



MICROBIOLOGY

Taxonomic and functional diversity from Antarctic ice-tephra microbial community: ecological insights and potential for bioprospection

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Abstract: Antarctic active volcanoes can disperse pyroclastic minerals at long distances, transporting nutrients and microorganisms to the surrounding glacial environment. The sedimented volcanic materials – called tephra – may interact with glacier ice and produce a unique environment for microbial life. This study aimed to describe the microbial community structure of an Antarctic glacier ice with tephra layers in terms of its taxonomic and functional diversity. Ice samples from Collins Glacier (King George Island) containing tephra layers of Deception Island volcano were analyzed by a whole shotgun metagenomic approach. Taxonomic analysis revealed a highly diverse community dominated by phyla Bacteroidetes, Cyanobacteria and Proteobacteria. The dominant genera were *Chitinophaga* (13%), *Acidobacterium* (8%), and *Cyanothece* (4%), being all of these known to include psychrotolerant and psychrophilic strains. Functional diversity analysis revealed almost complete carbon, nitrogen and sulfur biogeochemical cycles. Carbohydrate metabolism of the ice-tephra community uses both organic and inorganic carbon inputs, where photosynthesis plays an important role through CO₂ fixation. Our results also demonstrate a biotechnological potential for this glacial community, with functional annotations for styrene degradation and carotenoid pigment genes. Future metatranscriptomic studies shall further reveal the active strategies and the biotechnology potential of extremophiles from this unique ice-tephra microbial community.

Key words: Antarctica, bioinformatics, glacier ice, metagenomics, tephra.

INTRODUCTION

The Antarctic continent is among the 85% of the Biosphere in which temperatures are permanently cold – mostly close to or below 0°C (Moliné et al. 2014). Although it presents heterogeneous conditions among niches, the extremes are a constant in this continent. Terrestrial landscapes currently considered as ice-free areas are limited to around 0.3% of the total Antarctic continent (Convey et al. 2008), and average annual precipitation rarely exceeds 200

mm (Carvalho et al. 2018). Thus, almost the whole continent imposes cold desert conditions for life (Núñez-Montero & Barrientos 2018). In fact, Antarctica can be considered the coldest, driest, and windiest continent on Earth (Bratchkova & Ivanova 2011). Furthermore, Antarctica's huge ice sheet increases the reflection of incoming solar radiation, intensifying cold conditions and further escalating the incidence of ultraviolet (UV) radiation (Selbmann et al. 2014, Marizcurrena et al. 2017).

This exceptional combination of extremes harshened the development of life and led the Antarctic continent to be dominated by microorganisms (Cavicchioli 2015, Carvalho et al. 2018). Besides temperature, moisture, and radiation, antarctic-inhabiting microorganisms are also challenged with low nutrient availability and high salinity (Arenz & Blanchette 2011), which, in combination, selected for a higher presence of extremophiles — organisms thriving under extreme environmental conditions (Bendia et al. 2018, Duarte et al. 2019). As a result, novel cold-adapted enzymes, antibiotics, and even anti-cancer drugs were discovered by studying Antarctic extremophilic microorganisms in the last few years (Nichols et al. 2002, Marx et al. 2007, Núñez-Montero et al. 2019, Silva et al. 2020).

Due to the highly dynamic tectonics of the continent, the Antarctic geological history is full of volcanic events since the early Mesozoic Era (Panter 2021, Smellie 2021). Antarctica has four active volcanoes, among which Deception Island volcano have received special attention due to recent eruptions in 1967, 1969, and 1970 (Pedrazzi et al. 2014). As a result of these eruptions, volcanic dust and pyroclastic materials were dispersed by wind and eventually deposited into the surrounding glacial ice, forming ash layers known as tephra, representing evidence of past volcanic activities (Geyer et al. 2017). For instance, during an expedition to Collins Glacier at King George Island (about 120 km from Deception Island), Chinese researchers identified “dirty” bands within several ice cores collected at 80.2 m (Jiankang et al. 1999). Mineralogical and microstructure analysis of these bands revealed characteristics with significant correlation to volcanic ashes dating to the end of 1970 (Jiankang et al. 1999). From a microbiological point of view, these volcanic eruptions result in the dispersal of rich inorganic nutrients over long distances, notably sulfur and nitrogen

compounds. Also, endemic microorganisms from the volcano are supposed to propagate to other environments. As such, the interaction of glacial ice and volcanic tephra combine into a unique ecosystem with specific selective pressures for microbial communities. However, currently little is known about the effects of volcanic eruptions on polar communities of microorganisms (Bendia et al. 2018).

