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Research Article

Polyphenolic substrates and dyes degradation by yeasts from 25 de Mayo/King George Island (Antarctica)

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Abstract

Antarctica offers a range of extreme climatic conditions, such as low temperatures, high solar radiation and low nutrient availability, and constitutes one of the harshest environments on Earth. Despite that, it has been successfully colonized by 'cold-loving' fungi, which play a key role in decomposition cycles in cold ecosystems. However, knowledge about the ecological role of yeasts in nutrient or organic matter recycling/mineralization remains highly fragmentary. The aim of this work was to study the yeast microbiota in samples collected on 25 de Mayo/King George Island regarding the scope of their ability to degrade polyphenolic substrates such as lignin and azo dyes. Sixty-one yeast isolates were obtained from 37 samples, including soil, rocks, wood and bones. Molecular analyses based on rDNA sequences revealed that 35 yeasts could be identified at the species level and could be classified in the genera *Leucosporidiella*, *Rhodotorula*, *Cryptococcus, Bullera* and *Candida*. *Cryptococcus victoriae* was by far the most ubiquitous species. In total, 33% of the yeast isolates examined showed significant activity for dye decolorization, 25% for laccase activity and 38% for ligninolytic activity. Eleven yeasts did not show positive activity in any of the assays performed and no isolates showed positive activity across all tested substrates. A high diversity of yeasts were isolated in this work, possibly including undescribed species and conspicuous Antarctic yeasts, most of them belonging to oligotrophic, slow-growing and metabolically diverse basidiomycetous genera. Copyright © 2013 John Wiley & Sons, Ltd.

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Introduction

The Antarctic continent offers a range of extreme climatic conditions and constitutes one of the harshest environments conditions on Earth (low temperature, low humidity, high radiation, etc.) (Nedialkova and Naidenova, 2004). The exposed land area in Antarctica comprises < 2% of the land mass of the continent, including both continental and maritime regions. The soil habitats span a wide range of moisture and organic carbon contents (Connell *et al*., 2008). In recent decades the Antarctic regions have been investigated for the presence and exploitation of psychrophilic bacteria, archaea, algae and, more rarely, fungi (Ruisi *et al*., 2007; Kostadinova *et al*., 2009).

25 de Mayo/King George Island is the largest island within the South Shetland archipelago, northwest of the Antarctic Peninsula in the maritime Antarctic. The local climate is typical of the peri-Antarctic islands: humid and windy, cool with an average temperature of 1–3 °C in the warmest month and –7 °C in the coldest month, and very few sunny days indeed (Kostadinova *et al*., 2009).

Studies on these islands indicate that minimal soil temperature in winter are commonly buffered by overlying snow and remain above about –5 °C to 9 °C, even though short-term minimal air temperatures may be much lower. Similarly, short-term maximal soil temperatures in the range 14–26 °C are typically experienced (Krishnan *et al*., 2011).

Psychrophilic and psychrotolerant fungi significantly contribute to soil microbial biomass, playing a key role in decomposition cycles in cold ecosystems (Margesin *et al*., 2007; Xin and Zhou, 2007). These organisms present several adaptations in their membranes, enzymatic systems and genes of great biotechnological potential (Margesin and Schinner, 1994).

Although meta-genomic-based studies offer the opportunity to find an array of species that are not culturable, they may identify some individuals 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. Connell *et al*. (2008), showed that 43% of Antarctic yeast isolates were assigned to undescribed species, reflecting the lack of knowledge regarding cultivable yeasts that colonize the Antarctic soils.

Reactive dyes are among the most recalcitrant synthetic dyes against biodegradative processes and are considered a worldwide problem. Their pollution hazard is primarily based on carcinogenic or toxic components, such as aromatic amines and related compounds. Also, due to light absorption, they could significantly reduce photosynthetic activity in water bodies (Meehan *et al*., 2000; Stolz, 2001).

Most dye decolorization studies are nowadays focused on the employment of white rot fungi (WRFs), including *Phanerochaete chrysosporium* and *Trametes versicolor* (Yang *et al*., 2005). It is widely assumed that these WRFs could degrade synthetic dyes through their oxidative and non-specific ligninolytic enzyme system, which includes mainly lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase (Lac) enzymes (see e.g. Solís *et al*., 2012; Koyani *et al*., 2013). However, lignin has an extremely low nitrogen content when compared with reactive azo dyes.

The current study was designed to isolate yeasts from soils of the Potter Peninsula, 25 de Mayo/King George Island in Antarctica, and to study the polyphenolic substrates and dyes degradation.

Methods

Soil sampling and fungal isolation

Soil samples were collected during the 2011–2012 austral summer (January–March 2012) near the Argentinean scientific research station, Carlini (ex-Jubany) Base, located on the Potter Caleta, 25 de Mayo Island (62°14′18″S, 58°40′00″W).

Samples were collected from a range of locations around the Caleta, including an ornithogenic site; near to nesting birds (Punta Stranger); on the beach, near Refugio Elefante, two human-impacted areas (under the main dining room and near the gas oil tanks); and a largely pristine and naturally vegetated area (Tres Hermanos hill).

Samples (around 10 g) were taken from soil at a depth of 0–10 cm, using a sterile spatula. After collection, the samples were stored in sealed sterile bags or sterile flasks and immediately returned to the research station, where they were refrigerated at 4 °C, and subsequently treated for incubation and isolation.

