
03 MD3	165.72356	-78.34693	30	45.44	0.00	2.99	9.46	439	0.2	8.24
03 T14	162.58627	-77.68990	100	25.48	0.50	0.07	9.0	334	0.2	3.3
03 T21	163.08686	-77.58961	410	10.04	0.29	0.12	9.4	190	0.1	3.4
03 T23	163.11427	-77.59987	25	8.27	2.52	0.13	10.0	793	0.4	3.9
03 T30	163.3677	-77.5600	130	3.28	0.28	0.50	10.1	414	0.2	3.8
03 YB1	163.35742	-77.62176	125	6.46	1.33	0.01	9.9	119	0.1	3.5
03 YB2	163.36777	-77.62318	140	6.39	1.03	0.03	9.6	105	0.1	3.6
03 YB3	163.37953	-77.62327	175	6.25	0.91	0.18	9.9	161	0.1	3.5

cols [50]. Most isolates were deposited into the Centraalbureau voor Schimmelcultures (CBS; <http://www.cbs.knaw.nl>) in Utrecht, The Netherlands. These isolates are referred to by their CBS designations (Table 2). Several isolates have not yet been recovered after a freezer malfunction and are listed with ANT numbers (Table 2).

DNA Extraction and Analysis

DNA was extracted from individual colonies of representative cultures using DNEasy Plus DNA extraction kit (Qiagen) following manufacturer's directions. The concentration of recovered DNA was determined using a picogreen dsDNA quantification kit (Molecular Probes) modified for use with a Turner Designs Picofluor handheld fluorometer following manufacturer's instructions. Extracted DNA from fungal isolates was used as a template in PCR reactions. The ribosomal DNA for the ITS and 5.8s regions, between primers ITS5 and ITS4 [49], was amplified as previously described [13]. The resultant amplicons were purified and sequenced using previously described methods [13] except that Sybresafe gel stain (Invitrogen) was used as a safer alternative to ethidium bromide with UV visualization. The subsequent chromatograms were edited using Vector NTI for Mac OS X (Invitrogen Life Science) or Sequencher (v 4.1 Gene Codes Corporation) and compared with sequences from the international GenBank database (<http://www.ncbi.nlm.nih.gov/>) using BLASTn search. The ITS sequences were

submitted to GenBank (Table 2). Isolates of *Dioszegia* sp 1 (CBS 10623 and ANT 03-101) and *Clavispora lusitaniae* (CBS 10625) were difficult to identify due to poor sequence quality and potential polymorphisms within the ribosomal regions. Intra-strain ITS polymorphisms have been described in other basidiomycetes fungi [48] and *Clavispora lusitaniae* has been shown to have highly variable ribosomal genes [29]. PCR products were generated from single colonies of each of these isolates and were separated on a 1.8% low-melt agarose gel (NuSieve GTG, FMC Biopolymers) in $1\times$ TAE buffer (40 mM Tris-acetate, 1 mM EDTA), visualized with Sybresafe (Invitrogen), and the DNA bands were excised. The isolated PCR products were ligated into pCR4-TOPO[®] (Invitrogen), transformed into chemically competent *E. coli* OneShot[®] TOP 10 cells (Invitrogen) and selected on LB ampicillin agar plates by standard methods [37]. Plasmid DNA was isolated from individual colonies with a Qiagen plasmid isolation kit (Qiagen) following the manufacturer's instructions and used as a sequencing template with primers and methods described above. Identification of species was based on GenBank matches to previously described species. Nutrient utilization analysis [27] was used for confirmation of identification and discrimination for members of *Dioszegia*, *Leucosporidium*, and *Cryptococcus*. The ribosomal D1/D2 and/or ITS sequences are presently available in the GenBank database for all described yeasts [28, 38], allowing for rapid identification of isolates as well as

