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MICROBIOLOGY

Biobank of fungi from marine and terrestrial Antarctic environments

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Abstract: Harsh and extreme environments, such as Antarctica, offer unique opportunities to explore new microbial taxa and biomolecules. Given the limited knowledge on microbial diversity, this study aimed to compile, analyze and compare a subset of the biobank of Antarctic fungi maintained at the UNESP's Central of Microbial Resources (CRM-UNESP). A total of 711 isolates (240 yeasts and 471 filamentous fungi) from marine and terrestrial samples collected at King George Island (South Shetland Islands, Antarctica) were used with the primary objective of investigating their presence in both marine and terrestrial environments. Among the yeasts, 13 genera were found, predominantly belonging to the phylum Basidiomycota. Among the filamentous fungi, 34 genera were represented, predominantly from the phylum Ascomycota. The most abundant genera in the marine samples were *Metschnikowia*, *Mrakia*, and *Pseudogymnoascus*, while in the terrestrial samples, they were *Pseudogymnoascus*, *Leucosporidium*, and *Mortierella*. Most of the genera and species of the CRM-UNESP biobank of Antarctic fungi are being reported as an important target for biotechnological applications. This study showed the relevance of the CRM-UNESP biobank, highlighting the importance of applying standard methods for the preservation of the biological material and associated data (BMaD), as recommended in national and international standards.

Key words: Extremophile, taxonomy, microbial biobank, biotechnology.

INTRODUCTION

Antarctica's harsh conditions (e.g. low temperatures, freezing and thawing cycles, strong winds, high sublimation and evaporation rates, high radiation incidence, and long periods of darkness) limit the development of many life forms (Onofri et al. 2007). The diversity in Antarctica tends to be lower, and in some systems, biogeochemical cycles and food chains are exclusively formed by microorganisms (Vincent 2000, Duarte et al. 2018a).

Microorganisms and their biomolecules drive Bioeconomy in several socioeconomic sectors, with the production and development of raw materials, their conversion into products, and the recycling of by-products and waste (Kircher 2022). According to Antranikian & Streit (2022), any technological advancements will need to use the billions of microbial catalysts, pathways, cells, consortia, and compounds, based on a sustainable, biobased circular economy. The main purpose of sustainability is to raise the standard of living without increasing the use of resources beyond sustainable global levels (Murray et al. 2013).

The discovery of high value-added biomolecules can be intensified by the exploration of microbiological material from extreme environments, since, in addition to

the lack of knowledge about this material, adaptations to the environment can represent new metabolic pathways and biomolecules of biotechnological interest (Duarte et al. 2018b, Lo Giudice & Gugliandolo 2019, Giovanella et al. 2020). In this sense, prospecting, characterizing, and producing biomolecules of microbial origin is advantageous, since microbial resources are recovered from environmental samples, preserved *ex-situ,* and used repeatedly, without the need for new environmental intervention.

Microbial biobanks (also known as Microbial Culture Collections or Biological Resource Centers) underpin the development of biotechnology, maintaining, providing, and studying biological material and associated data (BMaD) within legal standards and with quality control. Additionally, as repositories and suppliers of microbial diversity and associated information, microbial biobanks have a relevant role in promoting the Convention on Biological Diversity, especially the Nagoya Protocol on Access and Benefit Sharing – ABS (Sette et al. 2013).

Using non-compliant microbiological material (e.g. misidentified microorganisms and/or contaminated cultures) in R&D and industrial or environmental processes can cause problems (Sette et al. 2013), including the waste of time and financial resources. The low reproducibility rates in research in the field of life sciences were reported by Freedman et al. (2015), indicating that the lack of reproducibility exceeds 50%, generating a loss of more than 25 billion dollars per year in the phase of preclinical studies and contributing to delays in the development of therapeutic drugs. The authors conclude that the use of high-quality biological products and reagents, as well as operating procedures within the requirements of good laboratory practices, play a central role in improving reproducibility.

To guarantee the quality of the biological material preserved in microbial biobanks and used in R&D and biotechnological processes, the Organization for Economic Cooperation and Development (OECD) published the OECD Best Practice Guidelines for BRC (OECD 2007). These OECD guidelines served as one of the references for the development of international technical standards for biobanks within the scope of Biotechnology (ISO 20387: 2018 Biotechnology - Biobanking), specifying general requirements for the competence, impartiality, and consistent operation of biobanks, including quality control requirements to ensure that biological materials and associated data are of appropriate quality for their intended use (ISO 2018).

The biobank Central of Microbial Resources at UNESP (CRM-UNESP) was officially created in 2013 and is registered at the World Data Centre for Microorganisms (WDCM 1043). Among the microbial resources in CRM-UNESP there is a collection of fungi (filamentous and yeasts) of Antarctic origin with approximately 2,800 isolates, considering both the research collection (BMaD to be known and exploited) and the main collection (already characterized BMaD), which have been used in studies on microbial diversity, resistance to adverse conditions, and biotechnological application conducted by the Laboratory of Environmental and Industrial Mycology (LAMAI) (Institute of Biosciences - UNESP, Rio Claro, SP, Brazil).

The aim of the present study was to compile, analyze and compare the BMaD of part of the biobank of Antarctic fungi (711 isolates obtained from marine and terrestrial samples collected at King George Island, South Shetland Islands, Antarctic Peninsula) considering their presence in marine and terrestrial Antarctic environments, as well as to indicate their potential for biotechnological applications.

MATERIALS AND METHODS

Biobank of Antarctic fungi: biological material

The Antarctic fungi used in the present study (240 yeasts and 471 filamentous fungi) were obtained from different Antarctic samples collected at eight sites in King George Island, South Shetland Islands, Antarctic Peninsula (Figure 1). Sampling was performed during the summer in 2010 and the summer in 2015 in the XXVIII and the XXXVII Brazilian Antarctic Operations (OPERANTAR), respectively (Table I). Comandante Ferraz Antarctic Station, Ullmann Point and Refuge II were common sites for marine sediment collection during both expeditions. Sampling and the isolation of filamentous fungi from the samples collected during OPERANTAR XXVIII were conducted as described by Duarte et al. (2018a). Sampling at Collins Glacier and the isolation of the fungi were performed as described by Santos et al. (2020). All other samplings and fungal isolations were conducted as reported by Wentzel et al. (2019).

The fungal isolates belong to the collection of the Laboratory of Environmental and Industrial Mycology (LAMAI), which is associated with the microbial biobank Central of Microbial Resources (CRM-UNESP) of the São Paulo State University (UNESP, Brazil). They are maintained by the following preservation methods: cryopreservation at -80 °C (filamentous fungi and yeasts) and Castellani (filamentous fungi), and lyophilization (part of the yeast collection).

Data associated with the microbiological material maintained at CRM-UNESP are stored in the information system for microbial culture collections (microSICol software).