For yeast isolation purposes, samples were subjected to two parallel procedures. A portion of each soil sample was excised under sterile conditions, using a sterile spoon or spatula, and directly spread onto Petri plates containing culture medium (see below). Simultaneously, another portion of the same sample was homogenized in an orbital shaker with a minimal volume of YM 1/10 medium (composition in g/l: yeast extract 0.3, malt extract 0.3, peptone 0.3, dextrose 0.5) for 3 h at 200 rpm and 15 °C; 100 ml of the resulting homogenate was spread onto Petri plates with the same medium plus 20% agar-agar. The plates were then incubated at 15 °C for 18–25 days under natural lighting conditions. Actively growing colonies were then taken from the plates and subcultured onto fresh YM 1/10 agar plates as individual isolates.

Yeast isolates are deposited in the Microbiological Resources Center Culture Collection (MIRCEN) of PROIMI-CONICET Institute, San Miguel de Tucumán, Argentina.

rDNA amplification, sequencing and analysis

The divergent domain at the 5′ end of the LSU rDNA gene (around 600 bp) was symmetrically amplified with primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5′-GGTCCGTGTTTCAA

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GACGG) according to standard methods, as described by Kurtzman (2011). An additional first step (97 °C, 10 min) was included before standard cycling conditions.

Sequences were analysed, and edited if necessary, using Invitrogen Vector NTI Advance 10.3.0 software (Invitrogen, San Diego, CA, USA). All isolates were sequenced and their DNA sequences were submitted to GenBank under Accession Nos listed in Table 1. Strain identification was performed by comparison with the GenBank (only type strains) and AFToL databases. Arbitrarily, a $\geq 99\%$ identity criterion was employed to identify strains at the species level. Taxonomy was checked against Kurtzman (2011). Sequences showing 96–99% identity were tentatively identified to the genus level. Sequences showing 96% identity were considered unidentified.

Qualitative assays for ligninolytic enzymes

Precultivation on basal medium

Yeasts inocula were precultivated on basal medium (BM; composition in g/l: KH_2PO_4 1, yeast extract 0.01, $C_4HI_2N_20_6$ 0.5, $CuSO_4{\cdot}5H_2O$ 0.001, $MgSO_4{\cdot}7H_2O$ 0.5, $Fe_2(SO_4)_3$ 0.001, $CaCl_2{\cdot}2H_2O$ 0.01, $MnSO_4{\cdot}H_2O$ 0.001) (Pointing, 1999) supplemented with 0.4% w/v glucose and solidified with 1.6% w/v agar. This preculture was carried on in order to limit any nutrient carry that could interfere with assay results interpretation.

Ligninolytic screening on solid media was performed on Petri dishes containing basal medium supplemented with 0.25% w/v lignin (lignin, alkali, low sulphonate content; Aldrich) and 1.6% w/v agar. Yeast were inoculated and incubated for 10–20 days at 15 °C in darkness. After incubation, the plates were flooded with a standard staining solution, 1% w/v aqueous solution of $FeCl_3$ and $K_3[Fe(CN)_6]$, according to Pointing (1999). Phenols in undegraded lignin will stain blue-green, with clear zones around colonies indicating oxidation of phenolic components.

Textile dye decolorizing ability

Decolorization screening on solid media was performed on Petri dishes containing 20 ml BM, 1.6% agar and a mixture of Vilmafix® Blue RR-BB (CI, Reactive Blue 221), Vilmafix® Red 7B-HE (CI, Reactive Red 141), Vilmafix® Black B-V(CI, Reactive Black 5) and Vilmafix® Yellow 4R-HE (CI, Reactive Yellow 84) to 200 mg/l (ppm) final concentration. Plates were inoculated with actively growing yeast from YM–agar, incubated at 15 °C and examined for decolorization during 10–20 days of cultivation. As controls, plates without dye were also inoculated (Pajot *et al*., 2008).

Laccase activity screening

Organisms were screened for laccase activity, using guaiacol and syringaldazine as indicator compounds. Screening of laccase-producing organisms was done on BM–agar plates with 0.02% guaiacol (Wang *et al*., 2010; Kumar *et al*., 2011a, 2011b). Yeast strains were inoculated in sterile Petri plates containing the supplemented medium and were incubated at 15 °C for 10 days. In the presence of guaiacol, an intense reddish-brown colour was produced in the medium around laccase-producing organisms (Viswanath *et al*., 2010; Ang *et al*., 2010). Similarly, syringaldazine was oxidized to a purple-coloured compound in the presence of laccases (Wang *et al*., 2010).

Statistical analysis

All statistical analysis were performed using Minitab Statistical Software, v. 16.0. Correlations between enzymatic abilities were calculated from two-by-two contingency tables. Categorical variables were analysed using χ^2 test. All comparisons were done using two-tailed tests and $p < 0.01$ was considered significant.

Results and discussion

Isolation

Samples from different areas of 25 de Mayo/King George Island were processed as described in Methods. Suspensions from each sample were seeded onto YM–acid–agar plates and incubated in triplicate at 15 °C. After 15–20 days of incubation, isolates were grouped according to their colony characteristics, such as pigmentation, texture, elevation, size and time of appearance. In this way, 61 morphotypes were ultimately recovered as pure cultures and deposited at the MIRCEN culture collection.

