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Fungal diversity in Antarctic lignocellulosic substrates and their production of enzymes and lipids with potential industrial applications

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Abstract

Antarctica is characterized by extreme conditions including low temperatures, strong winds, desiccation, high UV radiation, high salinity, freeze-thaw cycles and pH variations. As a result, the resident diversity is dominated by extremophilic microorganisms with adaptations that enable their survival and attract significant biotechnological interest. The present study aimed to recover culturable fungi from different lignocellulosic substrates obtained on Deception Island, maritime Antarctica, and evaluate their ability to produce enzymes and lipids of interest. A total of 47 fungal isolates were recovered from different substrates, representing 16 genera and 23 taxa of the phyla Ascomycota and Basidiomycota. The most abundant genus was *Coniochaeta*, followed by *Cadophora*, *Pseudogymnoascus*, *Mrakia* and *Leucosporidium*. The fungal community detected in this study displayed high diversity, richness, and dominance indices. The highest number of fungi produced amylase degradation halos, followed by inulinase and cellulase. However, inulinase was produced the highest number of good-producing fungi. The two strains isolated of the yeast, *Solicoccozyma terricola,* were able to produce intracellular lipid at low temperatures. Our data indicates the presence of a high diversity and dominance of decomposer taxa. Some of these fungi may have been introduced in wood originally imported for the construction of whaling station or research station buildings, or arrived on Deception Island in different ways. The wood substrates may also have served as bait for the resident mycobiota. The spectrum of enzymatic activity of the cultured fungi corroborates previous studies, confirming the importance of these enzymes for microorganism survival in Antarctica's habitats. The enzymes produced may have biotechnological potential as more sustainable alternatives in industrial processes involving enzymes active at low temperatures. The oleaginous yeast, *S. terricola*, demonstrated growth across a wide temperature range, which may favor its presence in Antarctica's cold but also variable temperature soils. This species also displays biotechnological potential as a potential lipid source, for instance for use as biofuel feedstock.

Keywords Antarctica, Bioprospecting, Biotechnology, Extremophiles

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Introduction

Antarctica is often described as an untouched continent due to its geographical isolation and extreme conditions [1]. However, despite these conditions, the continent is not completely isolated from biological transfer, as both macro- and microorganisms can reach the region, primarily through the air for the latter but also through oceanic transfer [2–5]. Dispersal of microorganisms and other propagules does not guarantee their survival and establishment but is necessary for these to occur [6]. One contemporary concern about the introduction of microorganisms to Antarctica is their potentially harmful implications, such as those of fungi responsible for the deterioration of wood used in the construction of buildings and other structures [7, 8].

The huts used by sealers and whalers in the South Shetland Islands in the 19th and early 20th centuries, and later more widely used to house researchers in the mid- to late 20th century were constructed with wood, often imported from Europe, North America, or South America and built in Antarctica [8, 9]. This suggests that fungi originally from temperate areas may have inadvertently been imported at the same time. Furthermore, animal fodder, human provisions and other items imported from beyond Antarctica during the early exploitation and exploration eras would also have facilitated the introduction of saprophytic microbes [7].

Cell walls in wood are composed of varying amounts of cellulose, hemicellulose and lignin. Microorganisms that degrade these polymers produce extracellular enzymes responsible for breaking down the cell wall [10]. Different physical, chemical and morphological changes occur in wood according to the growth characteristics of specific microorganisms and the type of degradation system used [11]. According to Blanchette et al. [10], white rot fungi can degrade any component of the cell wall, including lignin, leaving it with a white appearance. Brown rot fungi preferentially degrade cellulose in the early stages of wood colonization, causing the residual wood to turn brown and break into cubic pieces when dry. Another category of wood-degrading fungi is soft-rot fungi, which form erosions in the secondary wall but do not degrade the middle lamella [12, 13]. The cavities formed in the wood and the degradation of cellulose decrease the strength of the residual wood in concert with a loss of carbohydrates and a consequential increase in the concentration of lignin [10].

Augustyniuk-Kram et al. [14] demonstrated that fungal spores could be transported into Antarctica through the clothing and equipment of tourists and researchers. The commonly found genera in their study were *Penicillium*, *Cladosporium*, *Alternaria* and *Geotrichum*, which are cosmopolitan and ubiquitous, occurring globally including in the alpine and polar regions. However, determining whether these taxa are truly new arrivals to Antarctica is challenging. Multiple reports are available of representatives of *Cladosporium*, *Penicillium* and *Aspergillus* in both contemporary soil and air sampling studies and in deep layers of polar ice originally deposited thousands to hundreds of years ago [15–22]. Furthermore, the excellent ecophysiological adaptations of these microorganisms to enable survival in extreme environments such as Antarctica are well-known [3].

Enzymes are proteins that act as highly efficient biocatalysts in the metabolic reactions of an organism. They reduce the free energy of the reaction and provide a high rate of product formation without being consumed during the process [23]. Although plants and animals are also sources of these enzymes, microbial enzymes are often more attractive in a biotechnological context due to their wide variety of catalytic activities, higher yields, rapid growth in economical culture media, ease of genetic manipulation and consistency of supply [24]. High catalytic efficiency of enzymes in the temperature range of 0–20°C reduces the risk of microbial contamination and can shorten processing time in industrial or domestic applications, eliminating the need for expensive heating equipment and systems [25]. Therefore, cold-adapted extremophilic microorganisms have become important targets for researchers since their enzymes are stable under environmental conditions considered adverse for most organisms. Enzymes active at low temperatures are also used in the food, pharmaceutical, textile, paper and biofuel production industries [26]. With this background, our study focused on characterizing the diversity of cultivable fungi present in lignocellulosic substrates present around Whalers Bay, Deception Island, Antarctica, which face various extreme conditions. We also evaluated their capacity to produce enzymes and lipids of potential biotechnological interest useful in industrial processes.