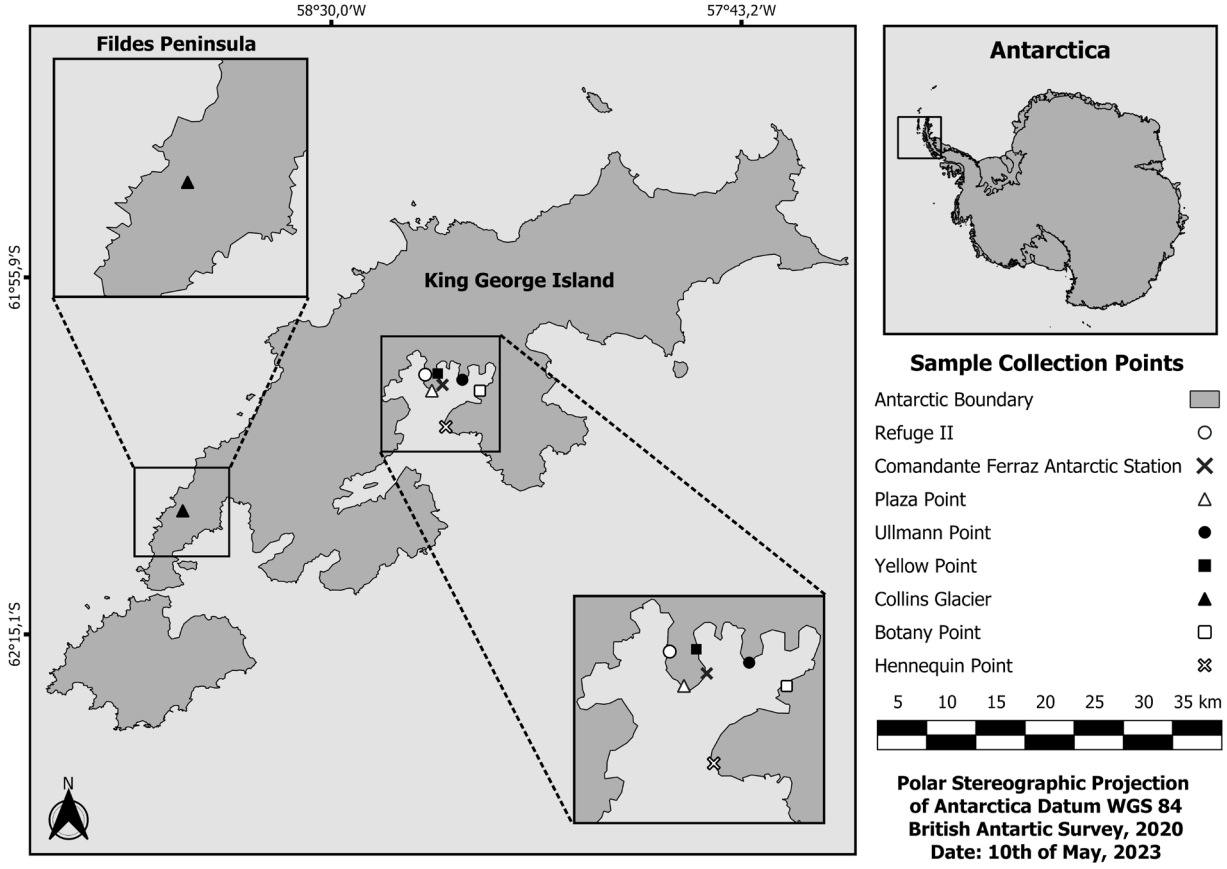

Figure 1. Sampling sites in King George Island (Admiralty Bay and Fildes Peninsula), South Shetlands Archipelago, Maritime Antarctica.

Table I. Data related to the expeditions, samples of origin, and sites of the filamentous fungi isolates. The yeasts were collected from the same samples and sites during OPERANTAR XXXIII. The samplings were performed at King George Island, Maritime Antarctica.

Expedition	Sample	Site	Geographic coordinate
OPERANTAR XXVIII (Summer 2010)	Ascidia (Marine invertebrate)	Comandante Ferraz Antarctic Station	62º05'130'S 58º23'536'W
	Starfish (Marine invertebrate)	Comandante Ferraz Antarctic Station	62º05'130'S 58º23'536'W
	Nacella sp. (Marine invertebrate)	Comandante Ferraz Antarctic Station	62º05'130'S 58º23'356'W
	Sea urchin (Marine invertebrate)	Comandante Ferraz Antarctic Station	62º05'130'S 58º23'356'W
	Amphipoda (Marine invertebrate)	Plaza point	62º05'S 58º24'W
	Isopod (Marine invertebrate)	Plaza point	62º05'S 58º24'W
	Salpa sp. (Marine invertebrate)	Plaza point	62º05'S 58º24'W
	Marine sediment	Comandante Ferraz Antarctic Station	62º05'130'S 58º23'356'W
	Marine sediment	Refuge II	62º04'373'S 58º25'335'W
	Marine sediment	Ullmann Point	62º05'015'S 58º20'987'W
OPERANTAR XXXIII (Summer 2015)	Soil	Collins Glacier (Fildes Peninsula)	62°09'821'S 58°55'373'W
	Soil	Yellow Point	62°04'479'S 58°23'726'W
	Soil (associated with the root of *Deschampsia antarctica*)	Hennequin Point	62°07'216'S 58°23'677'W
	Soil (associated with the root of *Coobanthus quitensis*)	Hennequin Point	62°07'216'S 58°23'677'W
	Soil (associated with the root of *Deschampsia antarctica*)	Plaza point	62°05'363'S 58°24'691'W
	Soil (associated with the root of *Coobanthus quitensis*)	Plaza point	62°05'363'S 58° 24'691'W
	Marine sediment	Botany Point	62°05'734'S 58°19'919'W
	Marine sediment	Comandante Ferraz Antarctic Station	62°05'130'S 58°23'356'W
	Marine sediment	Ullmann Point	62°05'015'S 58°20'987'W
	Marine sediment	Refuge II	62°04'373'S 58°25'335'W

Biobank of Antarctic fungi: associated data analyses

Data referring to the type of substrate, type of environment, number of isolates, and genera, both for yeasts and filamentous fungi, were used to create the graphs using the program Sigma Plot 10.0.

All isolates have been previously characterized using molecular taxonomy, employing the marker ITS for the identification of filamentous fungi and 28S-rDNA (region D1/D2) for yeast identification, as reported by Duarte et al. (2018a), Wentzel et al. (2019), Santos et al. (2020) and Farias et al. (2022). One representative of each taxon from the same Antarctic sample (substrate) and site (type of environment) (Table I) was used for the generation of the phylogenetic trees. The Genbank codes of these sequences are included in the trees of the filamentous fungi and yeasts (Figures 5 and 6, respectively).