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Table 1. Colony description, samples and sampling site characterization, temperature at sampling site and extracellular enzyme activities of isolates

No.	Presumptive identification	Colony colour/aspect	Sample location			Substrates	*T* (°C)	Activities		
			Sampling site	Latitude	Longitude			Dec	Lacc	Lig
1	*Leucosporidiella creatinivora*	White	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of PUF	8.4	–	–	+
2	Unindentified basidiomycetous	Cream	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of wood	3.3	–	–	–
3	*Rhodotorula glutinis*	Pink	Under Principal House	62°14'15.14'S	58°39'58.20'W	Air	NDA	+	–	–
4	*Cylindrobasidium* sp.	White	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of wood	4.4	–	+	–
5	Unidentified ascomycetous	White	Beach at Peñon 1	62°14'20.32'S	58°40'45.55'W	Rest of bone	9.0	–	+	–
6	*Cryptococcus victoriae*	White	Beach near the foot of the glacier	62°13'53.42'S	58°38'39.60'W	Lichen attached to stone	4.5	–	–	–
7	*Rhodotorula laryngis*	Pink	Man-impacted soil	62°14'17.60'S	58°40'7.99'W	Soil	3.0	+	–	+
8	*Dioszegia* sp.	Orange	Tres Hermanos hill	62°14'39.27'S	58°40'23.52'W	Algae	6.0	–	+	+
9	Unidentified basidiomycetous	Cream	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of wood	8.4	–	–	–
10	*Rhodotorula laryngis*	Pink	Beach at Peñon 1	62°14'20.32'S	58°40'45.55'W	Rest of bone	9.0	–	–	+
11	*Rhodotorula pallida*	Cream	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of PUF	6.4	–	–	+
12	*Mrakia* sp.	White	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Dry grass	10.3	–	–	–
13	*Rhodotorula* sp.	White	Beach at Peñon 1	62°14'20.32'S	58°40'45.55'W	Rest of wood	9.9	–	–	–
14	*Candida* sp.	White	Man-impacted soil	62°14'17.60'S	58°40'7.99'W	Soil	3.0	–	–	–
15	*Debariomyces hansenii*	White	Man-impacted soil	62°14'15.72'S	58°39'55.22'W	Soil	2.8	–	+	+
16	Unidentified ascomycetous	White	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	11.6	+	+	–
17	*Bullera armeniaca*	Orange	Soil near the Lake	62°15'9.12'S	58°39'25.57'W	Soil0	3.6	+	+	–
18	*Mrakia* sp.	Cream	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Dry grass	8.3	+	–	+
19	*Rhodotorula laryngis*	Pink	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of wood	8.4	+	–	+
20	*Rhodotorula* sp.	White	Beach at Peñon 1	62°14'20.32'S	58°40'45.55'W	Rest of bone	9.0	+	–	–
21	*Rhodotorula* sp.	Black/white	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of wood	3.3	+	+	–
22	Unidentified basidiomycetous	Black/white	Rocks at the foot of the glacier	62°14'20.78'S	58°39'35.08'W	Rest of wood	3.3	–	–	–
23	*Leucosporidiella creatinivora*	Cream	Beach near the foot of the glacier	62°13'53.42'S	58°38'39.60'W	Lichen attached to stone	4.5	+	+	–
24	*Cryptococus* sp.	Cream	Under Argentinean Lab	62°14'16.39'S	58°40'7.13'W	Air	NDA	–	–	–

25	*Debariomyces hansenii*	White	Under Dallman Lab	62°14'15.52'S	58°40'0.91'W	Air	NDA	–	–	–
26	*Rhodotorula mucilaginosa*	Pink	Under Dallman Lab	62°14'15.52'S	58°40'0.91'W	Air	NDA	–	+	+
27	*Rhodotorula laryngis*	Pink	Man-impacted soil	62°14'17.60'S	58°40'7.99'W	Soil	3.0	–	+	+
28	*Cryptococcus victoriae*	Cream	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	2.9	–	–	–
29	*Rhodotorula laryngis*	Pink	Beach near the foot of the glacier	62°13'53.42'S	58°38'39.60'W	Lichen/soil	3.0	–	–	+
30	*Cryptococcus gilvescens*	Cream	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Dry grass	10.3	–	+	–
31	*Rhodotorula* sp.	White	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	11.6	–	+	+
32	*Rhodotorula laryngis*	Pink	Beach at Peñon 1	62°14'20.32'S	58°40'45.55'W	Rest of bone	3.6	–	–	+
33	*Cryptococcus victoriae*	Cream	Soil near the Lake	62°15'9.12'S	58°39'25.57'W	Soil	6.0	–	–	–
34	*Debariomyces hansenii*	White	Man-impacted soil	62°14'15.72'S	58°39'55.22'W	Soil	2.8	+	+	–
35	*Debariomyces hansenii*	White	Man-impacted soil	62°14'15.72'S	58°39'55.22'W	Soil	2.8	–	–	–
36	*Cryptococcus* sp.	Cream	Man-impacted soil	62°14'17.60'S	58°40'7.99'W	Soil	3.0	–	–	+
37	Unidentified basidiomycetous	Orange	Under Argentinean Lab	62°14'16.39'S	58°40'7.13'W	Air	NDA	–	–	+
38	*Cryptococcus victoriae*	Cream	Tres Hermanos hill	62°14'39.27'S	58°40'23.52'W	Algae	6.0	–	–	–
39	*Sporobolomyces* sp.	Pink	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Lichen	6.0	–	–	–
40	*Cryptococcus victoriae*	White	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Moss	10.1	–	–	–
41	*Cryptococcus* sp.	Pink	Under Argentinean Lab	62°14'16.39'S	58°40'7.13'W	Air	NDA	–	–	+
42	*Rhodotorula creatinivora*	White	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Lichen	6.0	–	–	+
43	*Cryptococcus victoriae*	White	Tres Hermanos Hill	62°14'39.27'S	58°40'23.52'W	Algae	5.4	+	–	–
44	*Cryptococcus victoriae*	Cream	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Lichen	6.0	–	–	–
45	*Rhodotorula glutinis*	Pink	Under Principal House	62°14'15.14'S	58°39'58.20'W	Air	NDA	+	–	+
46	*Rhodotorula laryngis*	Pink	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Lichen	6.0	–	–	+
47	*Cryptococcus victoriae*	Cream	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	3.0	–	–	–
48	Unidentified ascomycetous	Black/white	Under Dallman Lab	62°14'15.52'S	58°40'0.91'W	Air	NDA	–	–	–
49	*Cryptococcus victoriae*	Cream	Under Dallman Lab	62°14'15.52'S	58°40'0.91'W	Air	NDA	–	–	–
50	*Cryptococcus victoriae*	Cream	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	2.9	–	–	–
51	*Rhodotorula laryngis*	Pink	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	2.9	+	–	+
52	*Rhodotorula* sp.	White	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Grass/penguin feces	3.6	–	–	–
53	*Cryptococcus* sp.	Cream	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	2.9	+	–	–
54	*Cryptococcus* sp.	Pink	Beach at Peñon 1	62°14'20.32'S	58°40'45.55'W	Rest of bone	3.6	+	–	+