Table 2 Species isolated from Southern Victoria Land soil

Species	Site isolated	Isolate number	GenBank accession	Closest match	Percent match
<i>Clavispora lusitaniae</i> ^a	03YB2	CBS 10625	EU149777	AY321475	99
<i>Cryptococcus nyarrowii</i>	03T21	CBS 10614	EU149778	AF400697	100
	03T23	CBS 10632	EU149780		
	03YB2	CBS 10740	DQ402536		
	03YB2	ANT 03-028	EU149779		
	03DJ1	CBS 10631	EU149783	AF444372	99
<i>Cryptococcus saitoi</i>	03Lab1	CBS 10628	EU149782		
	03Lab2	CBS 10630	DQ402537		
	03Lab6	CBS 10618	EU149781		
<i>Cryptococcus</i> sp 1	03YB1	ANT 03-149	EU149784	AB035045	94
<i>Cryptococcus carnescens</i>	03 G5	CBS 10755	EU149786	AB035050	99
	03CW1	CBS 10634	EU149785		
<i>Cryptococcus albidosimilis</i>	03Lab8	CBS 10619	EU149787	AF145325	100
<i>Cryptococcus vishniacii</i>	03Lab3	CBS 10616	EU149788	AF145320	100
<i>Debaryomyces hansenii</i> ^a	03Lab1	CBS 10629	EU149790	EF222227	100
	03Lab4	CBS 10751	EU149791		
	03T23	CBS 10686	EU149789		
<i>Dioszegia</i> sp 1	03CW2	CBS 10623	EU149792	AB049613	95
	03YB1	ANT 03-101	EU149793		
<i>Dioszegia</i> sp	2 03CW2	CBS 10637	EU149798	AF444379	91
	03T30	CBS 10750	EU149795		
	03YB1	CBS 10624	EU149799		
	03YB2	CBS 10613	EU149794		
	03YB2	CBS 10635	EU149797		
	03YB3	CBS 10615	EU149796		
	03YB3	CBS 10626	EU149800		
	03YB3	CBS 10627	EU149801		
<i>Leucosporidium</i> sp 1	03MD3	CBS 10633	EU149802	AF444529	87
	03T14	CBS 10684	EU149803		
	03T30	CBS 10641	EU149804		
<i>Leucosporidium</i> sp 2	03MD3	CBS 10638	EU149805	AF444529	96
	03CW1	CBS 10639	EU149806		
<i>Leucosporidium</i> sp 3	03MD3	CBS 10620	EU149807	AF444529	85
<i>Leucosporidium</i> sp 4	03MD3	CBS 10636	EU149808	AF444529	97
	03YB2	CBS 10640	EU149809		
<i>Mrakia stokesii</i>	03T30	CBS 10622	EU149810	AF144486	100
<i>Rhodosporeidium kratochvilovae</i>	03Lab6	CBS 10617	DQ402534	AF444520	100
<i>Rhodotorula laryngis</i>	03T23	CBS 10621	EU149811	AF444617	98
<i>Rhodotorula mucilaginosa</i>	03NB35	CBS 10685	DQ402533	AF444635	99
	03SP24	CBS 10752	EU149812		

Representative isolates with ITS GenBank accession numbers are listed. Isolates currently in the CBS collection are noted using the CBS accession number. The accession number of the closest match to described species listed in GenBank are shown

^a Members of Ascomycota

assignment of potential new species. Based on ITS sequence, several isolates represent potential previously undescribed species. Manuscripts for species descriptions and nutrient utilization are in development with coauthors and will appear separately.

Described species (from GenBank) and unknown sequences (from this work) were aligned with ClustalW followed by parsimony analysis with a heuristic search in random stepwise-addition (PAUP4.01b). Bootstrap analyses

were based on full heuristic search with TBR branch swapping. Bootstrap values are reported on the branches (only if >50%). The Ascomycetes *Debaryomyces hansenii* (GenBank accession number EF222227) and CBS 10686 (from this work) were used as an out-group. CBS10625 (from this work), which matched almost perfectly the Ascomycetes *Clavispora lusitaniae*, was not used as an additional out-group because of the very short ITS region presented by this species.