The ITS (filamentous fungi) and 28S-rDNA (yeasts) sequences were aligned using MAFFT v.7 (Katoh & Standley 2013) and edited using Aliview v.1.28 (Larsson 2014). The evolutionary history was inferred using the Maximum Likelihood in MEGA 11 (Tamura et al. 2021). The nucleotide substitution model used was generated in jModelTest 2 v.2.1.10 (Darriba et al. 2012) using the Akaike Information Criterion (AIC) with 95% confidence. The model used for yeasts was GTR + G and for filamentous fungi, GTR + I + G. The bootstrap consensus tree inferred from 1000 replicates was chosen to represent the evolutionary history of the analyzed taxa (Felsenstein 1985).

To analyze the correlation among the sampled locations, the function 'corr()' from the Pandas library (Reback et al. 2020) in Python 3 was used, employing the 'Pearson' method. The matplotlib library (Hunter 2007) and the seaborn library (Waskom 2021) in Python 3 were used to generate the heatmap.

RESULTS

Fungi from King George Island

The fungi used in the present study, from the biobank CRM-UNESP, encompass 240 yeasts and 471 filamentous fungi obtained from Antarctic marine and terrestrial samples collected at King George Island. The yeasts used in the present work had their 28S rDNA (D1/D2 region) previously sequenced and identified by Wentzel et al. (2019) and Farias et al. (2022). DNA sequencing of the ITS region has been previously used to identify filamentous fungi (Duarte et al. 2018a, Wentzel et al. 2019, Santos et al. 2020).

The compiled data showed that a total of 13 yeast genera were isolated from the 5 different substrates distributed throughout King George Island (Admiralty Bay and Fildes Peninsula), including root-associated soil, soil, glacier retreat soil, intertidal sediment, and marine sediment (Figure 2a and b).

As shown in Figure 2a, the most represented genus was *Metschnikowia* (29.58%), followed by *Mrakia* (16.67%), *Leucosporidium* (15.83%), *Goffeauzyma* (11.25%), *Holtermanniella* (8.33%), *Glaciozyma* (5.41%), *Meyerozyma* (3.75%) and *Cystobasidium* (3.75%). In contrast, the genera *Camptobasidium*, *Candida*, *Naganishia, Phenoliferia* and *Vishniacozyma* were the least dominant, together representing less than 5.5 %.

Metschnikowia was exclusively detected in marine sediment samples. The second most abundant genus, *Mrakia*, was primarily recovered from marine sediment, yielding 36 isolates, whereas only four isolates were recovered from root-associated soil. Conversely, *Leucosporidium* was recovered from nearly all substrates, with the exception of glacier retreat soil, and exhibited higher abundance in terrestrial environments, particularly in root-associated soil. The genus *Goffeauzyma* showed a widespread distribution in both marine (13 isolates) and terrestrial substrates (14 isolates),

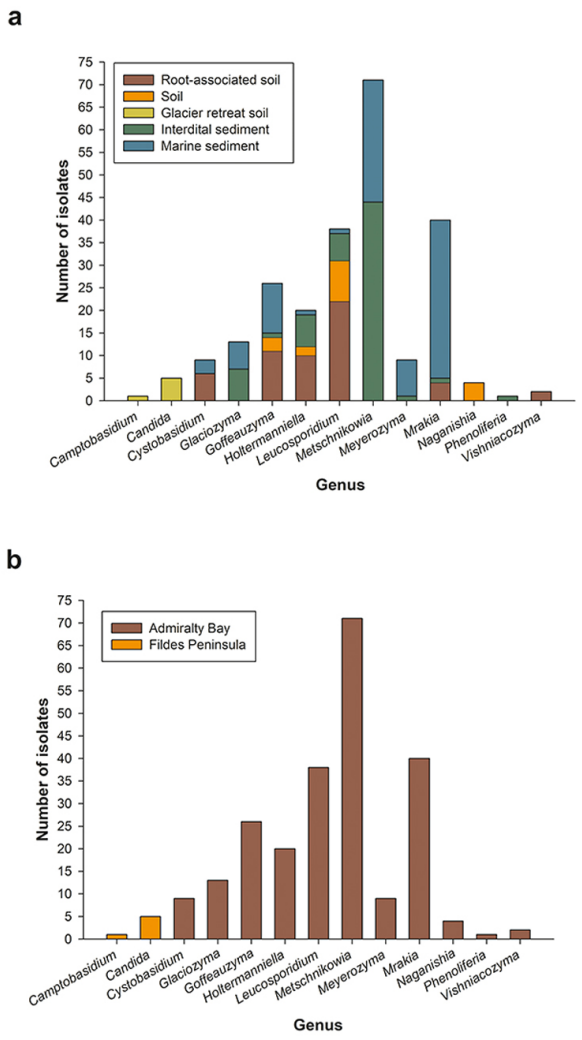

Figure 2. Number of yeast isolates per genera found in different types of samples (a) and sampling sites (b).

exhibiting a difference of one isolate between the two environments.

Four genera, namely *Phenoliferia*, *Glaciozyma*, *Metschnikowia*, and *Meyerozyma*, were exclusively isolated from marine substrates and accounted for 39.15% of the total yeast diversity in the CRM-UNESP biobank. In contrast, four other genera, *Camptobasidium*, *Candida*, *Naganishia*, and *Vishniacozyma*, were exclusively found in terrestrial samples, representing only 5% of the total yeast diversity in the biobank.

The analysis of the microbial distribution in different substrates revealed significant differences in the abundance of microorganisms in each environment. The highest abundance of yeasts was found in the marine sediment (38.75%), followed by intertidal sediment (28.33%) and root-associated soil (22.92%). Soil samples presented the lowest abundance of yeasts (7.5%).

Considering the geographical localization (Figure 2b), the great majority of the previously identified yeasts present in the CRM-UNESP biobank were isolated from Admiralty Bay (97.5%) in comparison with those isolated from Fildes Peninsula (2.5%).

Filamentous fungi exhibited a higher number of taxa in comparison with yeasts (Figure 3a). Among the 471 filamentous fungi, 34 genera were isolated from six substrates, namely root-associated soil, soil, glacier retreat soil, intertidal sediment, marine sediment, and marine invertebrates (Figure 3a) collected at King George Island (Admiralty Bay and Fildes Peninsula) (Figure 3b).

Pseudogymnoascus was the dominant filamentous fungal genus (Figure 3a), comprising 59.87% of the total isolates, followed by *Mortierella* (6.36%), *Pseudeurotium* (5.3%), and *Cadophora* (3.39%). Among these genera, *Pseudogymnoascus* was the most dominant in 5 out of the 6 types of substrates collected, except for the marine invertebrate. *Mortierella* was exclusively found in terrestrial samples (root-associated soil, soil, and glacier retreat soil), with the highest number of isolates obtained from glacier retreat soil (61.99%). *Pseudeurotium* and *Cadophora* were found in both marine and terrestrial samples, but *Pseudeurotium* was more abundant in terrestrial substrates (88% of the isolates obtained from glacier retreat soil), whereas *Cadophora* was more commonly found in marine samples (93.75% of isolates obtained from marine sediments and invertebrates).