(Continues)

Table 1. (Continued)

No.	Presumptive identification	Colony colour/aspect	Sample location			Substrates	*T* (°C)	Activities		
			Sampling site	Latitude	Longitude			Dec	Lacc	Lig
55	*Cryptococcus victoriae*	Cream	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	2.9	–	–	–
56	*Cryptococcus terricola*	White	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Dry grass	10.3	+	+	–
57	*Cryptococcus victoriae*	White	Punta Stranger	62°15'39.89'S	58°37'5.45'W	Grass/penguin feces	2.9	+	–	+
58	*Cryptococcus* sp.	Cream	Beach near the foot of the glacier	62°13'53.42'S	58°38'39.60'W	Lichen attached to stone	4.5	–	–	+
59	Unidentified ascomycetous	White	Under Dallman Lab	62°14'15.52'S	58°40'0.91'W	Air	NDA	–	–	–
60	*Exophiala* sp.	Black	Beach near Refugio Elefante	62°15'22.06'S	58°38'49.58'W	Dry grass	10.3	+	–	–
61	Unidentified ascomycetous	White	Beach near the foot of the glacier	62°13'53.42'S	58°38'39.60'W	Lichen	3.0	+	–	–

Activities: Dec, dye decolorization; Lacc, laccase activity (guaiacol oxidation); Lig, lignin degradation; +, positive reaction; –, negative reaction.

Yeasts are saprophytes, playing mainly a degradative role. Sub-Antarctic islands are home to a flora of mosses, liverworts, algae, cyanobacteria, lichens and phanerogams. Although primary productivity in this environment remains very low, a variety of yeast species have been reported from Antarctic sources and new species continue to be described (Vishniac, 2006).

Psychrophily, psychrotolerance and mesophily definitions form a continuum in which the boundaries are usually hard to establish. However, it is widely accepted that both psychrophilic and mesophilic organisms are able to grow at 15 °C. In this way, it is unsurprising that mesophilic yeasts, i.e. *Cryptococcus victoriae* and *Debaryomyces hansenii* (Vishniac, 2006), were also isolated.

Enzymatic activities

Eleven yeasts (17%; numbers 12, 13, 22, 24, 25, 28, 35, 47, 48, 55 and 59) did not show positive activity in any of the performed assays. Contrarily, none of the isolated yeasts showed positive activity across all four tested substrates (Table 1). No obvious correlations between taxonomy, enzymatic activity, collection sites or isolation substrate could be observed. In total, 33% of the yeast isolates examined showed significant activity for dye decolorization, 25% for laccase activity and 38% for ligninolytic activity.

Although guaiacol has been widely employed as a substrate for ligninolytic enzymes such as laccase (Klonowska *et al*., 2002), peroxidases (Kim and Shoda, 1999) and lignin peroxidase (Wong and Yu, 1999), no evidence of association was detected between guaiacol oxidation and lignin degradation ($\chi^2 \leq 1.91$, $p \geq 0.091$).

It is worth mentioning that 25 de Mayo/King George Island has a cold moist maritime climate and thin soils with a low organic matter content. However, no woody species occur and only lower plants (mosses and liverworts) are frequent, and only two vascular plant species, the Antarctic hairgrass (*Deschampsia antarctica*) and the Antarctic pearlwort (*Colobanthus quitensis*), are commonly reported in the South Shetland Islands (Bridge and Spooner, 2012).