The recent development of shotgun metagenomics has provided the opportunity to investigate the taxonomic and functional diversity of microbial communities (Gómez-Silva et al. 2019), further expanding our knowledge on metabolic pathways and the adaptation mechanisms required to thrive in extreme conditions. Additionally, the study of these communities may also reveal novel enzymes and metabolites with potential for medical, pharmaceutical and biotechnological applications. Therefore, this study aims to analyze the taxonomic and functional diversity of microbial communities from ice-tephra samples collected from Collins Glacier, King George Island, Antarctica.

MATERIALS AND METHODS

Ice sampling and decontamination

Ice samples were collected at the terminus of Collins Glacier, King George Island (62°10'4"S; 58°51'11"W) in January 2009 using an ice pick. The ice pick was used exclusively for microbiological sampling and was decontaminated with a 70% ethanol solution before use. A total of 5 blocks of ice were collected from the edge of the glacier, where three distinct layers of pyroclastic sediment (tephra) were visible (Figure 1). These tephra layers were identified as sedimented fragments of volcanic origin, more specifically, from the last three eruptions of Deception Volcano (circa 130 km from King George Island)



Figure 1. Photograph of the Collins Glacier (Fildes Peninsula, King George Island) during field sampling activity. Ice-tephra layers are indicated by arrows.

that occurred between 1967-70 (Jiankang et al. 1999, B.R. Mavlyudov, Institute of Geography of the Russian Academy of Sciences, *personal communication*). The retrieved ice samples were packed in autoclaved high-density polyethylene (HDPE) sacks and stored at -20°C until arrival in the Comandante Ferraz Antarctic Station (Estação Antártica Comandante Ferraz – EACF – Brazil). Samples were transported to Brazil in deep-freezers at -20°C aboard the Oceanographic and Supply Ship (Navio de Apoio Oceânico – NApOc) Ary Rongel. Upon arrival, all samples were inspected for cracks, microfractures, melting, and other damages that may occur during transportation. Damaged ice samples were discarded and the remaining samples were used in this study.

Ice samples containing tephra layers (ice pieces of 6 to 8 kg each) were decontaminated before the DNA extraction using a modified protocol based on Rogers et al. (2004). Briefly, a

1000 W electric-heated rod was used to remove contaminants from the outer ice surface. The remaining pieces of ice were immersed in a cold 5% sodium hypochlorite solution for 10 s, followed by three 200 mL rinses with cold sterile MilliQ water. The now surface-sterile ice samples were placed inside a new sterile HDPE sack and melted at 4°C overnight. The melted ice sample (total of about 1,800 mL) was filtered in sterile $0.22\ \mu\text{m}$ membranes. Fragments of the tephra layer remained on the membrane and were considered part of the ice sample. Finally, the filtered membranes were stored at -20°C until used for DNA extraction.

All procedures were taken inside a positive pressure laminar flow hood using autoclaved materials, including gloves, filters, and glassware. All solutions were either autoclaved or filtered through $0.22\ \mu\text{m}$ membranes. Also, to avoid excessive loss of ice by ablation and microfractures on the samples surface, all solutions were cold-stored at 4°C overnight before use.

Total DNA extraction and metagenomic sequencing

The ice total DNA was extracted using the UltraClean Water DNA Isolation Kit (Mo Bio, USA) with the following modifications: the original filtering membrane from the kit was replaced by the $0.22\ \mu\text{m}$ membranes used to filter the melted ice; the kit WD5 solution (10 mM Tris) was replaced by autoclaved MilliQ water pre-heated to 60°C before use. These modifications were applied to optimize the final DNA yield. After extraction, the total DNA was purified and concentrated to a final volume of $10\ \mu\text{L}$ with DNA Clean & Concentrator Kit (Zymo Research, USA) following the manufacturer's protocol. The final DNA concentration and purity were determined with Qubit dsDNA BR kit (Thermo Fisher Scientific, USA). Due to the small cell concentration in the

ice samples, a low DNA concentration (~ 3.7 ng μL^{-1}) was obtained for metagenomic sequencing. Therefore, the total DNA was uniformly amplified with Illustra GenomiPhi V2 DNA Amplification Kit (Cytiva, USA), which uses an isothermal strand displacement amplification approach.