Furthermore, 10 genera were found solely in glacier retreat soil samples, including

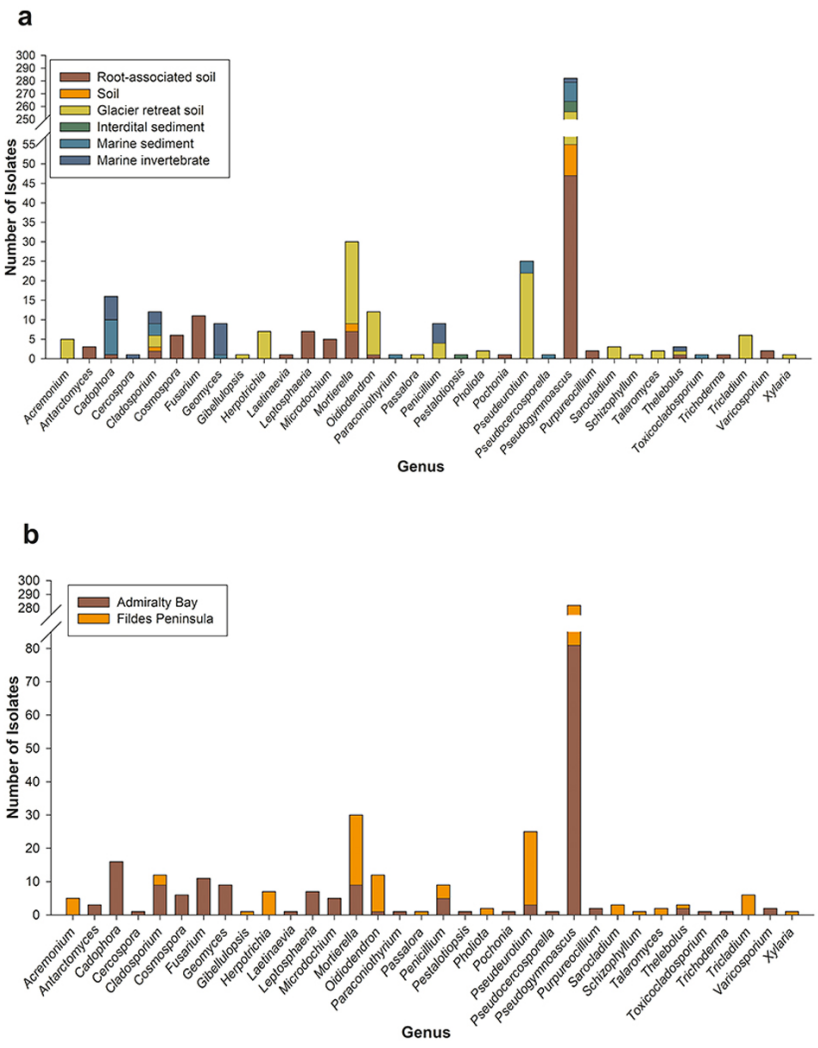

Figure 3. Number of filamentous fungi isolates per genera found in different types of samples (a) and sampling sites (b).

Acremonium, *Gibellulopsis*, *Herpotrichia*, *Passalora*, *Pholiota*, *Sarocladium*, *Schizophyllum*, *Talaromyces*, *Tricladium*, and *Xylaria*, representing 6.12% of the isolated filamentous fungi.

In addition, 10 genera were exclusively found in root-associated soil, namely *Antarctomyces*, *Cosmospora*, *Fusarium*, *Laetinaevia*, *Lepstosphaeria*, *Microdochium*, *Pochonia*, *Purpureocillium*, *Trichoderma*, and *Varicosporium*. These genera represented 8.4% of the total filamentous fungal diversity among the studied collection.

A total of 22 genera were exclusively found in the terrestrial samples: *Acremonium*, *Antarctomyces*, *Cosmospora*, *Fusarium*, *Gibellulopsis*, *Herpotrichia*, *Laetinaevia*, *Lepstosphaeria*, *Microdochium*, *Mortierella*, *Oidiodendron*, *Passalora*, *Pholiota*, *Pochonia*, *Purpureocillium*, *Sarocladium*, *Schizophyllum*, *Talaromyces*, *Tricladium*, *Trichoderma*, *Varicosporium*, and *Xylaria*. Together they represent 23.42% of the total filamentous fungal isolates.

Considering the marine samples, 6 genera were found exclusively in this environment. These genera include *Cercospora*, which was found only in marine invertebrates, as well as *Geomyces*, *Paraconiothyrium*, *Pestalotiopsis*, *Pseudocercosporella*, and *Toxicocladosporium*.

Together, these genera represent 2.96% of the total diversity of filamentous fungi isolated.

Significant differences were observed in the abundance of filamentous fungi among the different substrates. Glacier retreat soil exhibited the greatest abundance of filamentous fungi (61.99%), which was significantly higher than in root-associated soil (20.80%) and marine sediment (7.21%). Filamentous fungi isolated from marine invertebrates represented (5.73%) of the total, while soil samples accounted for (2.33%). The intertidal sediment presented the lowest abundance among all substrates collected (1.91%).

Admiralty Bay presented the highest diversity of filamentous fungi (Figure 3b). Of the 36 genera that were isolated, 24 were either exclusively or non-exclusively found in Admiralty Bay. However, the number of isolates obtained from Fildes Peninsula was higher than Admiralty Bay, with a higher abundance of *Pseudogymnoascus*, particularly in the glacier retreat soil samples collected there (Figure 3b).

Pearson correlation

The Pearson correlation coefficients confirmed that yeast groups were positively correlated with the type of environment (marine/marine and terrestrial/terrestrial), except for the glacier retreat soil, which had no correlation with isolates from both terrestrial and marine environments. Marine sediment was positively correlated with intertidal sediment, and the root-associated soil had a positive correlation with soil (Figure 4a).