Molecular identification of yeasts

According to the adopted approach, 85% of the isolates could be satisfactorily identified to the genus level, representing eight basidiomycetous

Table 2. Molecular identification of yeast isolate

		AFToL/Wasabi	NCBI					
No.	**Accession No.**	**Closest match**	**Accession No.**	**Identity (%)**	**Closest match**	**Accession No.**	**Identity (%)**	**Presumptive identification**
1	KC713824	*Leucosporidium scottii* CBS 614	AY646098	99	*Rhodotorula creatinivora* CBS 8620^T	AF189925.1	100	*Leucosporidiella creatinivora*
2	KC580663	*Sydowia polyspora* CLS-10	AY544675	95	*Rachicladosporium pini* CPC 16770^T	JF951165	88	Unindentified basidiomycetous
3	KC713825	*Rhodotorula glutinis* CBS 20^T	AY646097	99	*Rhodotorula glutinis* ATCC 32765^T	AF335985.1	99	*Rhodotorula glutinis*
4	KC713826	*Cylindrobasidium laeve* HHB 8633^T	DQ234541	97	*Campanella subdendrophora* ATCC 42449^T *Psilocybe chionophila* C659^T	AY445115 AF261605	89 89	*Cylindrobasidium* sp.
5	KC580664	*Metschnikowia bicuspidata* CBS 5575	FJ176876	94	*Metschnikowia bicuspidata* NRRL YB-4993^T	JQ689032	94	Unidentified ascomycetous
6	KC713827	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^T	AF363647	99	*Cryptococcus victoriae*
7	KC713828	*Occultifur externus* CBS 8732^T	AY745723	92	*Rhodotorula laryngis* CBS 2221^T	AF189937	99	*Rhodotorula laryngis*
8	KC713829	*Cryptococcus humicola* PYCC 3387^T	DQ645514	89	*Dioszegia changbaiensis* AS 2.2309^T	AY242819	97	*Dioszegia* sp.
9	KC713830	*Exidiopsis calcea* CBS 463.62	AY885162	93	*Cryptococcus arrabidensis* CBS 8678^T	AF181535	95	Unidentified basidiomycetous
10	KC713831	*Occultifur externus* CBS 8732^T	AY745723	92	*Rhodotorula laryngis* CBS 2221 ^{T}	AF189937	99	*Rhodotorula laryngis*
11	KC713832	*Occultifur externus* CBS 8732^T	AY745723	92	*Rhodotorula pallida* CBS 320^T	AF189962	99	*Rhodotorula pallida*
12	KC713833	*Mrakia frigida* CBS 5266^T	DQ831016	99	*Mrakia gelida* CBS 5272^T *Mrakia psychrophilia* AS 2.1971^T	AF189831 EU224266	99 99	*Mrakia* sp.
13	KC713834	*Kriegeria eriophori* CBS 101449	AY745728	96	*Rhodotorula psychrophila* PB19 (CBS 10440^T) *Rhodotorula glacialis* A19 (CBS 10436^T)	EF151252 EF151258	99 99	*Rhodotorula* sp.
14	KC580665	*Debaryomyces hansenii* (2) AFToL ID 1077	NDA	91	*Candida subhashii* UAMH 10744^T *Candida sake* NRRL Y-1622^T	EU836708 U45728	98 99	*Candida* sp.
15	KC580666		NDA	91	*Debaryomyces hansenii* var. *hansenii* CBS 1795^T	AJ508559 AJ508560	99 99	*Debariomyces hansenii*

(Continues)

Table 2. (Continued)

		AFToL/Wasabi	NCBI					
No.	Accession No.	Closest match	Accession No.	Identity (%)	Closest match	Accession No.	Identity (%)	Presumptive identification
		Debaryomyces hansenii (2) AFToL ID 1077			*Debaryomyces hansenii* var. *fabryi* CBS 1796^T			
16	KC580667	*Debaryomyces hansenii* (2) AFToL ID 1077	NDA	91	*Nadsonia commutata* NRRL Y-7950^T	U73598	98	Unidentified ascomycetous
17	KC713835	*Cryptococcus humicola* PYCC 3387^T	DQ645514	89	*Bullera armeniaca* CBS 7091^T	AF189883	99	*Bullera armeniaca*
18	KC713836	*Mrakia frigida* CBS 5266^T	DQ831016	99	*Mrakia gelida* CBS 5272^T *Mrakia psychrophilia strain* AS 2.1971^T	AF189831 EU224266	99 99	*Mrakia* sp.
19	KC713837	*Occultifur externus* CBS 8732^T	AY745723	93	*Rhodotorula laryngis* CBS 2221^T	AF189937	99	*Rhodotorula laryngis*
20	KC713838	*Kriegeria eriophori* CBS 101449	AY745728	96	*Rhodotorula arctica* JCM 13290^T *Bensingtonia yamatoana* CBS 7243^T	AB478858 AF189896	99 99	*Rhodotorula* sp.
21	KC580668	*Sydowia polyspora* AFToL ID 178	AY544675	90	*Rhodotorula arctica* JCM 13290^T	AB478858	99	*Rhodotorula* sp.
22	KC580669	*Sydowia polyspora* AFToL ID 178	AY544675	90	*Rachicladosporium pini* CPC 16770^T	JF951165	88	Unidentified basidiomycetous
23	KC713839	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	97	*Leucosporidiella creatinivora* CBS 8620^T	AF189925	100	*Leucosporidiella creatinivora*
24	KC713840	*Exidia uvapassa* AFToL-ID 461 (2)	AY645056	93	*Cryptococcus liquefaciens* CBS 968^T	AF181515	98	*Cryptococus* sp.
25	KC580670	*Debaryomyces hansenii* (2) AFToL ID 1077	NDA	98	*Debaryomyces hansenii* var. *hansenii* CBS 1795^T *Debaryomyces hansenii* var. *fabryi* CBS 1796^T	AJ508559 AJ508560	99 99	*Debariomyces hansenii*
26	KC713841	*Rhodotorula mucilaginosa* PYCC 5166^T	DQ832198	99	*Rhodotorula mucilaginosa* ATCC 32763^T	AF335986	99	*Rhodotorula mucilaginosa*
27	KC713842	*Occultifur externus* CBS 8732^T	AY745723	92	*Rhodotorula laryngis* CBS 2221^T	AF189937	99	*Rhodotorula laryngis*
28	KC713843	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^T	AF363647	99	*Cryptococcus victoriae*
29	KC713844	*Occultifur externus* CBS 8732^T	AY745723	91	*Rhodotorula laryngis* CBS 2221^T	AF189937	99	*Rhodotorula laryngis*

