Fungi in the Antarctic cryosphere: using DNA metabarcoding to reveal fungal diversity in glacial ice from the Antarctic Peninsula region

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Abstract

We assessed fungal diversity present in glacial of the Antarctic Peninsula using DNA metabarcoding through high throughput sequencing (HTS). We detected a total of 353,879 fungal DNA reads, representing 94 genera and 184 taxa, in glacial ice fragments obtained from seven sites in the north-west Antarctic Peninsula and South Shetland Islands. The phylum *Ascomycota* dominated the sequence diversity, followed by *Basidiomycota* and *Mortierellomycota*. *Penicillium* sp., *Cladosporium* sp., *Penicillium atrovenetum*, *Epicoccum nigrum*, *Pseudogymnoascus* sp. 1, *Pseudogymnoascus* sp. 2, *Phaeosphaeriaceae* sp. and *Xylaria grammica* were the most dominant taxa, respectively. However, the majority of the fungal diversity comprised taxa of rare and intermediate relative abundance, predominately known mesophilic fungi. High indices of diversity and richness were calculated, along with moderate index of dominance, which varied among the different sampling sites. Only 26 (14%) of the total fungal taxa detected were present at all sampling sites. The identified diversity was dominated by saprophytic taxa, followed by known plant and animal pathogens and a low number of symbiotic fungi. Our data suggest that Antarctic glacial ice may represent a hotspot of previously unreported fungal diversity; however, further studies are required to integrate HTS and culture approaches to confirm viability of the taxa detected.

Introduction

Despite its generally extreme conditions, Antarctica hosts diverse environments. Microorganisms dominate many of the most extreme environments of the continent [1, 2, 3]. Antarctica's continental ice sheets contain the largest volume of glacial ice, inherently characterized by unfavorable conditions to life, including low temperatures, low water activity, low nutrient availability and, in their surface layers, exposure to high levels of solar radiation [4, 5].

Fungi are amongst the microorganisms reported from the Antarctic cryosphere [6]. However, despite their recognised importance for ecosystem functioning in Antarctica and elsewhere, few studies have attempted to recover and identify fungal species from glacial ice and, until now, few species, mainly representing the phyla *Ascomycota*, *Basidiomycota* and *Mortierellomycota*, have been characterized from this environment [7, 8, 9, 10, 11, 12].

Glacial ice is formed through the precipitation, accumulation, compaction and recrystallization of snow. It can contain spores and mycelial fragments of fungi deposited from the air column, both on contemporary and palaeo timescales [5, 12, 13, 14]. Sonjak et al. [15] suggested that viable fungal cells obtained from Arctic and Antarctic glacial ice may range in age from 10,000 to 140,000 years, which represent aeolian transport of propagules of both local and distant origin. In addition, Rosa et al. [16] studied air samples in the Antarctic Peninsula region and showed the presence of fungi in the airspora, supporting the possibility of dispersion in different geographic scales around Antarctica. To date, very few studies have addressed fungal diversity present in Antarctic glacial ice, with those available being based on cultivation techniques [5]. Furthermore, fungal diversity present in Antarctic glacial ice has not

been assessed using cutting edge modern DNA metabarcoding techniques. In the current study, we assessed the fungal diversity, richness, abundance and distribution in glacial ice sampled in the different Antarctic sites using DNA metabarcoding high throughput sequencing (HTS).