Table 3 Species specific primers used in this study

Target species	Species-specific primer sequence	Size of predicted fragment
Dioszegia sp 1	Dsp2F- TTCTATTATCTTTCTACAC	435 bases
Dioszegia sp 2	Dsp2F- TATATCCATAACACCTGTG	427 bases
Leucosporidium sp 1	Lsp1F- TAGTAGTCGGCCTTGGGAAGT	516 bases
Leucosporidium sp 2	Lsp2F- TAGAAAGTTGGGATTACAAA	517 bases
Leucosporidium sp 3	Lsp3F- ACTTTTGTAATCGAAGCAA	506 bases
Leucosporidium sp 4	Lsp4F2- AAAGTTGGGACTACACATT	514 bases

Primers target the ITS region of *Dioszegia* and *Leucosporidium* species

Development of PCR Primers for Species Identification

For more rapid identification of multiple isolates, we utilized a three-primer PCR method comprising a universal primer set used for all isolates and a species-specific third primer [12, 16]. Primers ITS 5 and ITS 4 [49] are the flanking outer primers that also function as internal PCR controls. The third species-specific PCR primer binds internally on the fragment produced by the two flanking primers. A positive result is signaled by the occurrence of a smaller band, correlated in size to a predicted fragment. Species-specific PCR primers were developed to discriminate among *Dioszegia* and *Leucosporidium* species, respectively. Primers were designed by comparison of ITS sequences from this study with GenBank submissions. Sites in the ITS1 region with highly variable sequence were targeted for species-specific primer development. Primer design was achieved using the program Oligo version 4 for Mac OS 9 (Molecular Biology Insights; Table 3). PCR amplifications and visualizations were carried out as previously described [12] using 3.5% I.D.NA agarose (Cambrex) and Sybresafe gel stain (Invitrogen). To confirm that the correct fragment was indeed amplified, the smaller species-specific amplicon from each primer set was excised and sequenced as described above.

Data Analysis

Statistical analysis was performed using StatView version 5.0.1 (SAS). Principal component analysis (PCA) was used to condense the data set containing seven abiotic habitat predictor variables (soil moisture, soil pH, soil EC, elevation, distance to the marine coast, distance to the nearest glacier, and distance to the nearest lake or stream) measured at each site. Soil EC was used in the PCA rather than salinity since it was directly measured and salinity was calculated from EC values. We then plotted the first two principal components to better visualize abiotic similarities/dissimilarities among sites. Multiple linear regression using stepwise selection was used to model the relation between abiotic variables (PC1 and PC2 scores) and yeast biodiversity (the number of species present at a given site). Statistical significance was determined as $P < 0.10$.