30	KC713845	*Cryptococcus gastricus* CBS 8636^{T}	DQ645512	99	*Cryptococcus gilvescens* CBS 7525^{T}	AF181547	99	*Cryptococcus gilvescens*
31	KC713846	*Leucosporidium scottii* CBS 614	AY646098	98	*Rhodotorula creatinivora* CBS 8620^{T}	AF189925.1	98	*Rhodotorula* sp.
32	KC713847	*Occultifur externus* CBS 8732^{T}	AY745723	93	*Rhodotorula laryngis* CBS 2221^{T}	AF189937	99	*Rhodotorula laryngis*
33	KC713848	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^{T}	AF363647	99	*Cryptococcus victoriae*
34	KC580671	*Debaryomyces hansenii* (2) AFToL ID 1077	NDA	99	*Debaryomyces hansenii* var. *hansenii* CBS 1795^{T}	AJ508559	99	*Debariomyces hansenii*
					Debaryomyces hansenii var. *fabryi* CBS 1796^{T}	AJ508560	99	
35	KC580672	*Debaryomyces hansenii* (2) AFToL ID 1077	NDA	99	*Debaryomyces hansenii* var. *hansenii* CBS 1795^{T}	AJ508559	99	*Debariomyces hansenii*
					Debaryomyces hansenii var. *fabryi* CBS 1796^{T}	AJ508560	99	
36	KC713849	*Cryptococcus gastricus* CBS 8636^{T}	DQ645512	92	*Cryptococcus liquefaciens* CBS 968^{T}	AF181515	98	*Cryptococcus* sp.
37	KC713850	*Cryptococcus humicola* PYCC 3387^{T}	DQ645514	89	*Dioszegia changbaiensis* AS 2.2309^{T}	AY242819	95	Unidentified basidiomycetous
38	KC713851	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^{T}	AF363647	99	*Cryptococcus victoriae*
39	KC713852	*Sporobolomyces roseus* PYCC 4463^{T}	DQ832234	98	*Sporobolomyces koalae* JCM 15063 2^{T}	EU276011	99	*Sporobolomyces* sp.
40	KC713853	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^{T}	AF363647	99	*Cryptococcus victoriae*
41	KC713854	*Cystoflobasidium infirmominiatum* CBS 323^{T} (3)	DQ645524	98	*Cryptococcus macerans* CBS 2206^{T}	AF189848	98	*Cryptococcus* sp.
						AF189832	98	
					Cystoflobasidium bisporidii CBS 6346^{T}			
42	KC713855	*Leucosporidium scottii* CBS 614	AY646098	99	*Rhodotorula creatinivora* CBS 8620^{T}	AF189925.1	100	*Rhodotorula creatinivora*
43	KC713856	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^{T}	AF363647	99	*Cryptococcus victoriae*
44	KC713857	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^{T}	AF363647	99	*Cryptococcus victoriae*
45	KC713858	*Rhodotorula glutinis* CBS 20^{T}	AY646097	99	*Rhodotorula glutinis* ATCC 32765^{T}	AF335985.1	100	*Rhodotorula glutinis*
46	KC713859	*Occultifur externus* CBS 8732^{T}	AY745723	92	*Rhodotorula laryngis* CBS 2221^{T}	AF189937	99	*Rhodotorula laryngis*
47	KC713860	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^{T}	AF363647	100	*Cryptococcus victoriae*
48	KC580673	Unidentified (4)	–	85	*Neofusicoccum grevilleae* CPC 16999^{T}	JF951157	85	Unidentified ascomycetous

(Continues)

Table 2. (Continued)

		AFToL/Wasabi	NCBI					
No.	Accession No.	Closest match	Accession No.	Identity (%)	Closest match	Accession No.	Identity (%)	Presumptive identification
49	KC713861	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^T	AF363647	99	*Cryptococcus victoriae*
50	KC713862	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^T	AF363647	99	*Cryptococcus victoriae*
51	KC713863	*Occultifur externus* CBS 8732^T	AY745723	92	*Rhodotorula laryngis* CBS 2221^T	AF189937	99	*Rhodotorula laryngis*
52	KC713864	*Leucosporidium scottii* CBS 614	AY646098	98	*Rhodotorula creatinivora* CBS 8620^T	AF189925.1	98	*Rhodotorula* sp.
53	KC713865	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	97	*Cryptococcus carnescens* CBS 973^T	AB035054	97	*Cryptococcus* sp.
54	KC713866	*Cryptococcus humicola* PYCC 3387^T	DQ645514	90	*Cryptococcus festucosus* VKM Y-2930^T	AY462119	97	*Cryptococcus* sp.
55	KC713867	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^T	AF363647	99	*Cryptococcus victoriae*
56	KC713868	*Cryptococcus gastricus* CBS 8636^T	DQ645512	93	*Cryptococcus terricola* KCTC 7837^T	AF257276	99	*Cryptococcus terricola*
57	KC713869	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	98	*Cryptococcus victoriae* CBS 8685^T	AF363647	99	*Cryptococcus victoriae*
58	KC713870	*Cryptococcus* sp. CBS 681.93 (1)	AY646103	93	*Cryptococcus carnescens* CBS 973^T	AB035054	97	*Cryptococcus* sp.
59	KC580674	*Metschnikowia bicuspidata* CBS 5575	FJ176876	95	*Metschnikowia bicuspidata* NRRL YB-4993^T	JQ689032	94	Unidentified ascmycetous
60	KC776212	*Dactylospora lobariella* AFToL ID-2135	NDA	96	*Exophiala tremulae* UAMH 10998^T *Exophiala salmonis* CBS 157.67^T	JF951155 AY213702	96 96	*Exophiala* sp.
61	KC776213	*Cudoniella clavus* AFToL-ID 1660	DQ470944	98	*Hyphozyma variabilis* CBS 523.79^T	AF353596	91	Unidentified ascomycetous