Methods

Ice sampling

Three 'bergy bits' (glacial ice fragments) each of approximately 20 kg mass, were collected adjacent to the ice fronts of seven marine terminating glaciers in the South Shetland Islands and the north-west Antarctica Peninsula during the austral summer season in December 2015 and December 2016 (Fig. 1). Each was collected using sterile suits and gloves to minimize contamination risk. In the microbiology laboratory on board the Brazilian polar research vessel *Admiral Maximiano*, each sample was broken into smaller pieces, and surface decontamination carried out using 5% sodium hypochlorite (10 s), sterilized distilled water (10 s), and exposure to ultraviolet radiation (10 min) [12, 17]. The samples were melted and a total of 12–15 L of the resulting water filtered through 47 mm diameter (Millipore) membranes (three membranes per sampling site, each using 4–5 L) until each membrane became saturated. Membranes were then stored at -20°C until DNA extraction in the laboratory of Polar Microbiology and Tropical Connections of Universidade Federal of Minas Gerais, Brazil. Physico-chemical parameters (temperature, conductivity, resistivity, total dissolved solids, oxidation-reduction potential, pH, salinity) of the melted samples from each site were measured using a Hanna multi-parameter probe HI 9828 (Hanna Instruments, USA). A map showing the sample collection locations was generated using QGIS software (version 3.14.15; <https://www.QGIS.org>) and the SCAR Antarctic Digital Database (ADD version 7.0; <http://www.add.scar.org>).

DNA extraction, data analyses and fungal identification

The three membranes resulting from filtering the melted ice from each sampling site were processed together in order to increase DNA yield. Total DNA was extracted using 0.5 mL extraction buffer [sodium dodecyl sulfate (SDS) 10%], left at 55°C for 18 h, followed by 165 µL NaCl (5 M) and 165 µL cetyltrimethylammonium bromide (CTAB, 10%), then 600 µL chloroform was added and the mixture centrifuged (Eppendorf/Germany) at 13,000 rpm for 10 min. The supernatant was cleaned using the QIAGEN DNeasy PowerClean cleanup Kit. Extracted DNA was used as a template for generating PCR-amplicons. The internal transcribed spacer 2 region (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification [18, 19]. PCR-amplicons were generated using the universal primers ITS3 and ITS4 [20] and were sequenced at Macrogen Inc. (South Korea) on an Illumina MiSeq sequencer, using the MiSeq Reagent Kit v3 (600-cycle) following the manufacturer's protocol.

Raw fastq files were filtered using BBDuk version 38.34 (BBMap – Bushnell B. – sourceforge.net/projects/bbmap/) to remove Illumina adapters, known Illumina artefacts, and the PhiX Control v3 Library. Quality read filtering was carried out using Sickle version 1.33 -q 30 -l 50 [21], to trim 3' or 5' ends with low Phred quality score, and sequences shorter than 50 bp were also discarded. The

remaining sequences were imported to QIIME2 version 2019.10 (<https://qiime2.org/>) for bioinformatics analyses [22]. The qiime2-dada2 plugin is a complete pipeline that was used for filtering, dereplication, turn paired-end fastq files into merged, and removal of chimeras [23]. Taxonomic assignments were determined for amplicon sequence variants (ASVs = taxa) using the qiime2-feature-classifier [24] classify-sklearn against the UNITE fungal ITS database version 8.2 [25] and trained with Naive Bayes classifier and a confidence threshold of 98.5%. Fungal classification followed Kirk et al. [26], Tedersoo et al. [27], MycoBank (<http://www.mycobank.org>) and the Index Fungorum (<http://www.indexfungorum.org>).

Diversity, distribution and ecological analysis

To quantify species diversity, richness, and dominance, we used the following indices: (i) Fisher's α , (ii) Margalef's, and (iii) Simpson's, respectively, to assess alpha diversity. In addition, the Sorensen and Bray-Curtis similarity indices were used to assess beta diversity among the fungal assemblages. The relative abundance of the ASVs was used to quantify the fungal taxa present in the glacial ice sampled as described by Rosa et al. [28]. Fungal ASVs with relative abundance > 10% were considered dominant, ASVs with relative abundance of 1–10% intermediate and ASVs with < 1% minor (rare) components of the fungal community. All of the results were obtained with 95% confidence, and bootstrap values were calculated from 1,000 iterations. Taxon accumulation curves were obtained using the Mao Tao index. All diversity index calculations and t test were performed using PAST, version 1.90 [29]. To prepare Kronar charts, QIIME2 taxonomy classifications and the table of taxa abundance were converted to tsv and biom format, respectively. The table of fungal abundance was converted to tsv by using biom convert and combined with taxonomy classification with a custom script `krona_qiime.py` (https://github.com/lokeshbio/Amplicon_course/blob/master/krona_qiime.py). The Krona Tools (v. 2.7.1) [30] program, `ktImportText.pl`, was used to provide interactive visualization of identified fungi species. Venn analysis to compare the fungal diversity obtained from the different sampling locations was carried out using the program available at <http://bioinformatics.psb.ugent.be/webtools/Venn/>. The functional assignments of fungal ASVs at species and generic levels were assessed using FunGuild [31].