Methods

Sampling

Fragments of wood, fabric, rope and local soil were collected (n = 12 samples in total) from various points in Whalers Bay, Deception Island (Fig. 1), during the Brazilian Antarctic Operation XXXVII in December 2018. They were immediately stored in sterilized plastic bags (Whirl-pak®, Nasco, Atlanta, USA). After collection, the samples were kept at −20°C while being transported to the Laboratory of Polar Microbiology and Tropical

Fig. 1 Satellite imagery indicating the location of Whalers Bay, Deception Island, South Shetland Islands, maritime Antarctica. **a** Antarctic continent with the Antarctic Peninsula highlighted by a red rectangle; **b** Antarctic Peninsula with Deception Island (ID) highlighted by a red square; **c** Deception Island with Whalers Bay (WB) highlighted by a red square. Source: Google Earth, 2022; **d** Whalers Bay area, Deception Island, maritime Antarctica. The photographs illustrate the types of lignocellulosic materials collected. Photographs: L.H. Rosa

Connections at the Federal University of Minas Gerais and maintained at this temperature until processing.

Fungal isolation

The 12 samples were thawed and kept at 4 °C until fungal isolation. Yeast malt (YM) liquid medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 0.02% chloramphenicol) and YM solid medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% agar, 0.02% chloramphenicol, 0.01% ampicillin) were used. The YM liquid medium was used for enrichment to obtain the greatest possible diversity of fungi from the samples. For isolation, 5 cm fragments of wood, fabric or rope, and 1 g of soil samples, were inoculated into 50 mL tubes containing 7 mL of YM liquid medium, which were incubated under agitation in a shaker at 150 rpm for 10 d at 15 °C. Serial dilution was then performed in saline solution (0.85% NaCl) and dilutions between 10^{-4} and 10^{-6} were selected. Then, 100 µL of each sample was plated in triplicate on solid YM medium and, finally, the plates were incubated at 15 °C for 20 d.

Selective isolation of fermenting yeasts

Five-centimeter substrate fragments and 1 g of soil from each of the 12 samples were inoculated into sterilized 50 mL conical tubes containing 10 mL of a medium specifically designed for isolating fermenting yeasts, composed of 0.67% YNB (Yeast Nitrogen Base - Difco, Detroit, USA), 1% raffinose, 8% ethanol and 0.02% chloramphenicol. The tubes were incubated at 10 °C and 30 °C for 25 d. Then, 1 mL from each sample was inoculated into new tubes containing the same medium at the same temperatures. After 15 d, the liquid from each sample was streaked onto Petri dishes containing YM agar and incubated at the respective temperatures for 10 d.

Fungal preservation

The fungal colonies obtained were grouped based on their macromorphological characteristics (colony color, surface texture, edge appearance, pigment production), and each colony was purified separately on Petri dishes containing YM agar. The purified fungi were incubated at 15 °C for 7–21 d, preserved, and deposited in the Microorganism and Cell Collection of the Federal University of Minas Gerais (UFMG) under the code UFMGCB. The yeast isolates were inoculated into tubes containing 2 mL of GYMP broth (1% malt extract, 0.2% dibasic potassium phosphate, 2% glucose, 0.5% yeast extract, 0.02% chloramphenicol) and incubated for 48 h at 15 °C. Then, 800 µL of this broth was transferred in duplicate to sterilized

cryotubes containing 200 µL of pure glycerol. The filamentous fungal isolates were preserved in duplicate in Castellani solution [27] containing distilled water and kept at room temperature. In addition, 10 fragments of fungal mycelium were preserved in sterilized cryotubes, also in duplicate, containing 1 mL 15% glycerol solution before being stored at −80°C.

Fungal identification and diversity

A polyphasic approach based on morphological and molecular marker analyses was used for the identification of the isolated fungi. Total DNA extraction followed the protocol of Rosa et al. [21]. Fungi were grouped according to their colony's morphologies and the banding patterns of microsatellite regions amplified via fingerprinting (PCR-MST) using the oligonucleotide $(GTG)_5$ [28]. For filamentous fungi, the internal transcribed spacer region (ITS1-5.8S-ITS2) of the ribosomal DNA gene was amplified using the primers ITS1 and ITS4 [29]. For yeasts, the D1/D2 domain of the rDNA 26S gene was amplified using primers NL1 and NL4 [30]. The amplicons of these marker regions (ITS, D1/D2) were evaluated using 1% agarose gel electrophoresis, purified and sequenced by the Sanger method using an ABI automated system (Applied Biosystems Life Technologies, Massachusetts, USA). The generated nucleotide sequences were submitted for phylogenetic analysis through alignment and comparison of their similarities with the sequences of reference fungal species deposited in GenBank using the BLASTn program (Basic Local Alignment Search Tool) available on the NCBI portal (http://www.ncbi.nlm.nih.gov/blast/). Fungi with sequence e-value = 0, query coverage and identity ≥ 99% were considered to be the same species as identified in the database. Consensus sequences of the proposed taxa have been deposited in GenBank (accession codes given in Table 1). To estimate taxonomic diversity, the isolates were used as a proxy for the relative abundance of fungal taxa present in the material sampled. These were used to evaluate the diversity, richness and dominance of the taxa, using the indices: Fisher's α, Margalef's, and Simpson's, respectively. Species accumulation curve was obtained using the Mao Tao index. All results were obtained with 95% confidence, and bootstrap values of 1,000 replicates using PAST v1.9 software [31].