In contrast, for the data on the filamentous fungi (Figure 4b), there was a positive correlation between the different types of environments (marine/terrestrial) and among the same types of environments (marine/marine and terrestrial/terrestrial), except for isolates from marine invertebrate samples, which presented

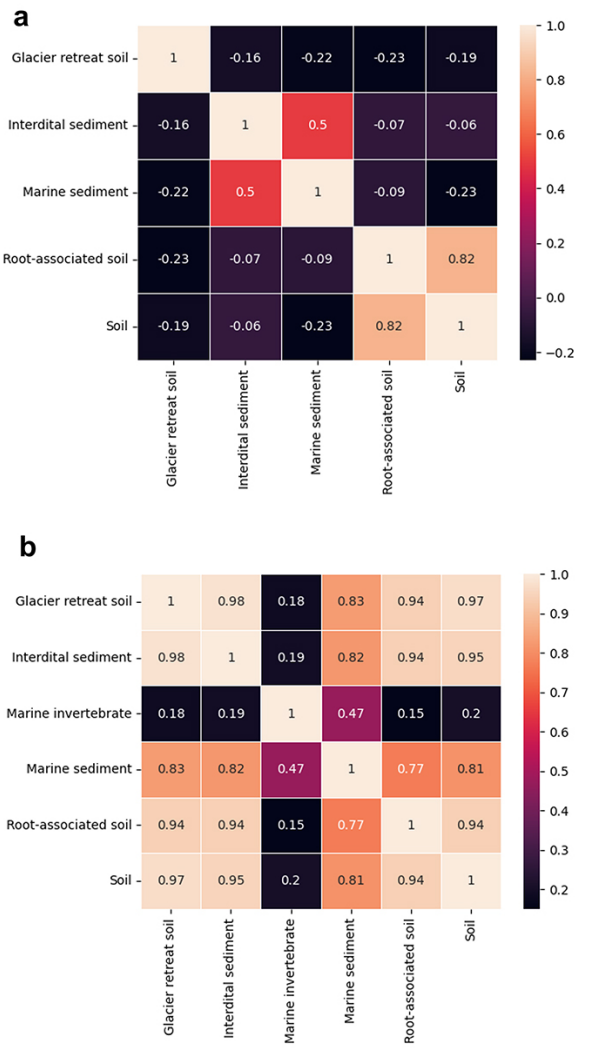

Figure 4. Heatmap showing the pairwise Pearson correlation coefficients (R) of the sampling sites for yeasts (a) and filamentous fungi (b). A Pearson's r value of 1 indicates a total positive correlation, a value of -1 indicates a total negative correlation, and a value of 0 indicates no correlation. p value adjusted to <0.05.

only a significant positive correlation with marine sediment.

Phylogenetic position

One representative of each yeast and filamentous fungi taxa from the same sample and site was used to generate phylogenetic trees (Figures 5 and 6).

Among the 13 Antarctica-derived yeast genera, 11 belong to the phylum Basidiomycota (Classes

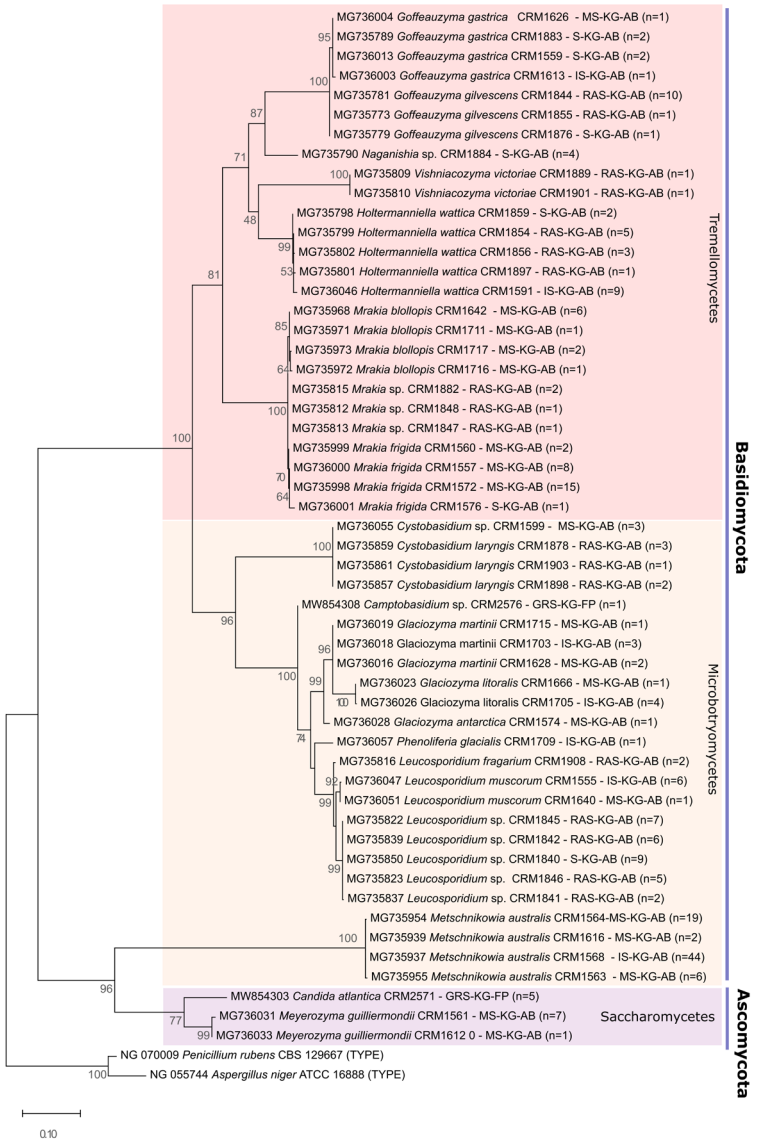

Figure 5. Phylogenetic analysis of the 28S rDNA (region D1/D2) sequences of the yeasts from the different samples and sites. The evolutionary history was inferred using the Maximum Likelihood method and the nucleotide substitution model GTR +G. The percentage of the trees in which the associated taxa cluster (bootstrap of 1000 replicates) is shown next to the branches.

Tremellomycetes and Microbotryomicetes). Considering the basidiomycetous yeasts of the Class Tremellomycetes, the genus *Goffeauzyma* was represented by the species *G. gastrica* (from marine sediment, intertidal sediment and soil) and *G. gilvescens* (from root-associated soil and soil). *Vishniacozyma* was represented by the species *V. victoriae* (only from root-associated soil). *Holtermanniella*, by the species *H. wattica* (from soil, root-associated soil and intertidal sediment). The genus *Mrakia* was represented by the species *M. blollopsis* (isolated only from marine sediment) and *M. frigida* (from marine sediment and soil) and other non-identified species from root-associated soil. This Class was also represented by the genus *Naganishia* (non-identified species), whose representatives were isolated from soil. The genera of the Class Microbotryomicetes were represented by the species *Cystobasidium laryngis* (from root-associated soil) and one non-identified species from this genus isolated from marine sediment. This Class was also represented by a non-identified species of the genus *Camptobasidium* (from glacier retreat soil), *Glaciozyma martini*,

G. litoralis, *G. antarctica* and *Phenoliferia glacialis* (all isolated from marine or intertidal sediments), *Leucosporidium fragarium* (from root-associated soil), *L. muscorum* (from marine and intertidal sediments), and *Metschnikowia australis*, also from marine and intertidal sediments.