Results

Fungal taxonomy

We detected a total of 353,879 fungal DNA reads, representing 94 genera and 184 distinct taxa in glacial ice obtained from the seven sampling locations in the South Shetland Islands and north-west Antarctic Peninsula (Suppl. Table 1). The phylum *Ascomycota* was dominant in all fungal assemblages, followed by *Basidiomycota* and *Mortierellomycota*. A single *Mucoromycota* taxon (*Rhizopus arrhizus*) was detected at low abundance (Fig. 2). *Penicillium* sp., *Cladosporium* sp., *Penicillium atrovenetum*, *Epicoccum nigrum*, *Pseudogymnoascus* sp. 1, *Pseudogymnoascus* sp. 2, *Phaeosphaeriaceae* sp. and *Xylaria grammica* (*Ascomycota*) were the most dominant taxa (all with > 10% of DNA reads), in rank order. Further 30 taxa were detected at intermediate abundance (< 1% DNA reads). The majority of the fungal ASVs detected (146 taxa; 79.3%), were classified as rare. Thirty-seven taxa could only be assigned to higher taxonomic levels (phylum, class, order, family).

Diversity, distribution and ecology

The Mao Tao's rarefaction curves approached a plateau for all sampling locations, indicating that the DNA reads obtained gave a good representation of the fungal sequence diversity present at each (Supplementary Fig. 1). Alpha diversity indices across the sampled locations indicated generally high diversity (Fisher α) and richness (Margalef) and moderate dominance (Simpson) indices (Table 1), varying between the different sites. The sequence diversity detected in the Leonardo-Blanchard region (Antarctic Peninsula) was most diverse and rich, and included a wider range of dominant taxa, followed by those of Greenwich Island, when compared with the other sampling locations. The sequence diversity detected in the Sikorsky region (Antarctic Peninsula) displayed the lowest diversity indices. The Leonardo/Blanchard (Antarctic Peninsula) location had the lowest values of conductivity and salinity.

Table 1

Physicochemical parameters of melted glacial ice and diversity indices of fungal assemblages at the different sampling locations in the north-west Antarctic Peninsula and South Shetland Islands.

Parameters/diversity indices/density	Sampling locations						
	KG-ASH	GI-FA	GI-T	AP-S	AP-LB	AP-RW	LI-H
Temperature melted ice (°C)	15.6	16	9.6	15.6	14.5	16.1	10.1
Conductivity ($\mu\text{S cm}^{-1}$)	25	23.5	21	50	6	23	18
Resistivity ($\text{M}\Omega \text{ cm}^{-1}$)	0.04	0.06	0.04	0.08	0.16	0.06	0.10
Total dissolved solids (ppm)	12	11.5	11	25.5	3	11.5	9
Oxidation-reduction potential (mV)	520.1	540.7	750.4	509.5	188.8	540.7	494.7
pH	6.51	6.7	6.7	7.4	6.5	6.65	6.9
Salinity (ppt)	0.01	0.01	0.02	0.02	0	0.01	0
Total number of reads	47,622	47,560	44,715	48,164	34,608	93,545	37,665
Number of taxa	82	88	61	54	95	87	64
Fisher's α	9.64	10.45	6.96	6.01	11.91	9.46	7.51
Margalef	7.52	8.08	5.60	4.92	8.99	7.51	5.98
Simpson	0.84	0.84	0.73	0.80	0.85	0.78	0.78
KG-ASH = King George Island, Ajax-Stenhouse Glacier; GI-FA = Greenwich Island, Fuerza Aérea; GI-T = Greenwich Island, Troub; AP-S = Antarctic Peninsula, Sikorsky; AP-LB = Antarctic Peninsula, Leonardo-Blanchard; AP-RW = Arctowski Peninsula, Rozier-Woodbury; LI-H = Livingston Island, Huron.							