(1) Recorded at CBS as *Ustilentyloma fluitans*; (2) only the AFToL ID numbers could be retrieved from database; (3) strain recorded as type strain of *Rhodosporidium infirmo-miniatum* at CBS; (4) related to *Glonium circumserpens*; NDA, no data available.

and three ascomycetous genera. As we previously hypothesized, the isolation medium here employed, with its low carbon content, could have biased results towards oligotrophic, slow-growing, metabolically diverse yeasts, found mainly between basidiomycetous genera. Only 35 isolates could be identified to the species level, representing a scarce 59%.

Although exceptions have been found, a difference $> 1\%$ in the rDNA D1/D2 region could be employed as a species delimitation criterion for ascomycetous and basidiomycetous yeasts (Kurtzman and Suzuki, 2010; Scorzetti *et al*., 2002). Accordingly, yeasts showing 98% or lower identity with recognized species could represent new taxa and require further characterization studies.

Yeasts identified at the species level belong to four basidiomycetous genera (*Leucosporidiella*, *Rhodotorula*, *Cryptococcus* and *Bullera*) and one ascomycetous genus (*Candida*). *Cryptococcus* and *Rhodotorula* were found to be the most representative genera (three and four identified species, respectively; Table 2).

The high number of unidentified yeasts could be explained in the light of a concurrence of factors affecting the isolation scheme, including temperature, media composition, fast processing and exposure to light/dark cycles, among others. The use of solid media, where colony appearance and growth can be checked daily, certainly also played a key role in the isolation of yeasts with different growth profiles (Pajot *et al*., 2011).

Most of the species reported here are common to Antarctic soils and have also been profusely reported in the Arctic or near-Arctic regions (Vishniac, 2006) or associated with Alpine or Andean glaciers (Turchetti *et al*., 2007; De García *et al*., 2007), representing well cold-adapted yeasts.

The sampling sites at 25 de Mayo Island analysed represent a variety of moderate microenvironments, with temperatures in the range 2.8–11.6 °C. These habitats present significant stress challenges to the soil microbiota, including oligotrophy, chronically low temperatures, long- and short-term temperature variations and freeze–thaw cycles (Peck *et al*., 2007).

It is unsurprising that both mesophilic yeasts, such as *R. laryngis*, *R. pallida* or *R. mucilaginosa*, and recognized psychrophilic yeasts, such as *C. victoriae* and *C. sake*, have been isolated in this study, since it is widely accepted that both psychrophilic and mesophilic organisms are able to grow at the incubation temperature employed (15 °C) (Vishniac, 2006).

Conclusions

Sixty-one isolates could be retrieved from 25 de Mayo/King George Island and have been tested for textile dyes and lignin degradation. The identified yeasts belong to widely reported, cold-adapted yeast taxa, most of them belonging to oligotrophic, slow-growing and metabolically diverse basidiomycetous genera. The rationale for basidiomycetous yeast prevalence in Antarctic samples is not clear, but presumably it could be related to the oligotrophy of soil samples and, almost marginally, to the isolation scheme employed.

As previously emphasized, oligotrophic microorganisms are usually related to the ability to degrade a broad spectrum of substrates, whilst copiotrophic microorganisms are related to the efficient degradation of easily accessible substrates. In this context, textile dye decolorization has been widely associated with lignin degradation.

We want to emphasize that several unidentified yeasts could be isolated and are now available at MIRCEN, representing a significant resource in order to reach a deeper understanding of the physiology, genetics, ecology and biotechnological potential of Antarctic yeasts. Such availability certainly represents a priceless advantage over most metaphylogenomic methods.

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Conflict of interest

The authors declare no conflicts of interest.

References

Ang T, Ngoh G, Chua A. 2010. A quantitative method for fungal ligninolytic enzyme screening studies. *Asia Pac J Chem Eng* **6**(4): 589–595.

Bridge P, Spooner B. 2012. Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? *Fungal Ecol* **5**: 381–394.

Connell L, Redman R, Craig S, *et al*. 2008. Diversity of soil yeasts isolated from South Victoria Land, Antarctica. *Microb Ecol* **56**(3): 448–459.

De García V, Brizzio S, Libkind D, *et al*. 2007. Biodiversity of cold-adapted yeasts from glacial meltwater rivers in Patagonia, Argentina. *FEMS Microbiol Ecol* **59**(2): 331–341.