Glacier ice metagenome was sequenced at Life Sciences Core Facility (LaCTAD) from State University of Campinas (UNICAMP), Brazil. The metagenomic library was prepared using Nextera DNA Sample Preparation kit and sequencing reactions were carried out using Illumina HiSeq 2500 (paired-end 2x100 bp).

Bioinformatic analysis

Raw sequencing reads were analyzed through the MG-RAST pipeline (Meyer et al. 2008). Briefly, raw reads were preprocessed to trim low-quality data from the FASTQ files followed by a screening algorithm that removes all putative contaminant reads (i.e. reads matching the human and mouse genome). The remaining high-quality reads were submitted to FragGeneScan (Rho et al. 2010), a machine learning approach for gene calling. Ribosomal RNA sequences were detected and classified using vsearch (Rognes et al. 2016) against customized SILVA, Greengenes, and RDP databases. Protein coding sequences were clustered at 90% identity cutoff using CD-HIT (Fu et al. 2012). A representative of rRNA and protein clusters (the longest sequences) was identified through similarity analysis using sBLAT (Kent 2002) and DIAMOND (Buchfink et al. 2015) for rRNA and proteins, respectively. After similarity analysis, the pipeline performs feature annotations of taxonomic composition and putative functions using the M5nr database (Wilke et al. 2011), which in turn provides nonredundant integration with GenBank (Benson et al. 2013), eggNOG (Jensen et al. 2008), IMG (Markowitz et al. 2008), KEGG (Kanehisa 2002), SEED (Overbeek et al. 2005), and UniProt

(Magrane & Uniprot Consortium 2011) databases. Taxonomic annotation is calculated by Lowest Common Ancestor (LCA) algorithm (Huson et al. 2007), which solves uncertainties by providing the lowest taxonomic level that satisfies the minimum confidence value. For example, when multiple taxonomic annotations for a single feature exist in different databases, LCA sets the taxonomic hit to the common ancestor of all matched species.

RESULTS AND DISCUSSION

Volcanic eruptions are known for the dispersion and deposition of microorganisms, volcanic ash, heavy metals, and other crystalline particles (Witt et al. 2016). Regardless of being a challenging environment for the maintenance of life, Antarctic soils are inhabited by a distinct array of microorganisms, well-adapted to their demanding physicochemical conditions (Zaikova et al. 2019). Likewise, Antarctic ice can also consist of rich microbial communities, as demonstrated by our metagenomic data.

The DNA extraction resulted in 37 ng of DNA from about 1,800 mL of melted glacier ice (or 20.5 ng L^{-1}). This result is expected for a low-density microbial environment such as glacier ice (Miteva 2008) and contrasts the much higher DNA yield from other Antarctic environments such as a pond of glacial meltwater (600 ng L^{-1}), glacier forefield soils (1,400 ng kg^{-1}), and lake sediments (2,810 ng kg^{-1}) (Ferrés et al. 2015, Muangchinda et al. 2015, Strauss et al. 2012). A total of 16,746,302 reads were generated with an average length of 101 ± 5 bp. The quality filtering validated 14,186,417 (84%) sequences, which were distributed into 16,823 (0.12%) ribosomal RNA sequences, 6,914,275 (48.74%) predicted proteins with known function, and 7,255,319 (51.14%) proteins with unknown function.

Taxonomy

The LCA taxonomic analysis of the ice-tephra samples revealed a microbial community primarily composed of Bacteria (96%), followed by small fractions of Eukaryota (3%), Archaea (0.14%), and Viruses (0.01%). Within Bacteria (Figure 2a), phylum Bacteroidetes (36%) was the most abundant, followed by Cyanobacteria (33.4%), Proteobacteria (11.7%), Acidobacteria (8.1%), and Actinobacteria (2%). These phyla are well known as widespread organisms with

important functions in Antarctic soils (Cowan et al. 2010). Proteobacteria (α , β , and γ), Cyanobacteria, Bacteroidetes, Actinobacteria, and Acidobacteria are also described as major phyla in other glacial ecosystems (Bulat et al. 2004, Miteva et al. 2008). Our metagenomic data also revealed a high abundance of classes Sphingobacteria (15%), Acidobacteria (7%), and Cytophagia (5%), while *Chitinophaga* (13%) was the dominant genus, followed by *Acidobacterium* (8%), and *Cyanothece* (4%) (Figure 2b).