Enzymatic activity

The production of four extracellular enzymes (amylase, cellulase, inulinase, and pectinase) was evaluated in the fungi isolated. Fungi previously grown in YM agar were inoculated in culture media supplemented with inducing substrates appropriate to the specificity of the screened enzyme. For the filamentous fungi, a fragment of mycelia 5 mm in diameter was inoculated in the center of the plates with the inducers. For yeasts, a needle loop from each colony was inoculated directly into the YM agar containing the specific substrate for each enzyme. The enzymatic activity of the fungi was verified in the plates with the inducers after 7 d incubation at 15°C, in which halos of extracellular hydrolysis were detected (characteristic zones around the colonies) indicating positive activity for each enzyme. For amylase, 6.7 g L^{-1} YNB, 2 g L^{-1} soluble starch and 20 g L^{-1} agar were used and enzyme activity was determined by the addition of Lugol [32]. For cellulase, carboxymethylcellulose (5 g L^{-1}) replaced glucose as a carbon source in YM agar (1:10) and an orange halo was revealed after the addition of Congo red dye (2.5 g L^{-1} in Tris HCl buffer 0.1 M, pH 8) together with a 1 M NaCl solution [33]. For the analysis of inulinase production, the methodology described by [34] was used. The isolates were cultured in inulin agar (10 g L^{-1} inulin, 2 g L^{-1} yeast extract, 5 g L^{-1} peptone, 0.5 g L^{-1} $MgSO_4$, 0.5 g L^{-1} NaCl, 0.15 g L^{-1} $CaCl_2$, and 20 g L^{-1} agar, pH 6.0). Enzyme activity was determined by the addition of Lugol and inulin degradation was determined by the formation of a yellowish zone on a brown background. For pectinase, the isolates were cultured in YM medium (1:10), containing 10 g L^{-1} pectin, pH 7.0. To check enzyme activity, plates were flooded with 10 g L^{-1} CTAB (cetyltrimethylammonium bromide - Sigma-Aldrich, St. Louis, USA). Pectin degradation was indicated by a clear halo around the colony on a white background [33, 35]. To compare enzymatic production across the isolates, the Enzyme Index (EI) was calculated, which corresponds to the ratio between the diameter of the hydrolysis halo and the diameter of the colony in mm [36]. All assays were performed in triplicate and fungi with an EI ≥ 2.0 were considered good enzyme producers [37].

Growth profile of the yeast *Solicoccozyma terricola* at different temperatures

Due to its ability to grow in a medium containing a high concentration of ethanol (8%) and its identification as the species, *Solicoccozyma terricola*, which is reported to be a lipid-producing yeast [38], this strain was subjected to various temperatures and intracellular lipid production tests. To determine the optimal growth temperature, *S. terricola* was streaked on solid YM plates and incubated in duplicate at the following temperatures: 5, 10, 15, 20, 25, 30 and 37 °C. Growth analysis was performed visually after 3 and 7 d and recorded photographically.

Lipid production

The methodology for analyzing lipid production was adapted from [38]. The Antarctic strain of *S. terricola* was inoculated on YM agar plates and incubated for 3 d at

Table 1 Culturable fungi isolated from lignocellulosic samples obtained at Whalers Bay, Deception Island (South Shetland Islands, maritime Antarctica). These fungi were identified from sequenced rDNA regions by comparison with sequences in the NCBI GenBank database using BLASTn

Sample	Top results on BLASTn (GenBank accession number)	UFMGCB[a]	Density (UFC L^{-1})	Coverage (%)	Identity (%)	Nº bp analyzed[b]	Proposed taxon (GenBank accession number of sequences)
M11 (rope)	*Cadophora antarctica* (NR1563811)[c]	18941	>300	99	100	515	*Cadophora* sp. 1 (PV299234)
M7 (wood), M8 (fabric), M9 (soil), M10 (wood)	*Cadophora luteo-olivacea* (NR1659451)[c]	18937	>300	100	100	548	*Cadophora luteo-olivacea* (PV299235)
M1 (wood), M6 (wood), M9 (soil), M11 (rope), M12 (wood)	*Coniochaeta* sp. (MZ2623671)[c]	18950	>300	100	100	400	*Coniochaeta* sp. 1 (PV299236)
M2 (wood)	*Coniochaeta mutabilis* (MH8561221)[c]	18967	>300	97	100	361	*Coniochaeta* sp. 2 (PV299237)
M4 (wood)	*Coniochaeta luteoviridis* (MH8559481)[c]	18952	>300	97	100	461	*Coniochaeta* sp. 3 (PV299238)
M4 (wood)	*Pseudogymnoascus appendiculatus* (NR1378751)[c]	18970	>300	100	93	488	*Pseudogymnoascus* sp. 1 (PV299239)
M4 (wood), M7 (wood), M9 (soil), M11 (rope)	*Pseudogymnoascus pannorum* (MH8610381)[c]	18954	>300	96	99	446	*Pseudogymnoascus* sp. 2 (PV299240)
M8 (fabric)	*Mollisia* sp. (MK0880591)[c]	18942	>300	96	99	437	*Mollisia* sp. (PV299241)
M12 (wood)	*Cadophora caespitosa* (MT889936)[c]	18945	>300	100	99	370	*Cadophora* sp. 2 (PV299242)
M2 (wood)	*Ascocoryne laurisilvae* (PP391343)[c]	18953	>300	99	91	386	*Ascocoryne* sp. (PV299243)
M2 (wood)	*Purimyces orchidacearum* (NR198746)[c]	18964	>300	95	93	337	*Purimyces* sp. (PV299244)
M1 (wood)	*Graphium rubrum* (NR1452681)[c]	18969	>300	99	100	498	*Graphium rubrum* (PV299245)
M6 (wood), M9 (soil), M10 (woodl)	*Mrakia blollopis* (NG0577101)[d]	18971	>300	100	100	525	*Mrakia blollopis* (PV290918)
M1 (wood), M9 (soil), M11 (rope)	*Mrakia gelida* (KY1085851)[d]	18982	>300	100	100	524	*Mrakia gelida* (PV290919)
M2 (wood), M3 (wood)	*Leucosporidium creatinivorum* (NG0423751)[d]	18979	>300	100	100	512	*Leucosporidium* sp. (PV290920)
M1 (wood), M5 (wood)	*Goffeauzyma gastrica* (NG0582961)[d]	18993	>300	100	100	482	*Goffeauzyma* sp. (PV290921)
M9 (soil), M12 (wood)	*Solicoccozyma terricola* (NG0661871)[d]	18985	35	99	100	496	*Solicoccozyma terricola* (PV290922)
M8 (fabric), M12 (wood)	*Phenoliferia glacialis* (NG0583691)[d]	18983	>300	99	100	511	*Phenoliferia glacialis* (PV290923)
M9 (soil)	*Cystobasidium laryngis* (AF1899371)[d]	18992	>300	99	100	482	*Cystobasidium laryngis* (PV290924)
M11 (rope)	*Candida davisiana* (KY1064091)[d]	18988	>300	100	100	457	*Candida davisiana* (PV290925)
M7 (wood)	*Papiliotrema laurentii* (KY1087391)[d]	18973	>300	96	100	520	*Papiliotrema* sp. (PV290926)
M11 (rope)	*Naganishia friedmannii* (NG0694131)[d]	18994	>300	100	100	515	*Naganishia* sp. (PV290927)
M1 (wood)	*Coniochaeta luteoviridis* (NG0673481)[d]	18991	>300	98	100	459	*Coniochaeta* sp. 3 (PV290928)