Kim SJ, Shoda M. 1999. Purification and characterization of a novel peroxidase from *Geotrichum candidum* Dec 1 involved in decolorization of dyes. *Appl Environ Microbiol* **65**(3): 1029–1035.

Klonowska A, Gaudin C, Fournel A, *et al*. 2002. Characterization of a low redox potential laccase from the basidiomycete C30. *Eur J Biochem* **269**: 6119–6125.

Kostadinova N, Krumova E, Tosi S, *et al*. 2009. Isolation and identification of filamentous fungi from Island Livingston, Antarctica. *Biotechnol Biotechnol Equip* **23**(2): 267–270.

Koyani RD, Sanghvi GV, Sharma RK, Rajput KS. 2013. Contribution of lignin degrading enzymes in decolourisation and degradation of reactive textile dyes. *Int Biodeterior Biodegrad* **77**: 1–9.

Krishnan A, Alias SA, Wong CMVL, *et al*. 2011. Extracellular hydrolase enzyme production by soil fungi from King George Island, Antarctica. *Polar Biol* **34**(10): 1535–1542.

Kumar L, Arantes V, Chandra R, Saddler J. 2011a. The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. *Bioresour Technol* **103**(1): 201–208.

Kumar VV, Kirupha SD, Periyaraman P, Sivanesan S. 2011b. Screening and induction of laccase activity in fungal species and its application in dye decolorization. *Afr J Microbiol Res* **5**(11): 1261–1267.

Kurtzman CP. 2011. The Yeasts: A Taxonomic Study, vol **1**. Elsevier Science: Amsterdam.

Kurtzman CP, Suzuki M. 2010. Phylogenetic analysis of ascomycete yeasts that form coenzyme Q-9 and the proposal of the new genera *Babjeviella*, *Meyerozyma*, *Millerozyma*, *Priceomyces* and *Scheffersomyces*. *Mycoscience* **51**(1): 2–14.

Margesin R, Neuner G, Storey K. 2007. Cold-loving microbes, plants, and animals – fundamental and applied aspects. *Naturwissenschaften* **94**(2): 77–99.

Margesin R, Schinner F, 1994. Properties of cold-adapted microorganisms and their potential role in biotechnology. *J Biotechnol* **33**(1): 1–14.

Meehan C, Banat IM, McMullan G, *et al*. 2000. Decolorization of Remazol Black-B using a thermotolerant yeast, *Kluyveromyces marxianus* IMB3. *Environ Int* **26**(1): 75–79.

Nedialkova D, Naidenova M. 2004. Screening the antimicrobial activity of actinomycetes strains isolated from Antarctica. *J Cult Collect* **4**: 29–35.

Pajot HF, De Figueroa LIC, Spencer JF, Fariña JI. 2008. Phenotypical and genetic characterization of *Trichosporon* sp. HP-2023, a yeast isolate from Las Yungas rainforest (Tucumán, Argentina) with dye-decolorizing ability. *Antonie van Leeuwenhoek* **94**(2): 233–244.

Pajot HF, Delgado OD, de Figueroa LIC, Fariña JI. 2011. Unraveling the decolourizing ability of yeast isolates from dye-polluted and virgin environments: an ecological and taxonomical overview. *Antonie van Leeuwenhoek* **99**(3): 443–456.

Peck LS, Convey P, Barnes DK. 2007. Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. *Biol Rev* **81**(1): 75–109.

Pointing SB. 1999. Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. *Fungal Divers* **2**: 17–33.

Ruisi S, Barreca D, Selbmann L, *et al*. 2007. Fungi in Antarctica. *Rev Environ Sci Biotechnol* **6**(1): 127–141.

Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A. 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res* **2**: 495–517.

Solís M, Solís A, Pérez HI, *et al*. 2012. Microbial decolouration of azo dyes: a review. *Process Biochem* **47**(12): 1723–1748.

Stolz A. 2001. Basic and applied aspects in the microbial degradation of azo dyes. *Appl Microbiol Biotechnol* **56**: 69–80.

Turchetti B, Buzzini P, Goretti M, *et al*. 2007. Psychrophilic yeasts in glacial environments of alpine glaciers. *FEMS Microbiol Ecol* **63**(1): 73–83.

Vishniac HS. 2006. Yeast biodiversity in the Antarctic. The Yeast Handbook. Biodiversity and Ecophysiology of Yeasts, Peter G, Rosa C, (eds). Springer-Verlag: Berlin; 419–440.

Viswanath B, Chandra MS, Pallavi H, Reddy BR. 2010. Screening and assessment of laccase producing fungi isolated from different environmental samples. *Afr J Biotechnol* **7**(8): 1129–1133.

Wang C, Zhao M, Li D, *et al*. 2010. Isolation and characterization of a novel *Bacillus subtilis* WD23 exhibiting laccase activity from forest soil. *Afr J Biotechnol* **9**(34): 5496–5502.

Wong Y, Yu J. 1999. Laccase-catalyzed decolorization of synthetic dyes. *Water Res* **33**(16): 3512–3520.

Xin M, Zhou P. 2007. *Mrakia psychrophila* sp. nov, a new species isolated from Antarctic soil. *J Zhejiang Univ Sci B* **8**: 260–265.

Yang Q, Yediler A, Yang M, Kettrup A. 2005. Decolorization of an azo dye, reactive black 5, and MnP production by yeast isolate, *Debaryomyces polymorphus*. *Biochem Eng J* **24**: 249–253.