The beta diversity of the fungal assemblages varied across the different sampling locations (Fig. 3). The presence-absence-based Sorensen index showed that the most similar fungal assemblages were found at Greenwich Island, Traub and Livingston Island, Huron, followed by Antarctic Peninsula, Leonardo-Blanchard and King George Island, Ajax-Stain House. However, the abundance-related Bray-Curtis index indicated that the fungal assemblages from Greenwich Island, Fuerza Aérea and Antarctic Peninsula, Sikorsky showed the highest similarity.

The physicochemical properties of all the ice samples were generally similar, except for those from Antarctic Peninsula, Leonard-Blanchard and Antarctic Peninsula, Sikorsky, which displayed the extreme values of conductivity and total dissolved solids (Table 1; Supplementary Fig. 2). The fungal assemblage detected in the ice sampled in the Antarctic Peninsula, Leonard-Blanchard site, which had the lowest physicochemical parameters, included the lowest number of DNA reads and the highest number of ASVs, and had the highest diversity (Fisher α) and richness (Margalef) indices. In contrast, the fungal assemblage detected in the ice of Antarctic Peninsula, Sikorsky displayed the lowest values of the same diversity parameters. PCA analysis indicated that the conductivity, total dissolved solids, oxidation-reduction potential, pH and salinity showed negative correlation with the number of taxa, Fisher α , Margalef and Simpson indices. Twenty-six of the 186 fungal taxa detected were present at all sampling locations (Suppl. Table 2), while 82 taxa were detected at only a single location. Ecological functional assignments of the taxa detected at generic level are given in in Suppl. Table 3. Taxa of 94 genera were detected, with the most common group being saprophytic fungi, followed by plant and animal pathogens and a small number of symbiotic fungi.

Discussion

Fungal taxonomy

Glacial ice is considered an extreme and ultra-oligotrophic environment and one of the most challenging natural environments for life globally [2]. Representatives of Bacteria, Archaea and Fungi have been detected in glacial ice from different cold regions of the planet [15, 32]. However, among the microorganisms present in the glacial ice, fungi remain poorly known and few taxa have been reported to date [6, 14] in studies based on traditional culturing methods.

Many factors, such as extraction, PCR and primer bias, can influence the outcomes of metabarcoding studies and the numbers of reads obtained [33], thus leading to misinterpretation of absolute abundance [34]. However, Giner et al. [35] concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are linked with higher abundance [36, 37]. Therefore, for comparative purposes, we consider here the number of reads as a proxy for relative abundance. The current study, using a state-of-the-art HTS metabarcoding approach, focused on the detection of fungal DNA in environmental samples.

Our data revealed the presence of rich and diverse fungal sequence diversity in glacial ice collected from the seven different sampling locations. The total sequence diversity was dominated by a relatively small

number of taxa of the genera *Penicillium*, *Cladosporium*, *Epicoccum*, *Pseudogymnoascus* and *Xylaria*, all members of the *Ascomycota*. However, the majority of the diversity identified comprised intermediate and rare members of the phyla *Basidiomycota*, *Mortierellomycota* and *Mucoromycota*.