M1- Crooked hut, Biscoe House, Whalers Bay; M2- Roof to the right of the lake; M3- Whaler to the right of the roof; M4- Destroyed house to the left of the small vat; M5- Hut to the left of the blubber melting vats; M6- Biscoe House, Whalers Bay; M7- Destroyed hut next to the Biscoe House; M8- Cloth, Biscoe House; M9- Wood floor inside Biscoe House; M10- Hut next to the tanks; M11- Between the destroyed house to the left of the vat and the Biscoe House; M12- Whalers Bay hangar

[a] *UFMGCB* Collection of Microorganisms and Cells of the Federal University of Minas Gerais

[b] *bp* base pairs

Taxa subject to BLASTn analysis based on [c]ITS, and [d]D1-D2

20 °C, the optimum temperature for yeast growth determined in the experiment described above, to obtain the pre-inoculum. A colony was then inoculated in glass tubes containing 5 mL of GMY broth (40 g L^{-1} glucose, 3 g L^{-1} yeast extract, 8 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$), which were then incubated for 9 d at 20 °C without shaking. The cell concentration in the solution was standardized using a spectrophotometer with absorbance adjusted to 0.1 at a wavelength of 600 nm.

To evaluate intracellular lipid accumulation, a stock solution of Nile Red (1 mg in 10 mL of acetone) (Sigma-Aldrich, St. Louis, USA) was prepared, and 40 µL was added to a 2 mL tube containing 1 mL of the fungal culture. Then, 10 µL of the solution were inoculated on a slide and, after 5 min, the cells were observed under a Confocal Microscope Nikon C2+ (Thermo Fisher Scientific, San Francisco, USA), with a 60× objective lens under the immersion oil. Finally, the cells were photographed before and during fluorescence emission.

Results

Fungal identification and diversity

A total of 47 fungal isolates were recovered from the different substrates, representing 15 genera and 23 taxa of the phyla Ascomycota and Basidiomycota. The genera identified were *Ascocoryne*, *Cadophora*, *Candida*, *Coniochaeta*, *Cystobasidium*, *Goffeauzyma*, *Graphium*, *Leucosporidium*, *Mollisia*, *Mrakia*, *Naganishia*, *Papiliotrema*, *Phenoliferia*, *Pseudogymnoascus* and *Purimyces* (Table 1). The most abundant genus was *Coniochaeta*, followed by *Cadophora*, *Pseudogymnoascus*, *Mrakia* and *Leucosporidium*. In addition, from the selective media with 8% ethanol used to recover ethanol resistant yeasts, two distinct isolates were obtained from different wood fragments and soil samples. These yeast isolates were present in low density and were identified as the species *Solicoccozyma terricola*.

The fungal community detected in the lignocellulosic and soil samples displayed high diversity (Fisher's α = 17.80), richness (Margalef = 5.71), and dominance (Simpson = 0.93) indices. However, the community did not reach an asymptote in the Mao Tao index (Fig. 2), suggesting that further diversity remains to be discovered in the sampled lignocellulosic substrates.

Enzymatic activities

All 47 isolates were tested for production of amylase, cellulase, inulinase and pectinase enzymes (Table 2, Suppl. Figure 1). The highest number of fungi producing degradation halos related to amylase (35), followed by inulinase (34) and cellulase (33), while pectinase had the lowest number of producing isolates (12). Inulinase had the highest number of good-producing fungi (eight).