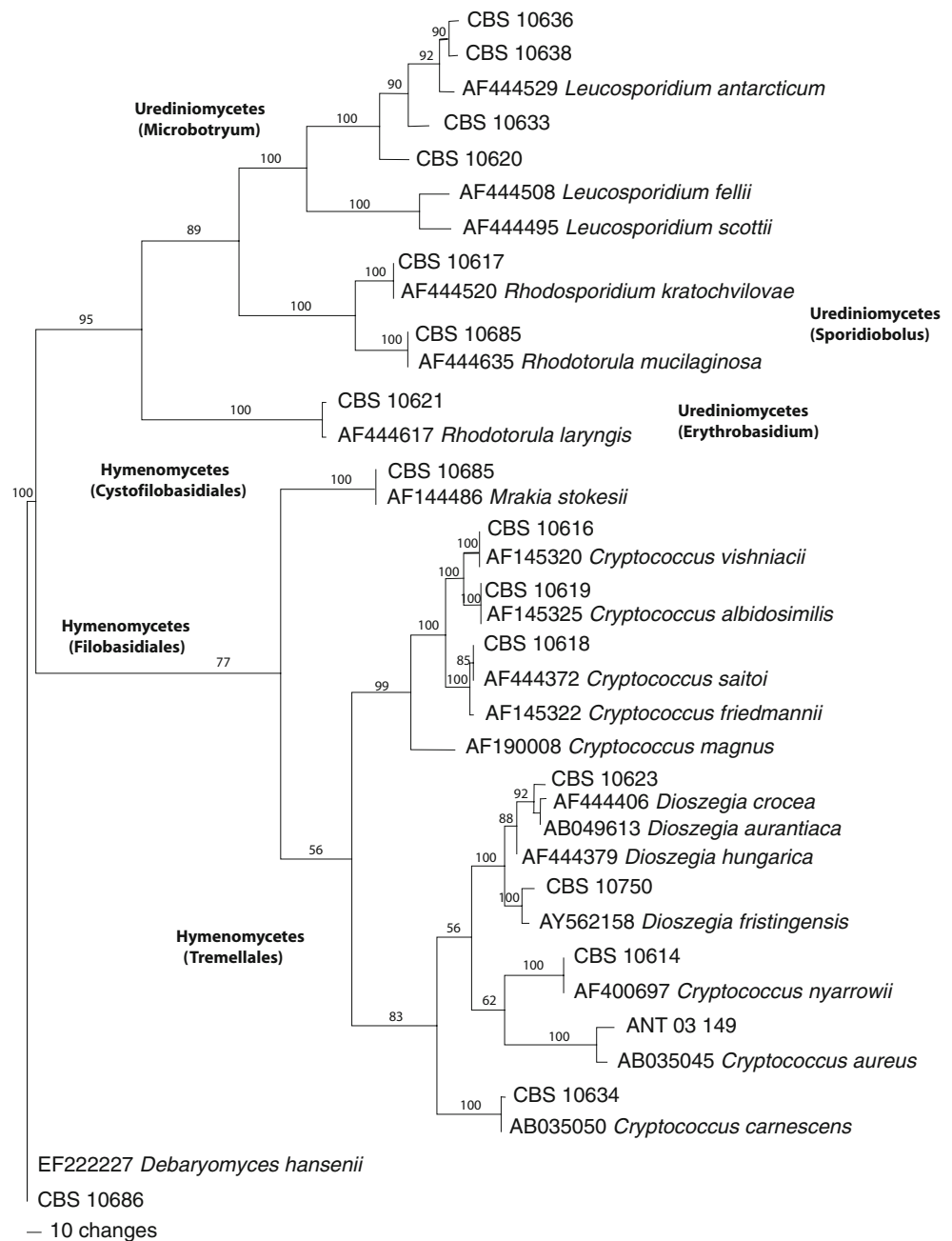
Results

Isolates of yeasts in this study were cultured from 20 sites (Fig. 1, Table 1) and were identified as 18 species from eight genera (Tables 2 and 4). Basidiomycetous species constituted

Table 4 Yeast species found at each site in this study

Site	Species found
03 DJ 1	<i>Cryptococcus saitoi</i>
03 G 5	<i>Cryptococcus carnescens</i>
03 Lab 1	<i>Cryptococcus saitoi</i> <i>Debaryomyces hansenii</i>
03 Lab 2 (Dauphin pond)	<i>Cryptococcus saitoi</i>
03 Lab 3	<i>Cryptococcus vishniacii</i>
03 Lab 4	<i>Debaryomyces hansenii</i>
03 Lab 6	<i>Cryptococcus saitoi</i> <i>Rhodospiridium kratochvilovae</i>
03 Lab 8	<i>Cryptococcus albidosimilis</i>
03 NB35	<i>Rhodotorula mucilaginoso</i>
03 SP 24	<i>Rhodotorula mucilaginoso</i>
03 CW1	<i>Cryptococcus carnescens</i> <i>Leucosporidium</i> sp 2
03 CW2	<i>Dioszegia</i> sp 1 <i>Dioszegia</i> sp 2
03 MD3	<i>Leucosporidium</i> sp 1 <i>Leucosporidium</i> sp 2 <i>Leucosporidium</i> sp 3 <i>Leucosporidium</i> sp 4
03 T14	<i>Leucosporidium</i> sp 1
03 T21	<i>Cryptococcus nyarrowii</i>
03 T23	<i>Cryptococcus nyarrowii</i> <i>Debaryomyces hansenii</i> <i>Rhodotorula laryngis</i>
03 T30	<i>Dioszegia</i> sp 2 <i>Leucosporidium</i> sp 1 <i>Mrakia stokesii</i>
03 YB1	<i>Cryptococcus</i> sp <i>Dioszegia</i> sp 1 <i>Dioszegia</i> sp 2
03 YB2	<i>Clavispora lusitaniae</i> <i>Cryptococcus nyarrowii</i> <i>Dioszegia</i> sp 2 <i>Leucosporidium</i> sp 4
03 YB3	<i>Dioszegia</i> sp 2