The phylum Ascomycota (Class Saccharomycetes) was represented by two species: *Candida atlantica* (from glacier retreat soil) and *Meyerozyma guilliermondii* (from marine sediment).

The 34 genera of filamentous fungi of Antarctic origin were distributed into three phyla: Ascomycota, Basidiomycota and Mortierellomycota. Ascomycota representatives comprised the most abundant group, with 31 genera distributed into four Classes (Leotiomycetes, Eurotiomycetes, Sordariomycetes, and Dothideomycetes) (Figure 6).

Considering the filamentous fungi of the Class Leotiomycetes, the genus *Laetinaevia* was represented by the species *L. cameoflavida*, from root-associated soil. The genus *Cadophora* was represented by three species, *C. luteo-olivacea* (from marine sediment and root associated soil), *C. malorum* (from marine sediment and marine invertebrate), and *C. fastigiate* (from marine sediment). Regarding the Class Sordariomycetes, the genus *Fusarium* was represented by two putative species, *F.* cf. *oxysporum* and *F.* cf. *avenaceum*, both from root-associated soil. *Purpureocillium lilacinum* and *Microdochium lycopodinum*, both from root-associated soil, were the only representatives of the genera *Purpureocillium* and *Microdochium*, respectively. The genus *Cladosporium*, of the Class Dothideomycetes, was represented by the species *C. halotolerant*, isolated from a marine invertebrate, but also by other non-identified (at the species level) isolates from different origins: soil, glacier retreat soil, root-associated soil, marine sediment, and marine invertebrate.

Basidiomycota representatives were solely of the Class Agaricomycetes. The genera *Pholiota* and *Schizophyllum* were represented by non-identified species from glacier retreat soil. *Mortierella* was the only genus of the Class Mortierellomycetes and the phylum Mortierellomycota, with no fully-identified species, but with isolates from soil, glacier retreat soil, and root-associated soil.

DISCUSSION

Fungi from King George Island

The nature and diversity of yeasts and filamentous fungi from marine and terrestrial Antarctic environments is still poorly understood. According to Duarte et al. (2018b), the growing number of new fungal taxa from Antarctica indicates an apparently hidden diversity in this environment. Antarctica is known for its extreme and harsh environmental conditions (Fell et al. 2006). Furthermore, the Antarctic marine environment is also influenced by various biotic and abiotic factors, such as ocean currents, wind patterns, and the presence of marine animals. These factors can create diverse microhabitats that promote the growth and survival of different microbial species (Ruisi et al. 2007, Convey & Peck 2019, Rosa et al. 2019). Studies have shown that yeasts are commonly found in marine environments, where they can be associated with various substrates, such as algae, seawater, and sediments (Buzzini et al. 2012, Ogaki et al. 2019, Rosa et al. 2019). In Antarctica, marine environments, particularly marine sediments, have been found to harbor a diverse range of yeasts (Fell 2012, Rosa et al. 2019). In this CRM-UNESP biobank comprised of strains isolated from Antarctica, a great diversity and abundance of yeasts in marine sediments collected from

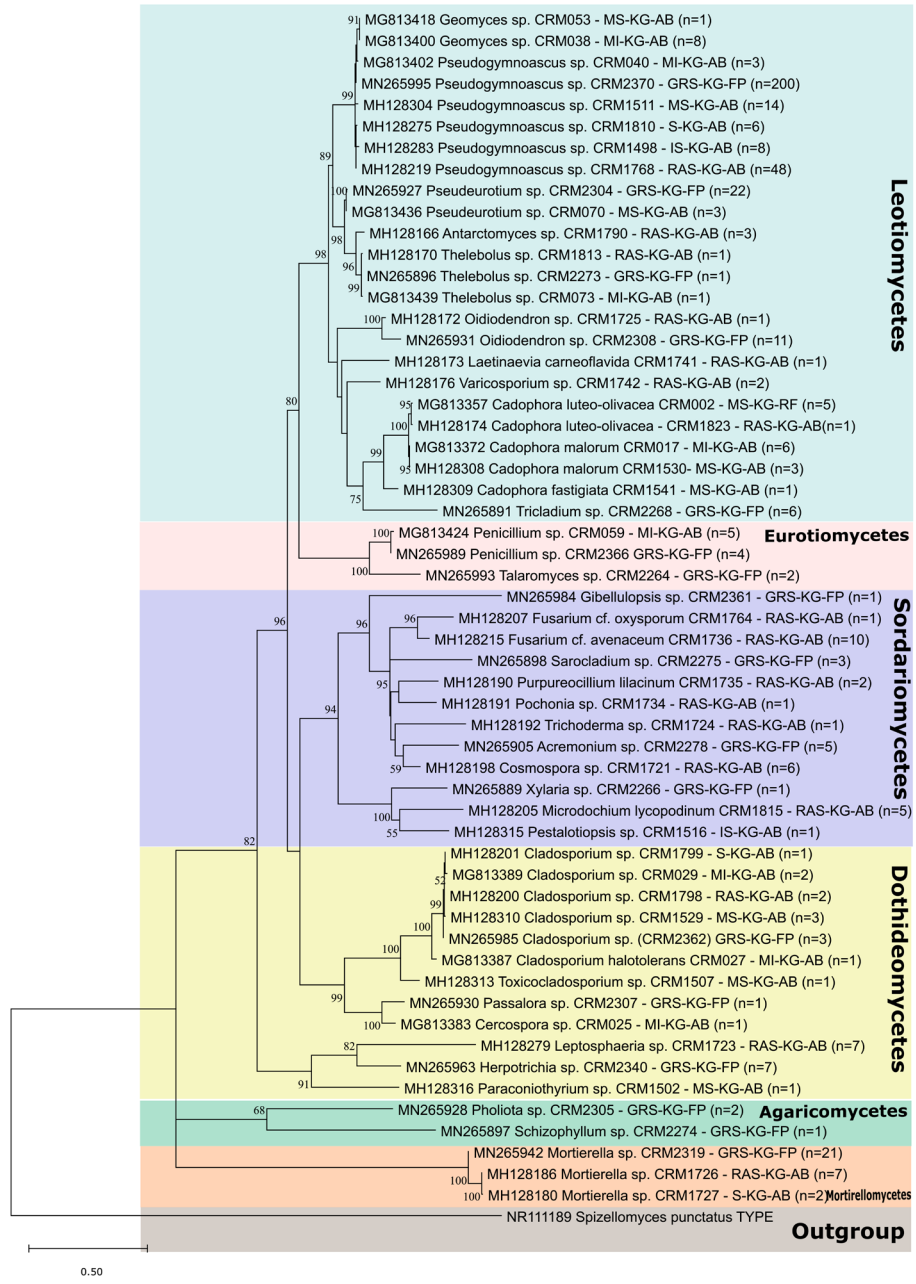

Figure 6. **Phylogenetic analysis of the ITS sequences of the filamentous fungi from the different samples and sites. The evolutionary history was inferred using the Maximum Likelihood method and the nucleotide substitution model GTR + I+G. The percentage of the trees in which the associated taxa cluster (bootstrap of 1000 replicates) is shown next to the branches.**