For amylase, 31 isolates of filamentous fungi presented a degradation halo and four were good producers. However, only two isolates were identified: *Coniochaeta* UFMGCB 18938 and *Mollisia* UFMGCB 18942. Four yeast isolates presented a halo for the enzyme, but no isolate had an EI ≥ 2. For cellulase, 25 isolates of filamentous fungi presented a degradation

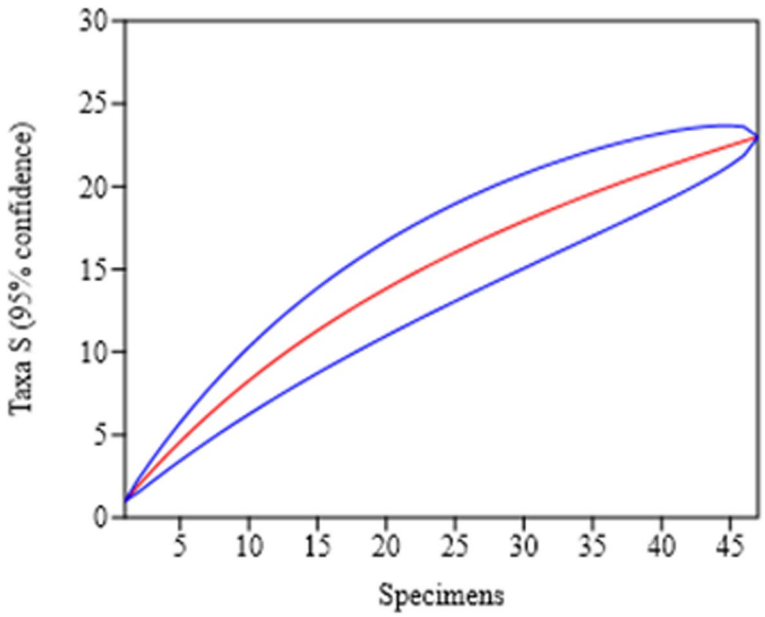

Fig. 2 Species accumulation curve (Mao Tao), with 95% confidence limits, of fungal amplicon sequence variants (ASVs) obtained from the lignocellulosic substrates analyzed

Table 2 Average enzymatic indices produced by fungal isolates obtained from lignocellulosic samples from Whalers Bay, Deception Island (South Shetland Islands, maritime Antarctica)

		Average enzymatic index (mm) (mean ± SD)			
Filamentous fungi	**UFMGCB code**	**Amylase**	**Cellulase**	**Inulinase**	**Pectinase**
Cadophora sp. 1	18941	1.4 ± 0.01	1.26 ± 0.01	-	-
Cadophora luteo-olivacea	18937	1.51 ± 0.01	-	1.55 ± 0.02	1.1 ± 0.01
C. luteo-olivacea	18949	1.52 ± 0.04	1.19 ± 0.05	1.48 ± 0	-
C. luteo-olivacea	18960	1.39 ± 0.35	1.16 ± 0.01	1.65 ± 0.05	-
C. luteo-olivacea	18961	1.6 ± 0.04	1.37 ± 0.05	1.87 ± 0.02	1.16 ± 0.01
C. luteo-olivacea	18962	1.49 ± 0.03	-	1.89 ± 0.03	-
Coniochaeta sp. 1	18938	**2.06 ± 0.47**	1.26 ± 0.1	-	-
Coniochaeta sp. 1	18948	1.48 ± 0.01	-	1.66 ± 0.15	-
Coniochaeta sp. 1	18950	1.58 ± 0.14	1.4 ± 0.07	1.51 ± 0.12	-
Coniochaeta sp. 1	18957	1.5 ± 0.07	1.38 ± 0.03	1.57 ± 0.07	-
Coniochaeta sp. 1	18958	1.3 ± 0.01	1.57 ± 0.1	1.55 ± 0.14	-
Coniochaeta sp. 1	18963	1.52 ± 0.13	1.65 ± 0.01	**2.03 ± 0.02**	-
Coniochaeta sp. 2	18966	1.42 ± 0.1	1.68 ± 0.03	-	-
Coniochaeta sp. 2	18967	1.85 ± 0.07	**2.7 ± 0.07**	**2.51 ± 0.04**	-
Coniochaeta sp. 3	18952	1.33 ± 0.08	1.52 ± 0.11	1.58 ± 0.01	-
Pseudogymnoascus sp. 1	18947	1.4 ± 0.05	-	1.74 ± 0.06	-
Pseudogymnoascus sp. 1	18970	1.23 ± 0.04	1.6 ± 0	1.56 ± 0.02	-
Pseudogymnoascus sp. 2	18965	1.34 ± 0.08	**2.27 ± 0**	-	-
Pseudogymnoascus sp. 2	18968	1.97 ± 0.04	1.9 ± 0.21	-	-
Pseudogymnoascus sp. 2	18954	1.8 ± 0.04	**2.14 ± 0.04**	-	-
Pseudogymnoascus sp. 2	18956	1.6 ± 0.03	1.78 ± 0.10	-	-
Mollisia sp.	18942	**2.28 ± 0.18**	1.89 ± 0.03	1.86 ± 0.19	-
Cadophora sp. 2	18945	1.37 ± 0	-	-	-
Ascocoryne sp.	18953	-	**2.03 ± 0.03**	-	-
Purimyces sp.	18964	1.98 ± 0.16	-	**2.57 ± 0.01**	-
Graphium rubrum	18969	1.5 ± 0.06	1.25 ± 0.03	1.7 ± 0.04	-
Yeasts					
Mrakia blollopis	18971	-	1.9 ± 0.04	-	**2.57 ± 0.01**
M. blollopis	18977	-	-	1.19 ± 0.01	-
M. blollopis	18981	-	**2.41 ± 0**	-	1.99 ± 0.07
Mrakia gelida	18972	-	-	-	**2.01 ± 0.07**
M. gelida	18974	-	1.16 ± 0.04	-	1.8 ± 0.15
M. gelida	18982	-	-	-	1.65 ± 0.16
Leucosporidium sp.	18975	-	-	1.7 ± 0.02	-
Leucosporidium sp.	18976	-	-	**2.37 ± 0**	-
Leucosporidium sp.	18979	1.73 ± 0.08	-	1.94 ± 0.01	-
Leucosporidium sp.	18980	-	-	**2.3 ± 0.15**	-
Goffeauzyma sp.	18993	1.54 ± 0.03	1.22 ± 0.02	1.39 ± 0	-
Goffeauzyma sp.	18978	-	-	1.37 ± 0.12	-
Solicoccozyma terricola	18984	-	-	1.28 ± 0.02	-
S. terricola	18985	-	-	-	-
Phenoliferia glacialis	18983	-	-	**2.46 ± 0.03**	1.33 ± 0.01
P. glacialis	18987	-	**2.11 ± 0.01**	-	**2.44 ± 0.12**
Cystobasidium laryngis	18992	-	-	-	-
Candida davisiana	18988	1.56 ± 0.085	1.89 ± 0	1.4 ± 0.04	-
Papiliotrema sp.	18973	-	-	-	-
Naganishia sp.	18994	-	-	-	**2.04 ± 0.1**