Figure 2 Phylogenetic tree depicting the relationship of yeast cultures isolated in this study to ITS GenBank sequences of published species. Parsimony analysis was conducted with a heuristic search in random step-wise-addition (PAUP4.01b). Bootstrap analyses were based on full heuristic search with TBR branch swapping

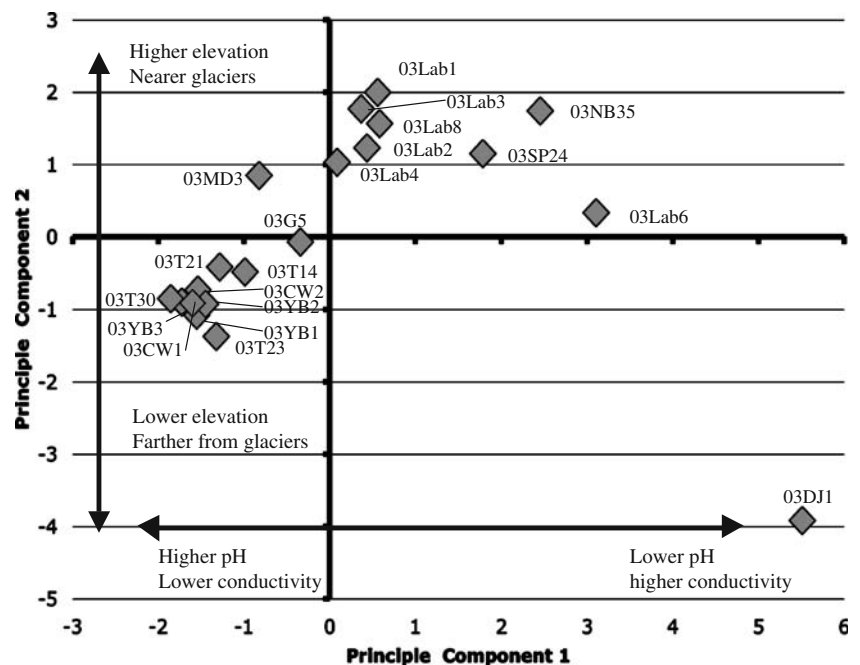


89% of the 18 species isolated. Forty-three percent of these Basidiomycetes are from potentially unidentified species. The ITS sequence data show a phylogenetic diversity of yeasts as depicted in Fig. 2. The tree was constructed to illustrate the relationship of yeast cultures isolated in this study to ITS GenBank sequences of published species. The top group, Urediniomycetes, [38] is divided into: *Microbotryum* (all the *Leucosporidium* species), *Sporidiobolus* (*Rhodotorula kratochvilovae*, *Rhodotorula mucilaginoso*), and *Erythrobasidium* (*Rhodotorula laryngis*, and CBS 10621). All the other in-group sequences represent Hymenomycetes [38] and

are divided in three groups: Filobasidiales (only *Cryptococcus* species), Tremellales (*Dioszegia* and *Cryptococcus* species), and Cystofilobasidiales (*Mrakia stokesii* and CBS 10622). The out-group comprises Ascomycetes (*Debaryomyces hansenii* and CBS 10686).

The species represented in this study are dominated by basidiomycetous yeasts (89%) with only two species representing ascomycetes (Table 2): *Debaryomyces hansenii* and *Clavispora lusitanae*. Six of the eighteen species belong to the genus *Cryptococcus* (33%) followed by four species of *Leucosporidium* (22%). Two *Dioszegia* and four

Figure 3 Principal components analyses of the abiotic variables used to characterize the relationships among environmental gradients across study sites. The implied gradients are indicated by arrows



Leucosporidium isolates may represent novel species. Members of both genera are known to be psychrophilic and therefore potentially active in the communities from which they were isolated. One *Cryptococcus* isolate may also represent a previously undescribed species.