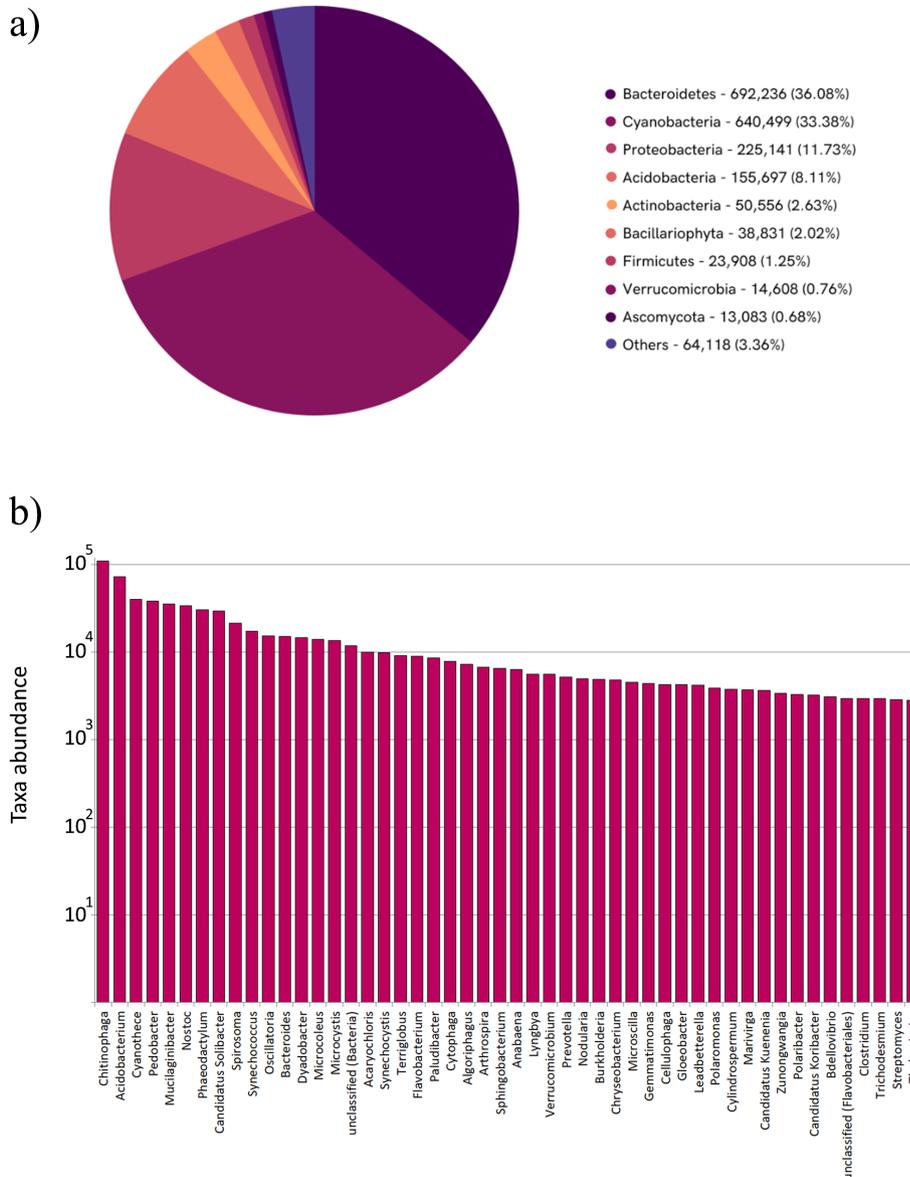


Figure 2. Distribution of microbial groups found in the glacier ice-tephra sample. a) Phyla abundance. b) Genus abundance, where only the top 50 most abundant are shown. The y-axis plots the abundances of annotations on a log scale.