Table 2 (continued)

Filamentous fungi	UFMGCB code	Average enzymatic index (mm) (mean ± SD)			
		Amylase	Cellulase	Inulinase	Pectinase
Coniochaeta sp. 1	18938	**2.06 ± 0.47**	1.26 ± 0.1	-	-
Coniochaeta sp. 1	18948	1.48 ± 0.01	-	1.66 ± 0.15	-
Coniochaeta sp. 1	18950	1.58 ± 0.14	1.4 ± 0.07	1.51 ± 0.12	-
Coniochaeta sp. 1	18957	1.5 ± 0.07	1.38 ± 0.03	1.57 ± 0.07	-
Coniochaeta sp. 1	18958	1.3 ± 0.01	1.57 ± 0.1	1.55 ± 0.14	-
Coniochaeta sp. 1	18963	1.52 ± 0.13	1.65 ± 0.01	**2.03 ± 0.02**	-
Coniochaeta sp. 2	18966	1.42 ± 0.1	1.68 ± 0.03	-	-
Coniochaeta sp. 2	18967	1.85 ± 0.07	**2.7 ± 0.07**	**2.51 ± 0.04**	-
Coniochaeta sp. 3	18952	1.33 ± 0.08	1.52 ± 0.11	1.58 ± 0.01	-
Coniochaeta sp. 3	18991	1.37 ± 0.09	1.36 ± 0.01	1.58 ± 0.03	-

UFMGCB = Collection of Microorganisms and Cells of the Federal University of Minas Gerais. Bold = fungal isolates considered good producers (mean enzymatic index ≥ 2)

halo and four were good producers, two assigned to *Ascocoryne* sp. UFMGCB 18953 and *Coniochaeta* sp. UFMGCB 18967, and two representing *Pseudogymnoascus*, UFMGCB 18965 and UFMGCB 18954. Eight yeast isolates presented cellulase halos, and two were good producers: one representative of the genus *Mrakia* (UFMGCB 18981) and one isolate of *Phenoliferia glacialis* UFMGCB 18987.

For inulinase, 23 isolates of filamentous fungi presented degradation halos and five were good producers. Two isolates represented the genus *Coniochaeta* (UFMGCB 18963 and UFMGCB 18967), one *Purimyces* (UFMGCB 18964), and two were unassigned (UFMGCB 18944 and UFMGCB 18955). Eleven yeast isolates presented halos and three were good producers, two representing the genus *Leucosporidium* (UFMGCB 18976 and UFMGCB 18980) and one isolate of *P. glacialis* (UFMGCB 18983).

For pectinase, of the two isolates of the yeast, *S. terricola*, obtained from the specific medium for isolating fermenting yeasts, only one isolate (UFMGCB 18984) presented a halo, but was not a good enzyme producer. Only three isolates of filamentous fungi presented a degradation halo for pectinase, and none were good producers. Among the yeasts, nine isolates presented a halo, and four were good producers, including two isolates of the genus *Mrakia* (UFMGCB 18971 and UFMGCB 18972), one of the genus *Naganishia* (UFMGCB 18994) and one of *P. glacialis* (UFMGCB 18987).

The filamentous isolate of *Coniochaeta* sp. 2 (UFMGCB 18967) was a good producer of cellulase and inulinase, with enzymatic indices of 2.70 ± 0.07 and 2.51 ± 0.04, respectively. The yeast isolate, *P. glacialis* UFMGCB 18987, was a good producer of cellulase and pectinase, with enzymatic indices of 2.11 ± 0.01 and 2.44 ± 0.12, respectively.

Temperature assays and lipid production by the yeast *Solicoccozyma terricola*

The two strains of the yeast *S. terricola* were evaluated for their ability to grow on YM agar medium at different temperatures after 3 and 7 days (Suppl. Figure 2). The yeasts showed growth between 5 and 25 °C but produced a greater number of strong colonies between 15 and 20 °C. After evaluating the optimal growth temperature for *S. terricola*, 20 °C was selected for the lipid production assay. In this assay, lipid bodies stained with Nile Red dye were visualized, indicating lipid production by the isolates UFMGCB 18984 (Suppl. Figure 3) and UFMGCB 18985 (Suppl. Figure 4).

Discussion

Fungal diversity

The historical buildings and structures in Whalers Bay on Deception Island were constructed in the early part of the 20th century associated with the whaling station that operated on the island. Some of these constructions were later repurposed, along with new buildings, to support the development of research operations in the mid-20th century. The import of wood for this purpose may have facilitated the introduction of exogenous species of fungi into Antarctica [7]. Such fungi may potentially include human and animal pathogens and as well as wood-damaging saprophytic fungi, challenging the long-term conservation of these structures [7, 39, 40]. All extant buildings and structures in Whalers Bay form part of the formally declared Antarctic Historic Monument (formal ref), with similar designations applied to various of the remaining 'age of exploration' hut in the Ross

Sea and East Antarctic coastline, as well as early scientific era research station buildings at various locations on the Antarctic Peninsula. Antarctic Treaty nations therefore have a duty to protect and ensure the integrity of these historic locations.