Labyrinth sites (upper Wright Valley) and the Taylor Valley sites have a diversity of habitats based on abiotic factors. The first two principal components of these abiotic habitat variables (PC1 represents a transition of pH and EC; PC2 represents a transition of elevation and distance to glaciers) accounted for about 70% (44.6% and 24.9%, respectively) of the total variance in the original data set. PC1 was correlated most positively with soil EC and most negatively with soil pH. PC2 was correlated most positively with elevation and most negatively with distance from glaciers. Figure 3 is a simple plot of PCA results using principle component scores (PC1 represents a gradient in pH and EC; PC2 represents a gradient in elevation and distance to glaciers) and illustrates that the sites clustered in the lower left of the graph have higher pH and lower EC (predominantly Taylor Valley sites) than those in the upper right section (predominantly Wright Valley sites). In addition, sites in the Wright Valley cluster are closer to glaciers and at higher elevations than the sites in the Taylor Valley cluster. The Don Juan Pond site (03DJ1) contains hyper-saline soil and represents an outlying point for the Wright Valley sites.

Yeast species found in this study were not distributed randomly across the landscape. The analysis of yeast distribution (using linear regression with PC1 and PC2 as predictor variables against the number of species found at each site)

revealed that yeast biodiversity was associated significantly with PC1 ($P=0.06$; overall model adjusted $R^2=0.11$). The sites with the highest biodiversity had higher pH values and lower EC (Fig. 3 and Table 4). Additionally, components of some clades were found only in specific locations. For example, *Cryptococcus albidosimilis*, *Cryptococcus saitoi*, and *Cryptococcus vishniacii* were found only in the Labyrinths and in the Don Juan Pond area in the upper Wright Valley, habitats similar to those where *Cryptococcus albidosimilis* [47] and *Cryptococcus vishniacii* [46] were originally isolated. All of these species belong to the albidus clade in the Filobasidiales [38] and cluster with 100% bootstrap value. The three other *Cryptococcus* species (*Cryptococcus carnescens*, *Cryptococcus nyarrowii*, and *Cryptococcus* sp 1) were found only in the Taylor Valley sites. These three species belong to the Tremellales, a highly diverse group. The most southern site near Mount Discovery (03MD3) was dominated by the four *Leucosporidium* species (Table 4).

Comparison of the ITS region of all *Leucosporidium* and *Dioszegia* species in GenBank, as well as our isolates revealed suitable target sequences for the generation of diagnostic-sized PCR fragments. Six species-specific PCR primers (Table 3) were tested against isolates from six ribotypes in our collection (Fig. 4) in a three-primer PCR assay. Species-specific amplicons were obtained from all appropriate primer sets, and concurrent ITS fragments were noted frequently. The presence of both species-specific and universal ITS amplicons in a reaction is dependant on the target-to-primer ratio. This ratio can be shifted by variation of the ITS gene copy number in each of the isolates. The