Bacteroidetes are composed of chemoorganotrophic bacteria, contributing to the carbon cycle mostly as polymeric carbon degraders (Aislabie et al. 2006). These phyla can use a wide variety of organic (mono-, di-, and polysaccharides) and inorganic (CO_2) compounds as energy sources, as well as ammonia (NH_3) and sulfide (H_2S) as nitrogen and sulfur sources, respectively (Smith et al. 2006). The high abundance of Bacteroidetes in our sample may reflect the presence of such simple carbon sources within this glacial ecosystem (Rime et al. 2016). The dominant genus, *Chitinophaga*, includes over 37 species (Lee et al. 2020) of chitinolytic bacteria able to degrade chitin and other complex carbon sources such as casein and gelatin (Pankratov et al. 2006, Cowan et al. 2010). Several *Chitinophaga* strains are psychrotolerant, able to grow at minimum temperatures of 4 to 10°C (Pankratov et al. 2006, Li et al. 2017, Jin et al. 2018). Additionally, some *Chitinophaga* species have a dormant stage that would also allow for survival in periglacial habitats (Vimercati et al. 2019).

Acidobacterium, the second most abundant genus in the ice community, is characterized by growth in acid environments within the range pH 2-6 (Kielak et al. 2016). *Acidobacterium* species are commonly associated with the dry soils of Antarctica and probably encompassing a role in biogeochemical cycles (Lee et al. 2008, Cowan et al. 2010), able to use several carbon sources including mono- (arabinose, dextrose, and xylose) and polysaccharides (xylan and agar), even in low concentrations (de Castro et al. 2013). The high abundance of *Acidobacterium* in our samples suggest the ice-tephra from King George Island is a relatively acidic environment. Indeed, glacial ice has liquid water veins, described as acidic and oligotrophic, that sustain microbial communities (Price 2000).

The cold-desert characteristics of Antarctica provides biological support for a wide variety of endolithic communities (Yung et al. 2014), with Cyanobacteria comprising a substantial part of biomass in such extreme habitats (de los Ríos et al. 2007). With the main role on carbon and nitrogen fixation (de la Torre et al. 2003), Cyanobacteria add structural biomass to the community (Cowan et al. 2010), and have been identified from nearly all endolithic ecosystems (de los Ríos et al. 2007). García-Lopez et al. (2021) found several genera of endolithic-colonizers cyanobacteria in volcanic rocks from glacier ice samples in the South Shetland Islands. In our samples, *Cyanothece*, *Nostoc*, *Synechococcus*, and *Oscillatoria* represented the main genera within the phylum Cyanobacteria. These genera are present in most Antarctic habitats (Pandey et al. 2004) and are well-known for their capacity for colonizing endolithic communities (de los Ríos et al. 2007, Yung et al. 2014), constituting great targets for the study of photosynthesizing organisms with psychrotroph behavior (Tang et al. 2019). The presence of Cyanobacteria in soils that are otherwise poor in nutritional content reflects in a more suitable environment for other phyla to grow (Niederberger et al. 2008), although the high content of cyanobacterial biomass may also derive from aerial distribution from water systems, i.e., Antarctic lakes (Adams et al. 2006, Aislabie et al. 2006). This phylum has a widespread distribution in Antarctica, with some endemic genera (Lee et al. 2012).

In this study, we obtained 225,141 reads (11.73%) from Proteobacteria, mostly from Alpha (3.56%), Beta (3.8%), and Gamma (2.45%) classes. The most abundant genera within the Alphaproteobacteria were *Bradyrhizobium* (1.78% of all Proteobacteria), *Rhodopseudomonas* (1.64%), and *Methylobacterium* (1.52%). In the Betaproteobacteria, genera *Burkholderia* (4.4%), and *Polaromonas* (2.69%) ranked as more

abundant, while in the Gammaproteobacteria genera *Pseudomonas* (3.2%), and *Xanthomonas* (2.3%) were found in higher proportion. *Polaromonas*, *Gemmatimonas*, *Burkholderiales*, and *Xanthomonas* are predominant genera in Antarctic soil (Niederberger et al. 2008, Wang et al. 2015). The psychrophilic genera *Polaromonas* and *Acidithiobacillus* are also found in pioneer communities exposed to volcanic activity (Fujimura et al. 2016).