King George Island were found, supporting these findings. *Metschnikowia* was exclusively detected in marine sediment samples, indicating its potential adaptation to marine environments. *Metschnikowia* species may have adapted to the specific conditions of the marine environment, as seen in the endemic species *M. australis*, which has only been found in Antarctica, and in previous studies it has been isolated from seawater, Antarctic krill, macroalgae, sponges and marine sediment (Fell & Hunter 1968, Donachie & Zdanowski 1998, Loque et al. 2010, Vaz et al. 2011, Vaca et al. 2013). *M. australis* has evolved to adapt to the unique conditions of the Antarctic marine environment, demonstrating a promising biotechnological potential. Furbino et al. (2014) demonstrated that *M. australis* can produce natural bioactive products with

selective antifungal activities against *Candida albicans*, *C. krusei*, and *C. sphaerospermum*, highlighting the importance of further research on this species for pharmaceutical applications.

The second most abundant yeast genus, *Mrakia*, was primarily recovered from marine sediment. The genus *Mrakia* has been observed to have a broad distribution in cold environments. As reported by Kurtzman & Fell (1998), this genus has been commonly isolated from Antarctic and Greenland soil, as well as other cold habitats. Additionally, Hua et al. (2010) demonstrated that *Mrakia frigida* has the ability to produce a killer toxin with potential applications in biotechnology. Specifically, their research showed that this toxin was effective against the yeast *Metschnikowia bicuspidata*, which is pathogenic for crabs. The biotechnological potential of *Mrakia* and its ability to thrive in cold environments make it an interesting target for further studies.

Leucosporidium, the third most abundant yeast genus studied from the CRM-UNESP biobank, has demonstrated adaptations for survival in cold environments. Lee et al. (2010) reported that the isolate *Leucosporidium* sp. AY30 can synthesize cryoprotectant macromolecules and produce extracellular ice-binding glycoproteins. Additionally, Deegenaars & Watson (1998) found evidence that some species of *Leucosporidium* (*L. fellii* and *L. scottii*) produce CSPs, which help maintain cellular homeostasis in response to rapid temperature changes.

The greater abundance of yeasts in Antarctic marine sediments can be attributed to the cold and poor-nutrient conditions prevailing in these environments. Yeasts possess adaptive mechanisms to tolerate extreme environmental conditions, such as low temperatures, high salt concentrations, and low nutrient availability (Buzzini et al. 2012). Furthermore, the organic matter content in marine sediments may be another contributing factor. Organic matter acts as a carbon and energy source for microbial growth and metabolism (James et al. 2022). Although marine sediment presented the highest abundance of yeasts in the CRM-UNESP biobank, the positive correlations found in the Pearson analysis for the yeasts indicate the preference for similar environmental conditions among them, as reported by Fuhrman et al. (2015).

Cold-adapted yeasts have developed various adaptation strategies to survive in extreme cold environments. One crucial adaptation strategy is the high synthesis of unsaturated fatty acids, which contributes to high plasma membrane fluidity, which is related to their degree of adaptability and survival in extremely cold environments (Buzzini et al. 2012). Most cold-adapted yeasts can proliferate at sub-zero temperatures by decomposing organic compounds and accumulating high concentrations of metabolites of the tricarboxylic acid cycle, glycerol, and trehalose, which are important cryoprotectants (Tsuji 2016). In addition, these yeasts have developed other adaptation mechanisms, such as synthesizing protecting proteins to respond to thermal stresses, synthesizing cryoprotectant macromolecules to reduce the presence of cytoplasm ice crystals, making subcellular, molecular and metabolic changes, reducing growth rates, and producing cold-active enzymes (Buzzini et al. 2012, Duarte et al. 2013, Segal-Kischinevzky et al. 2022, Tsuji 2016). The understanding of these adaptations may have important implications for biotechnological applications, such as the development of cold-resistant enzymes and bioremediation strategies (Duarte et al. 2018b).

A larger diversity and abundance of filamentous fungi from the CRM-UNESP biobank

was found in Antarctic terrestrial samples in comparison with marine environments. The variation in the diversity and abundance of these fungi in Antarctica is influenced by multiple factors, including soil properties, moisture content, temperature, and nutrient availability (Siciliano et al. 2014). Specifically, soil properties such as pH and organic matter content significantly affect the composition and diversity of filamentous fungal communities in Antarctica (Bahram et al. 2018). Moreover, nutrient availability, particularly carbon and nitrogen, is crucial for the growth and survival of filamentous fungi in this region (Bahram et al. 2018, Canini et al. 2020).

The Pearson analysis revealed a positive correlation between the different types of environments (marine/terrestrial) and among the same types of environments (marine/marine and terrestrial/terrestrial) (Figure 4b). According to Amend et al. (2019), many fungi found in marine environments are also found in terrestrial environments, even when these marine samples are collected in locations far from the coast. This occurs mainly because fungal spores can travel long distances by the wind and other weather events (Wang et al. 2021).

As decomposers, filamentous fungi play a crucial role in Antarctic ecosystems by contributing to biogeochemical carbon cycling, as well as returning important nutrients to the environment (Barone et al. 2022). The higher abundance of *Pseudogymnoascus* in Antarctic terrestrial environments is consistent with previous studies that have reported its prevalence in cold habitats, including the Arctic, alpine, Antarctic, and temperate ecosystems (Rosa et al. 2019). Representatives of the genus *Pseudogymnoascus* are distributed globally and are common in cold environments, some of them with psychrophilic nature (Wentzel et al. 2019, Santos et al. 2020) and pathogenic capabilities (Gomes et al. 2018). *Pseudogymnoascus* is widely distributed across marine and terrestrial Antarctica ecosystems (Wentzel et al. 2019, Santos et al. 2020, Rosa et al. 2021), highlighting its versatility and adaptability to various Antarctic habitats.

Several studies have reported the biotechnological potential of *Pseudogymnoascus* species, including their ability to produce bioactive metabolites (e.g. antibacterial, antifungal, trypanocidal, herbicidal, and antitumoral activities), as well as their potential for bioremediation and biodegradation of environmental pollutants (Furbino et al. 2014, Henríquez et al. 2014, Gonçalves et al. 2015, Gomes et al. 2018, Purić et al. 2018, Vieira et al. 2018, Díaz et al. 2019). Furthermore, recent studies have shown the potential of *Pseudogymnoascus* for the production of polyketides, a class of compounds with a wide range of biological activities, including anticancer, antifungal, and antibacterial properties (Shi et al. 2021).