The genera *Cladosporium* and *Penicillium* include well-known cosmopolitan species often detected in the airspora. In Antarctica, different species of *Cladosporium* have been detected in association with plants and soil [3]. Species of *Penicillium* are widespread across Antarctica and have been reported in studies of multiple terrestrial substrates including soils [38, 39, 40], permafrost [41, 42], associated with marine macroalgae [43], invertebrates [44], sediments [45, 46] and seawater [47]. 2017). *Penicillium atrovenetum* was detected as the dominant fungal sequence present in the gypsum encrustations and carbonate veins of rocks in a polar desert region of continental Antarctica [48]. Members of *Pseudogymnoascus* occur widely in cold polar, alpine, and temperate environments [49, 50, 51, 52]. In Antarctica, they have been reported from soils [49, 53, 54], associated with plants [55, 56, 57] and marine macroalgae [58], in freshwater lakes [45], and associated with lichens [59]. *Cladosporium*, *Penicillium* and *Pseudogymnoascus* sequences were detected as dominant fungal sequences present in air and snow samples from the South Shetland Islands [16, 28, 60]. The dominance of sequences in these genera in the glacial ice examined here is consistent with these fungi being abundant in the air, being deposited (possibly facilitated by snow precipitation) on the glacier surface and progressively incorporated in the glacial ice as it becomes compacted overtime. It is important, however, to note that metabarcoding methodologies detect the presence of DNA sequences, with identification still limited by the available sequence databases, and do not provide any confirmation of viability. Therefore, further specific studies are necessary to determine whether the fungal taxa detected are present in a viable form.

Members of the genus *Epicoccum* are commonly present in air, soil, decaying vegetation and as endophytes in living plant tissues, with some also being documented producers of bioactive compounds [61]. In Antarctica, species of *Epicoccum* have been documented in aerobiological studies on Signy Island, South Orkney Islands [62] and associated with Antarctic marine sponges [63]. The DNA of *Epicoccum nigrum* was also recently detected in rock surface gypsum encrustations and carbonate veins in the Ellsworth Mountains [48].

The genus *Xylaria* contains between 570–670 recognized species [64], but may include many more, yet to be described [65]. Species of *Xylaria* are important saprophytic fungi, found on decomposing wood in temperate and tropical ecosystems [66] and also as plant endophytes [67]. Additionally, members of the genus are amongst the most prolific secondary metabolite producers [64]. In Antarctica, *Xylaria* has been reported from soil exposed by glacial retreat on King George Island [68].

Diversity, distribution and ecology

Aside from the eight *Ascomycota* taxa classified as dominant in the current study, the majority of taxa were of rare or intermediate abundance, and were mostly known as mesophilic fungi. de Menezes et al. [12] reported culturable fungal diversity from the same glacial ice samples as examined here. They documented the presence of 27 taxa belonging to 14 genera. The number of taxa detected and diversity

ecological indices calculated using the metabarcoding approach in the current study were approximately seven times greater than those reported by de Menezes et al. [12] (Table 2). The use of metabarcoding revealed sequence diversity potentially representing a much richer and more diverse fungal community than previously appreciated, including 184 taxa belonging to the 98 genera, amongst which were fungi not previously reported from Antarctica. The fact that 37 taxa could only be assigned at higher taxonomic levels (phylum, class, order, family), provides reinforce the evidence that Antarctic environments are likely to host new and/or previously unreported fungal diversity.

Table 2

Comparison of diversity indices obtained in studies of fungal diversity detected by traditional culturing methods and DNA metabarcoding from glacial ice samples obtained from the north-west Antarctic Peninsula and South Shetland Islands

Sampling location	Number of taxa/ASVs		Fisher α		Margalef		Simpson	
	TCM ^a	HTS ^b	TCM	HTS	TCM	HTS	TCM	HTS
King George Island, Ajax-Stenhouse	4	82	1.12	9.64	0.82	7.52	0.61	0.84
Arctowski Peninsula, Rozier-Woodbury	1	87	-	9.46	-	7.51	-	0.78
Livingston Island, Huron	8	64	12.98	7.51	3.36	5.98	0.82	0.78
Greenwich Island, Traub	1	61	-	6.96	-	5.60	-	0.73
Greenwich Island, Fuerza Aerea	5	88	2.52	10.45	1.51	8.08	0.73	0.84
Antarctic Peninsula, Sikorsky	6	54	1.78	6.01	1.28	4.92	0.33	0.80
Antarctic Peninsula, Leonardo-Blanchard	3	95	0.95	11.91	0.65	8.99	0.55	0.85
^a Traditional culturing methods [12]; ^b DNA metabarcoding (current study).								