Despite the extreme environmental condition characteristics of the study region, the fungal diversity recovered from the lignocellulosic samples obtained here displayed high diversity indices, dominated by taxa recognized as decomposers of organic matter. In addition, the rarefaction curve indicates that this fungal community was not fully described by our data. These results are similar to those reported by [8], who analyzed the fungal diversity present in the same samples using a culture-independent metabarcoding (environmental DNA) approach. The data of both studies reinforce that the historically imported wooden structures and artifacts examined can act as baits for fungal taxa, especially decomposers, providing favorable microhabitats with abundant nutrients (organic matter).

The most frequent genus found in the current study was *Coniochaeta*. Some members of this genus are known to be pathogens of woody hosts, or root endophytes [41], while others can cause opportunistic human infections, such as endocarditis and septic shock [42]. *Coniochaeta* species have been reported from several Antarctic substrates, including wood from Deception Island [43] and Syowa Station [44] and soils from the McMurdo Dry Valleys [45].

Isolates of the genera *Cadophora*, *Pseudogymnoascus* and *Mrakia* were present in similar abundance. *Cadophora* species are frequently reported in soils and wood associated with Antarctic constructions [43, 46]. The species *C. luteo-olivaceae*, identified in the present study, was also reported by Held & Blanchette [43] in old buildings at Whalers Bay, indicating this group's ability to thrive in polar environments, especially when wood is present as a primary source of nutrients.

Pseudogymnoascus is a genus widely reported from the cold terrestrial and marine environments of the Arctic and Antarctic [5, 7, 47, 48]. Arenz et al. [19] suggested that representatives of *Pseudogymnoascus* play important roles in decomposition and nutrient cycling in Antarctica. Members of *Mrakia* have also been isolated from various extreme polar environments [49, 50]. Tsuji et al. [51] noted that *Mrakia* accounts for approximately 35% of the cultivable fungi isolated from lake sediments and soils of East Antarctica.

Representatives of the genus *Leucosporidium* are also well-known in polar regions, with species reported in Antarctic seawater [52], soils [53, 54], bryophytes and angiosperms [55]. Two isolates were identified as belonging to the genus *Goffeauzyma*, which includes cold-adapted species [56]. *Goffeauzyma* representatives have been found in Antarctic soil samples [57]. Two isolates of *P. glacialis* were identified; the species is a psychrophile and has previously been reported from biofilms collected on the Antarctic Peninsula [55] and in high densities in snow samples, including some collected on Deception Island [58].

The species *S. terricola*, previously known as *Cryptococcus terricola*, is a psychrotolerant oleaginous yeast found in soils of cold environments [59]. *Solicoccozyma terricola* has been reported in soils, mosses and lichens in Antarctica [60, 61] and in soil and glacier sediments in glacial environments, such as the Forni and Sforsellina glaciers in Italy [62].

Cystobasidium laryngis, formerly known as *Rhodotorula laryngis*, is a yeast isolated from decaying wood in the Faroe Islands [63], from the angiosperm *Deschampsia antarctica* [64], and from Antarctic lichens and soils [60, 65]. *Mollisia*, also reported in this study, is a cosmopolitan genus comprising of saprophytic species found in decaying plant tissues, especially wood and grasses [66]. Held & Blanchette [43] reported *Mollisia* species in decaying wood in Whalers Bay. The genus *Naganishia* comprises polyextremophilic species, such as *N. vishniacii*, which has been isolated from Antarctic soil samples [67]. *Naganishia* species are described as some of the most resistant organisms known to UV radiation, one of the extreme conditions found in Antarctica [68]. Additionally, *Cadophora*, *Mrakia*, *Cystobasidium* and *Mollisia* share the ecological role of including species reported as important endophytes in the roots of plants living in polar and alpine regions [41, 64, 66, 69].

The genus *Graphium* has a wide distribution, with species isolated from blocks of wood in the sea around Hong Kong [70]. Some species exhibit barotolerance, with the ability to survive in the extreme conditions of the deep sea [71]. *Graphium rubrum* has been reported in high densities in samples from various depths in the Southern Ocean [72]. *Candida davisiana* has been isolated from Antarctic soil samples [34, 71]. This species has also been reported to be resistant to UV radiation [61]. Representatives of the genus *Papiliotrema* have also been reported in Antarctica [73–75]. The species *P. laurentii*, previously considered saprophytic, has been associated with human infections in cancer patients and has shown an increase in cases in recent decades [76].

The genus *Ascocoryne* (syn. *Gliocladium*) was originally obtained as an endophyte from the Patagonian plant species, *Eucryphia cordifolia* [76, 77]. It has also been described as a producer of petroleum-like compounds when grown in a cellulose-based medium [78]. *Ascocoryne* representatives are common in various climatic zones, ranging from Antarctica to the tropics, and have

been reported from Scott's Discovery Hut on Ross Island, Victoria Land [79, 80]. *Purimyces* is a newly erected genus characterized by having root endophytic representatives of *Cattleya locatellii* and does not appear to have been reported from Antarctica [81].

Representatives of the genera *Coniochaeta* and *Cadophora* are the main causes of wood decomposition in buildings in Antarctica. They have been reported in various studies and commonly represent the largest number of isolates found, corroborating the results of the present study [43, 82–85]. The genera *Graphium* and *Mollisia* have also been associated with wood decomposition in Antarctic buildings [43, 84]. In addition, the abundance of *Cadophora* and *Coniochaeta* in the lignocellulosic substrates studied here, which include species recognized as important decomposers, might indicate that a portion of the resident fungal communities is adapting to warmer temperatures and potentially accelerating decomposition processes in Antarctic environments.

Enzymatic activities of Antarctic fungal isolates

Fungi in Antarctica possess physiological and metabolic adaptations that enable them to survive the extreme conditions in the region's various microhabitats, such as antifreeze proteins, exopolysaccharides, antioxidants, photoprotective pigments and enzymes active at low temperatures [74]. These enzymes have properties that can be useful in biotechnological processes, such as reducing energy costs in production processes and selective inactivation in complex mixtures due to their instability when exposed to heat [86].