Some new species of *Pseudogymnoascus* isolated from Antarctic samples have been recently described. These new species are considered endemic and/or highly adapted to the extreme conditions of the cold continent, and encompass *Pseudogymnoascus antarcticus* sp. nov., *Pseudogymnoascus australis* sp. nov., *Pseudogymnoascus griseus* sp. nov., and *Pseudogymnoascus lanuginosus* sp. nov. (Villanueva et al. 2021). The genus *Pseudogymnoascus* was predominantly detected in the glacier retreat soil, alongside the genera *Mortierella* and *Pseudeurotium*, which were also among the three most commonly recovered genera in the Antarctic samples. These findings suggest that glacier retreat soil may represent a hotspot for fungal diversity in Antarctica. The high abundance of filamentous fungi in glacier retreat soil can be attributed to

several factors. Firstly, the unique environmental conditions of glacier retreat soil, such as low temperatures and high moisture content, create favorable habitats for the growth and survival of filamentous fungi. Glacier retreat soil also provides a rich nutrient source for filamentous fungi. As the glacier retreats, it exposes previously ice-covered soil, a new environment for microorganisms to establish (Santos et al. 2020). This serves as a nutrient reservoir for filamentous fungi, promoting their growth and proliferation (Siciliano et al. 2014).

Furthermore, the physical and chemical properties of glacier retreat soil, such as its texture, pH, and mineral composition, can influence the diversity and abundance of filamentous fungi. Certain fungi may have specific adaptations that allow them to thrive in these soil conditions, leading to their increased abundance. Such adaptations have been demonstrated to present numerous biotechnological applications, including the production of long-chain polyunsaturated fatty acids (LCPUFAs), the promotion of plant growth, and the synthesis of phytoregulators. Some species of the genus *Mortierella*, the second most abundant genus of filamentous fungi found in the glacier retreat soil, are known for their ability to produce LCPUFAs, such as docosahexaenoic acid (DHA) and arachidonic acid (AA), which are essential for human health and commonly found in fish oil (Streekstra, 2010). Therefore, *M. alpina*, *M. renispora* and *M. parvispora* are potential alternative sources for LCPUFA production (Gomes et al. 2018, Streekstra, 2010). Two species of *Mortierella* (*M. antarctica* and *M. verticillata*) have also been shown to promote plant growth by producing indoleacetic acid, gibberellic acid, and ACC-deaminase (Ozimek et al. 2018). These plant growth regulators can enhance seed germination, root growth, and nutrient uptake, making *Mortierella* a potential biofertilizer for sustainable agriculture. Additionally, representatives of the genus *Mortierella* have been shown to produce secondary metabolites with antiparasitic (*M. parvispora*) and herbicidal (*M. amoeboidea*) properties (Gomes et al. 2018). These findings suggest that species of the genus *Mortierella* have significant biotechnological potential and can be further explored for various applications.

In addition to the diverse filamentous fungi found in glacier retreat soil, the roots of the two native Antarctic vascular plant species, *Deschampsia antarctica* and *Colobanthus quitensis*, also harbored a rich fungal diversity. A study conducted by Rosa et al. (2009) on filamentous fungi associated with these plants revealed a rich fungal diversity in the root-associated soil. The high fungal diversity in the root-associated soil of these plants could be attributed to their efficient nitrogen acquisition ability, as suggested by Hill et al. (2011).

Furthermore, Rosa et al. (2010) conducted a study on fungal endophytes associated with the leaves of *Colobanthus quitensis*, a dicotyledonous plant found in Antarctica, which also presented a significant diversity of fungal endophytes. Additionally, Santiago et al. (2012) investigated endophytic fungi associated with *Deschampsia antarctica* and *Colobanthus quitensis*, recovering a total of 564 isolates of endophytic fungi from these plants. The diversity of fungi associated with these plants may have important ecological implications, such as contributing to their ability to resist environmental stressors. Nonetheless, further research is needed to fully understand the diversity and ecological roles of fungi associated with the Antarctic flora.

Recent investigations have provided valuable insights into the fungal species that inhabit Antarctic environments. Poveda et al. (2018) reported the isolation of 27 filamentous fungi

from marine sponges collected in King George Island. The researchers screened these isolates for cold-active pectinases and discovered that eight of them exhibited pectinolytic activities at 15°C. Notably, *Geomyces* sp. F09-T3-2 displayed the highest levels of pectinolytic activity and showcased optimal performance at 30°C. A part of the CRM-UNESP biobank of Antarctic fungi has been screened for cold-adapted enzymes in previous studies. Representatives of the genera *Cadophora, Cladosporium, Cosmospora, Geomyces, Oidiodendron* and *Penicillium* were able to produce ligninolytic enzymes, while xylanase was produced by fungi of the genera *Cadophora* and *Penicillium,* and L-ASNase by representatives of *Cosmospora, Geomyces* and *Penicillium* (Duarte et al. 2018a). In another study, one *Pseudogymnoascus* from the CRM-UNESP biobank stood out in terms of protease production (Wentzel et al. 2019). Additionally, in the study reported by Kita et al. (2022), two *Penicillium* isolates from the CRM-UNESP biobank of Antarctic fungi (recovered from Antarctic marine sediments) showed promising results in the decolorization of a textile dye at low and moderate temperatures. These findings suggest that these fungi possess enzymatic capabilities that could be harnessed for environmental cleanup applications.

The production of bioactive compounds by the fungi residing in Antarctic marine sediments holds significant promise for medicine and biotechnology (Jasani et al. 2017, Henríquez et al. 2014). These compounds could serve as valuable resources for drug discovery, since they may possess unique properties and mechanisms of action. Furthermore, the ability of these fungi to adapt to extreme environmental conditions raises intriguing possibilities for developing biotechnological applications in challenging settings (Rédou et al. 2015).

Data from the present study revealed the presence of different fungal taxa (filamentous and yeasts) inhabiting the Antarctic marine and terrestrial environments. Some taxa were specifically found in marine samples and others, in the terrestrial ones. The majority of the yeasts recovered from the marine and terrestrial Antarctic samples belong to the phylum Basidiomycota, while the filamentous fungi from marine and terrestrial samples belong mainly to the phylum Ascomycota. Considering that representatives of many extremophilic genera and/or species related to those found in the CRM-UNESP biobank of fungi have demonstrated potential for application in different sectors of socio-economic relevance, Antarctic environments can be considered a prolific source of microbial resources for the development of biotechnology, which could move bioeconomy. The results showed the value of these genetic resources, highlighting the importance of their maintenance in culture collections (microbial biobanks) aligned with national and international regulations related to the preservation and distribution of biological material and associated data. Therefore, further studies focused on metabolites, enzymes, and resistance to adverse conditions, among others, can be performed, expanding our knowledge related to the ecological roles and biotechnological significance of the Antarctic microorganisms.

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