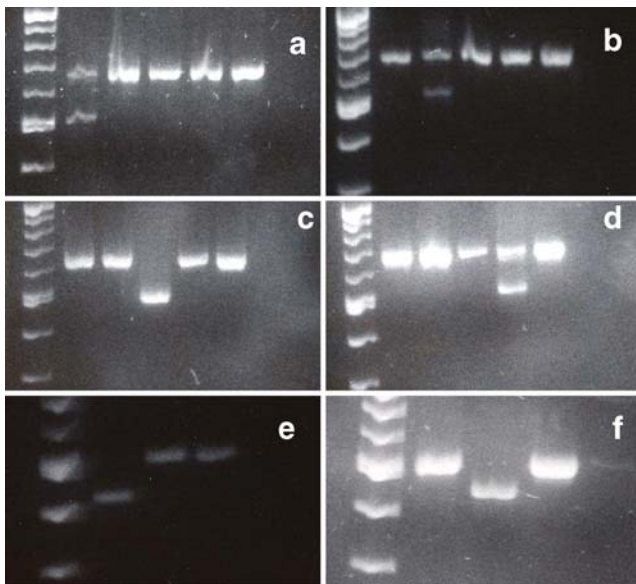


Figure 4 Agarose gel electrophoresis PCR products from four species of *Leucosporidium* (a–d) and two species of *Dioszegia* (e–f). Primers include two outer universal ITS primers (ITS5 and ITS4) [49] and an internal species-specific primer. Each panel is as follows: PCR products from reactions with *Leucosporidium* species are: (a) *Leucosporidium* sp 1 isolate CBS 10638 (b) *Leucosporidium* sp 2 isolate CBS 10639, (c) *Leucosporidium* sp 3 isolate CBS 10620 and (d) *Leucosporidium* sp 4 isolate number CBS 10636. Each set displays fragments as follows: lane 1, 100 base pair marker with the 500bp marker greater intensity; lane 2, *Leucosporidium* sp 1 primer; lane 3, *Leucosporidium* sp 2 primer; lane 4, *Leucosporidium* sp 3 primer; lane 5, *Leucosporidium* sp 4 primer; lane 6, no species-specific primer; lane 7, no DNA control. PCR products from reactions with *Dioszegia* species are: (e) *Dioszegia* sp 2 isolate CBS 10750 and (f) *Dioszegia* sp 1 isolate CBS 10623. Each set displays fragments as follows: lane 1, 100bp marker with the 500bp marker greater intensity; lane 2, *Dioszegia* sp 2 primer; lane 3, *Dioszegia* sp 1 primer; lane 4, no species-specific primers; lane 6, no DNA control and all three primers. All primer mixes showed great specificity with no detectable cross reactivity on non-target isolates

sequence of the smaller species-specific bands matched the predicted sequence in all cases, demonstrating specific priming and fragment amplification. All primer mixes showed great specificity with no detectable cross-reactivity on nontarget isolates.

Discussion

South Victoria Land habitats exhibit some of the most extreme conditions on earth, with very dry and cold locations, as well as some highly saline sites, yet a variety of yeasts were cultured from these soils. This study utilized a culture-dependant process with one medium and two temperatures. Despite this limited survey, 43% of all the basidiomycetous taxa isolated may represent novel species. In addition, several other isolates were not culturable after the soil was transported back to the University of Maine, thus not identified or included in this study. A more extensive examination of soil

samples with a broad array of media may generate more unique species and add to the list of yeasts that may participate in the active soil consortium. This result is not surprising for studies of yeasts in unique environments. For example, Gadanho et al. [22] found a high proportion of potentially novel species in Mid-Atlantic hydrothermal fields and in acidic-mine waste water systems [21].

A latitudinal study along the Antarctic peninsula showed that vegetation abundance and type were most significant for predicting fungal abundance [51]. Because the soils in this study have no vegetation of any kind, vegetation abundance and type cannot be used as a predictor. In an intercontinental-scale latitudinal study of yeast taxa distribution, Vishniac [44] found that temperature, rainfall (closely associated with net primary productivity—NPP), and EC could explain approximately 44% of the distribution of dominant yeast species. The same study found that the 32% of the distribution of clades in the most common orders were associated with rainfall and pH, and vegetation type could played the same role for orders [44]. The average soil moisture in this study was 5.7%, ranging from 1% (03DJ1) to 14.8% (03Lab6). There was no strong association found between soil moisture and yeast biodiversity; however, soil moisture in this study was taken at discrete time points and not as an average over the summer season. An abiotic factor that has been found to be important for other Antarctic desert soil inhabitants is moisture [52]. Although PC1 and PC2 do not find moisture to be an important component in itself, other factors, such as elevation, distance to glaciers, or distance to lakes or streams, certainly can affect the average soil moisture level over the entire year. An additional abiotic factor that fluctuates and was determined at only discrete time points is temperature. Yeast populations may rise and fall during the season in association with either moisture or temperature, and these data would not be captured in our model because of its “snap-shot” quality.

In a global study of soil bacterial diversity and richness, Fierer and Jackson [18] found that differences could best be explained by soil pH, with the lowest levels of bacterial diversity in acidic soils. The bacterial diversity study found that soils with pH values above pH 8.5 were rare, and the authors were not certain if the bacterial diversity would plateau at near normal pH values or continue to rise with higher pH values [18]. Most of the soil sampled in this study was basic, with pH values averaging pH 8.9 and ranging from pH 6.8 to 10.1, with the highest found in Taylor Valley. These values are well above the average soil pH found in Fierer and Jackson’s work [18]. The sites with higher pH values had the greatest yeast diversity, suggesting that overall soil microbe diversity may continue to rise rather than plateau with increasing pH. A previous study of fungal abundance (not diversity) in Taylor Valley Antarctic found

that filamentous fungi were associated with higher pH soils, but yeasts were more evenly distributed [13], demonstrating that yeasts were able to utilize a broader range of habitats in the polar desert soils. In this study, the association of yeast diversity (not abundance) with the PC1 factors of pH and EC may reflect the greater abundance of other organisms at those locations to participate in food-webs.