Functional Profiling

The functional analysis of the ice-tephra samples revealed 48% predicted proteins with known

functions. Among the 29 major metabolic classes within the Subsystems-based annotations (SEED database), Carbohydrate metabolism (13.88%) had the highest quantity of annotated reads, followed by clustering-based subsystems (CBSS), indicating functionally coupled genes with unknown function (12.08%). Amino acid (9.42%), Protein (8.09%), RNA (3.78%), and DNA metabolism (5.80%) were also major metabolic classes (Table I).

Carbohydrate metabolism in bacteria is extremely diverse, since carbohydrates are the main source of energy obtained and are also part of other cellular processes. The

Table I. Functional Diversity. Abundance of ice-tephra metagenomic reads assigned to the general SEED functional subsystems.

Functional Subsystem	No. of reads
Carbohydrates	237,448
CBSS	206,743
Amino Acids and Derivatives	161,270
Protein Metabolism	152,309
Miscellaneous	117,698
Cofactors, Pigments, Others	111,779
DNA Metabolism	99,246
Respiration	74,311
Cell Wall and Capsule	67,684
RNA Metabolism	64,795
Virulence, Disease and Defense	62,837
Nucleosides and Nucleotides	59,706
Membrane Transport	42,924
Fatty Acids, Lipids, and Isoprenoids	41,937
Stress Response	39,284
Cell Division and Cell Cycle	20,354
Phages, Prophages, Plasmids	19,170
Photosynthesis	17,057
Regulation and Cell signaling	15,535
Potassium, Nitrogen and Phosphorus metabolism	46,027
Metabolism of Aromatic Compounds	14,615
Sulfur Metabolism	12,054
Others (motility, iron and secondary metabolism and sporulation)	25,622

glacial habitat can vary as a sink or source of carbon, depending on the balance between autotrophs and respiration rates. The exact carbon substrate used for cold environments is still not determined (Hodson et al. 2008). Pentose phosphate pathway (2,869 reads) and the tricarboxylic acid (TCA) cycle (2,350 reads) were the two major components of central carbohydrate metabolism (Subsystems Level 3). Lactate (8,891 reads) and mixed acid (4,282 reads) were the most abundant fermentation pathways in our ice-tephra community. The biosynthesis of galactoglycans and related lipopolysaccharides (LPS) in Subsystems Level 2 CBSS was the main known cluster (12,666 reads), followed by fatty acids metabolic clusters (7,630 reads). Alterations in unsaturated fatty acids, decrease in fatty acid chain length and increase in chain branching maintain the fluidity of the cytoplasmic membrane at lower temperatures. LPS are also essential for surviving in cold environments (Kumar et al. 2002).

Cyanobacteria were found as the main responsible for carbon fixation and photosynthetic capacity within the ice-tephra community, with a total of 22,683 reads linked to CO₂ uptake (Figure S1 – Supplementary Material), the majority (8,712 reads) involving the Calvin-Benson cycle (Figure S1). Functional annotation pathways for the reductive carboxylate showed phosphoenolpyruvate synthase had the major number of reads (1,104), and the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) was also detected (502 reads). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes reversible oxidation and phosphorylation reactions in glycolytic metabolisms, presenting higher activity under lower temperatures (Kostadinova et al. 2011). GAPDH genes were found in the ice-tephra samples (2,612 reads) and were classified as Archaeal (genera *Thermoplasma*,

Methanococcus, and *Methanosarcina*) by comparison in the RefSeq database.

Nitrogen cycling is another important biogeochemical process in glacier ecosystems (Bendia et al. 2018). Genes related to several pathways of the nitrogen cycle were found in the ice-tephra samples, including nitrogen fixation, nitrification, and denitrification. However, the low number of reads (161) and genes (12) relating to nitrogen fixation suggests that this microbial community may use other inorganic molecules (ammonium or nitrate) or organic nitrogen compounds as nitrogen sources. As for nitrification, the ammonium-oxidizing bacteria *Nitrosococcus* (11,150 reads), *Nitrosomonas* (8,057 reads), and *Nitrospira* (5,567 reads) dominated the community. These nitrifying microorganisms are widespread in different aerobic ecosystems, including psychrotolerant species such as *Nitrospira lacus* (Urakawa et al. 2015). Interestingly, sequences annotated for ammonium-oxidizing archaea were also found (*Nitrosopumilus* sp., 334 reads), indicating their support in the nitrification process. Additionally, nitrite oxidation genes from *Nitrobacter* (7,974 reads), *Nitrospira* (2,707 reads), and *Nitrococcus* (1,562 reads) were present and suggest the complete potential of ammonium to nitrate oxidation in the ice-tephra environment. On the anaerobic route of the nitrogen cycle, cold-adapted denitrifying bacteria were found with higher abundance of *Dyadobacter* (63,180 reads), *Gramella* (40,774 reads), and *Pseudomonas* (24,021 reads). *Dyadobacter* and *Gramella* were reported in other cold ecosystems like Tibetan Glacier ice (Shen et al. 2013) and Antarctic marine sediments (Li et al. 2018), while genus *Pseudomonas* is commonly distributed worldwide and include several cold-adapted strains (Reddy et al. 2004). Genes for copper-containing nitrite reductase (84 reads), nitrous-oxide reductase (84 reads), and nitric-oxide reductase (80 reads) were