In the Antarctic lignocellulosic samples examined here, the genus *Coniochaeta* was classified as a good producer of amylase, inulinase and cellulase. Lopez et al. [87] and Henzelyová et al. [88] reported *Coniochaeta* species as cellulase producers. Our study appears to be the first report of *Coniochaeta* isolates capable of producing amylase and inulinase. Two isolates of the genus *Pseudogymnoascus* showed good cellulase production. Krishnan et al. [89] previously reported cellulase-producing *Pseudogymnoascus* species, highlighting that this genus plays an important role in decomposition processes in polar environments. Members of the genus *Mollisia* are known producers of amylase [90], as noted here. Only isolates of the genus *Ascocoryne* produced cellulase at a high rate. Other non-polar members of this genus also expressed high cellulase activity, a potential which has been commercially exploited [79, 91]. Our study provides the first report of an isolate of the genus *Piromyces* being a good producer of inulinase.

One isolate of the genus *Mrakia* was a good producer of cellulase, and two others were good producers of pectinase. Similar results have been reported for other members of this genus [60, 92]. Two isolates of the genus *Leucosporidium* were good producers of inulinase, consistent with a previous study [93]. The isolates of *P. glacialis* were good producers of cellulase, inulinase and pectinase, consistent with the findings of Pathan et al. [92] and Carrasco et al. [60] for cellulase and pectinase. However, our study appears to be the first to report isolates of this species producing inulinase. The only isolate of the genus *Naganishia* was a good producer of pectinase, consistent with [94].

All the enzymes detected in the lignocellulosic fungi recovered in our study showed activity at low temperatures, suggesting potential applications in various industrial sectors. Amylases might be used in the detergent industry (as cold-water detergents), food processing in cold saccharification, textile desizing, molecular biology (for laboratory use), and as animal feed additives (in cold climates). Inulinase is used for the production of fructooligosaccharides (FOS) at low temperatures (functional food), functional alcohol production (cold-fermentation wine or beer), and animal feed (cold climates) to boost the digestibility of inulin-rich feedstocks. Pectinase is used for fruit juice clarification at low temperatures (for better preservation of vitamins, flavor and color, extending the shelf life of juice), wine and cider production (cold fermentation) and textile processing (eco-friendly bioscouring). Cellulase is used in the textile industry (cold bio-polishing and biostoning), detergent industry (cold-wash laundry enzymes) and the paper and pulp industry (eco-friendly pulp processing).

Lipid production by the yeast *Solicoccozyma terricola*

Solicoccozyma terricola is a psychrotolerant oleaginous yeast, often isolated from soils in cold environments [59] including Antarctica [61]. Studies involving *S. terricola* focus on its ability to accumulate intracellular lipids, a potentially important characteristic for the food, cosmetics, textiles and fuel production industries [95].

Studies have assessed its ability to produce lipids, with the amount produced varying according to the temperature and carbon sources provided [38, 96, 97]. Tasselli et al. [95] reported that growing the species at 20 °C resulted in the highest lipid production with a lipid yield close to that found in palm oil.

Conclusions

Results obtained in this study confirm that the lignocellulosic substrates associated with structures in the historic whaling station and research station in Whalers Bay on Deception Island contain a range of culturable fungal taxa previously reported from Antarctica. Some of the taxa found appear to originate from other

regions and substrates, raising the possibility of these fungi being introduced into the region along with the materials and human communities involved in their construction. Our taxonomic and diversity results are consistent with those reported in the eDNA metabarcoding study of de Souza et al. [8], again suggesting that the high diversity and dominance of recognized decomposer taxa may have been introduced when wood was first imported, not least given the lack of wood-related substrates in the natural environment of Antarctica. However, these taxa may also have arrived on Deception Island as spores dispersed by air currents, birds or human activity, or these imported substrates may have acted as baits for already present mycobiota.

The enzymatic activities of the fungi found are consistent with previous studies, confirming the importance of these enzymes in the survival of microorganisms in extreme environments such as Antarctica. We detected a moderate diversity of fungi in the substrates examined in this study. We suggest that their biotechnological potential should now be explored as more sustainable alternatives to industrial processes involving enzymes active at low temperatures. Additionally, further genetic studies may be conducted to identify which genes are responsible for the enzymes detected in the Antarctic fungi. In particular, we identified *Coniochaeta* taxa as excellent producers of inulinase and amylase for the first time, along with members of the genus *Purimyces* and the species *Phenoliferia glacialis* for inulinase. The oleaginous yeast *S. terricola* demonstrated growth across a wide temperature range, highlighting its psychrophile characteristics, which may facilitate its presence in the Antarctic terrestrial environment. This yeast produced lipids at an optimal temperature of 20 °C, underscoring its biotechnological potential as a lipid source, for instance for use as biofuel feedstock. However, further detailed studies will be necessary to determine the metabolic lipid pathways and their potential use as biofuel and in other applications. Finally, our results show that the Antarctic ecosystem shelters important microbes with great potential for biotechnological applications, reinforcing the urgency to preserve the region to avoid biological extinction due to local climate changes.

Supplementary Information

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Supplementary Material 1.

Authors' contributions

E.A.A.T. and L.H.R. conceived the study. L.H.R. collected the samples. E.A.A.T., L.M.D.S. and C.A.R. performed the fungal identification. E.A.A.T., L.M.D.S. and C.R.C. performed the biological assays. E.A.A.T., L.M.D.S., C.A.R. P.C. and L.H.R. analyzed the results and wrote the manuscript. All authors read and approved of the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable. This manuscript does not include any individual person's data in any form.

Competing interests

The authors declare no competing interests.

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