The predominance of *Cryptococcus* species in soil, particularly arid soil, has been ascribed to their ability to produce polysaccharide capsules [44], but there may be additional reasons. Although yeasts, in general, utilize simple sugars, some basidiomycetous species may follow different assimilation patterns [27]. *Cryptococcus* species may be able to utilize available nutrients in oligotrophic systems while most of the ascomycetous species cannot. In oligotrophic ocean waters, basidiomycetous yeasts account for the vast majority of species [15, 32], as well as in oligotrophic glacial meltwater in Argentina [14]. A study of soil associated with historic Antarctic huts and a limited number of South Victoria Land Dry Valleys soil samples, Arenz et al. [2], found that *Cryptococcus* species accounted for 67% (4 out of 6) of the yeast species identified in the Dry Valley soil and 72% (13 of 18) in soils surrounding the historic huts. However, studies of yeasts from other locations did not show this predominance of basidiomycetous yeasts. For example, the yeasts isolated from Mid-Atlantic hydrothermal vents were dominated by ascomycetous genera (12 of the 19 identified species) [22]. Since hydrothermal vents are known to have rich animal and microbial life, it is possible that there is abundant organic carbon available for yeasts, thus allowing ascomycetous genera to proliferate. The finding of *Cryptococcus* species from specific clades in the Labyrinths of Wright Valley segregated from the species found in Taylor Valley is interesting. It is likely that the habitat conditions restrict the occurrence of these species to each valley. It is also possible that the lack of suitable sample size has given the impression of segregation. Resolution of this question and acquiring enough data to address the null hypothesis in a statistically significant manner requires further investigation of many more sites across the region.

Simple isolation of yeasts from soil does not a priori indicate that the organism in question is indigenous. To be considered indigenous or adapted to the Antarctic soils, a yeast must not only be able to grow at cold temperatures and under oligotrophic conditions, but must also be able to survive multiple freeze-thaw cycles [43, 45]. For example, *Clavispora lusitaniae* has been isolated from Antarctica only once before and in association with liquid waste at field camps [6]. This isolate may represent an organism that is not an active member of the community but was rather blown into the soil from another location or transported by humans. *Rhodotorula mucilaginosa* was isolated from the two sites of highest elevation (Sponsors Peak and a peak

above Niebelungen Valley). This species is a common cosmopolitan yeast and has been isolated from a variety of very different habitats including dry Antarctic mountain peaks, Mid-Atlantic hydrothermal vents [22], deep sea Pacific sites [32], and Arctic glaciers [10]. Higher UV input at the poles has led to the hypothesis that UV may be an important ecological factor effecting soil microbial distribution in continental Antarctica [41]. Fungi that have dark pigments may have a selective advantage in areas with high UV stress; thus, it is possible that the highly pigmented *Rhodotorula mucilaginosa* is an indigenous organism due to adaptability and pigmentation. All isolates of *Cryptococcus saitoi* were found in Wright Valley, whereas other yeasts, such as *Leucosporidium* species, were found at many sites (including dead sponges found on the glacier surface near Mt Discovery and Taylor Valley moss—data not shown) and may represent new species. These factors together strongly support the hypothesis that *Cryptococcus saitoi* and the *Leucosporidium* species are truly indigenous. No *Leucosporidium* species were reported in the historic hut study [2], again indicating that further and more extensive investigations are required to develop a list of yeasts that are active soil consortium members in the various polar desert habitats.

The development of a PCR assay to rapidly identify some of the common *Leucosporidium* and *Dioszegia* isolates found in South Victoria Land should allow for more stream-lined assessment of community structure in future work. Building from this and other studies [16], additional sets of PCR primers are under development for discrimination among the *Cryptococcus* species identified from these soils.

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