Sorensen and Bray-Curtis similarity indices indicated that the beta diversity of the fungal assemblages varied across the different sampling sites, which may be related with ice physicochemical properties. The fungal assemblage detected in the ice sampled in the Antarctic Peninsula, Sikorsky location formed an isolated group based on the fungal taxa present, as well as having the highest values of conductivity and total dissolved solids and the lowest number of ASVs, Fisher's α (diversity) and Margalef index values (richness). However, when the Bray-Curtis index was compared with the physicochemical parameters and alpha diversity indices no other correlations were detected. Our alpha and beta diversity data differed

from those reported by de Menezes et al. [12] in their study of culturable fungal diversity from the same glacial ice samples as examined here. The PCA analysis reported by de Menezes et al. [12] showed a positive correlation between pH and the diversity indices only at two sampling sites, differing from the current analyses, which identified a positive correlation between the lowest values of the physicochemical parameters and the highest diversity values.

Conclusions

Our data suggest that Antarctic glacial ice may host a hotspot of as yet unreported fungal diversity. Use of a DNA metabarcoding approach revealed the presence of high fungal sequence diversity including a small number of dominant fungi with capabilities for aerial dispersal and a high number of taxa of rare or intermediate abundance. The sequence diversity detected was dominated by saprophytic taxa, followed by known plant and animal pathogens and a small number of symbiotic fungi. The potentially high fungal diversity detected here in glacial ice samples emphasizes the need for further studies characterizing fungal communities of this extreme ecosystem using a combination of culturing and metabarcoding approaches.

Declarations

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Data Availability Statement

All raw sequences have been deposited in the NCBI database under the codes SRX9966699, SRX9966700, SRX9966701, SRX9966702, SRX9966703, SRX9966704, SRX9966705 and SRX9966706.

Author contributions

GCAM, LHR, JCS and PEASC conceived the study. GCAM and LHR performed fungal DNA extraction from ice. GCAM, LHR, PEASC, OHBZ, PC, MCS, JCS and CAR analyzed the results and wrote the manuscript. All authors read and approved of the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval The collections and studies performed in Antarctic Peninsula were authorized by the Secretariat of the Antarctic Treaty and by PROANTAR.

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Figures

Figure 1

Location of ice sample collections. (A) Antarctica showing the north-west Antarctic Peninsula and South Shetland Islands in the red rectangle and (B) the sites where the glacial ice samples were collected. Site 1 - Antarctic Peninsula, Sikorsky ($64^{\circ}12'S$; $60^{\circ}53'W$); Site 2- Antarctic Peninsula, Leonardo-Blanchard ($64^{\circ}42'S$; $61^{\circ}58'W$); Site 3 - Arctowski Peninsula, Rozier-Woodbury ($64^{\circ}45'S$; $62^{\circ}13'W$); Site 4 - Greenwich Island, Traub ($62^{\circ}29'31''S$; $59^{\circ}48'00''W$); Site 5- Greenwich Island, Fuerza Aérea ($62^{\circ}30'S$; $59^{\circ}38'W$); Site 6 - King George Island, Ajax-Stenhouse ($62^{\circ}06'S$; $58^{\circ}27'W$); Site 7 - Livingston Island, Huron ($62^{\circ}37'50''S$; $60^{\circ}06'50''W$).

Figure 2

Krona charts of (A) total fungal diversity across all sampling sites, (B) King George Island, Ajax-Stenhouse, (C) Greenwich Island, Fuerza Aérea, (D) Antarctic Peninsula, Sikorsky, (E) Antarctic Peninsula, Leonardo-Blanchard, (F) Arctowski Peninsula, Rozier-Woodbury, (G) Livingston Island, Huron and (H) Greenwich Island, Traub.

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