found in the metagenome, suggesting the possible conversion of nitrate to dinitrogen by the ice community. Members of the phylum Planctomycetes were present in the metagenome (0.61% of total Bacteria), however, no anammox genes were found. Similarly, cryoconite holes from Asian glaciers hold a relatively diverse community of nitrifiers and denitrifiers, but no anammox microorganisms are present (Segawa et al. 2014).

Genes for sulfur metabolism (3,255 reads) were also present in the ice-tephra samples. Microorganisms related to the sulfur cycle are those that best fit volcanic gradients, with a function on both oxidation and reduction of sulfur compounds. Oxidation of hydrogen sulfide (H_2S) under aerobic or anaerobic conditions generates key elements — elemental sulfur (S^0) and sulfate (SO_4^{2-}) — for the growth of photosynthesizing microorganisms (García-Lopez et al. 2021). Sulfate is also used by sulfur-reducing bacteria as a terminal electron acceptor during anaerobic respiration, which generates H_2S under strictly anaerobic conditions. Analysis of the sulfur cycle in the ice-tephra samples showed a relatively higher number of sequences related to sulfur assimilation (69.4%), rather than sulfur oxidation (16.8%) or sulfate reduction (13.8%). Interestingly, the balance of sulfur oxidation and sulfate reduction genes was also observed at the taxonomic level, with the main genera associated with sulfur oxidation (e.g., Proteobacteria *Paracoccus*, *Thiomonas*, and *Thiobacillus*) were as abundant as those related to sulfate reduction (e.g., *Desulfotomaculum*, *Desulfomicrobium*, and *Desulfococcus*). One exception was *Desulfovibrio*, which outnumbered both groups and showed a relative abundance of 0.21% of all Bacteria, while sulfur-oxidizing and sulfate-reducing bacteria were found between 0.02-0.08%. While this is not clear for our samples, the complete sulfur cycle

seems to occur in the ice-tephra, registering an important role for the above-mentioned genera. Nonetheless, microorganisms related to sulfur metabolism are believed to be dispersed to long distances as a consequence of explosive volcanic eruptions (García-Lopez et al. 2021), and should be further investigated to better understand their function in this community.

Cold adaptations in microorganisms include specific proteins for DNA replication, transcription, and translation. The low temperatures provide reduced thermal energy, inducing other physicochemical restrictions such as increased solvent viscosity and solubility of gases and increased osmotic stress (Collins & Margesin 2019). As enzymatic activities decrease in cooler temperatures, the ATP demand is reduced, thus resulting in the formation of reactive oxygen species (ROS) and requiring the activation of antioxidant defenses (Mykytczuk et al. 2013). For psychrophiles, the accumulation of solutes like glycine, betaine, and choline is important for overall osmotic balance (Goordial et al. 2016). A higher number of glycolytic proteins in psychrophilic compensate for the low efficiency of glycolytic enzymes in cold environments, including fructose-1,6-bisphosphatase (Mykytczuk et al. 2011), found in our study in major quantities (1,197 reads).

Photosynthetic pigments and carotenoids

Photosynthesis-related pathways were almost complete in our samples, with most of the enzymes for photosystems I and II, cytochrome b complex, photosynthetic electron transport, and ATPase (Figure 3a), besides the light-harvesting chlorophyll complex and secondary pigments of the antenna complex (Figure 3b) (Mackey et al. 2013). As the second most abundant Bacteria phylum in our samples, we can hypothesize that Cyanobacteria are the main microorganisms responsible for photosynthesis in these

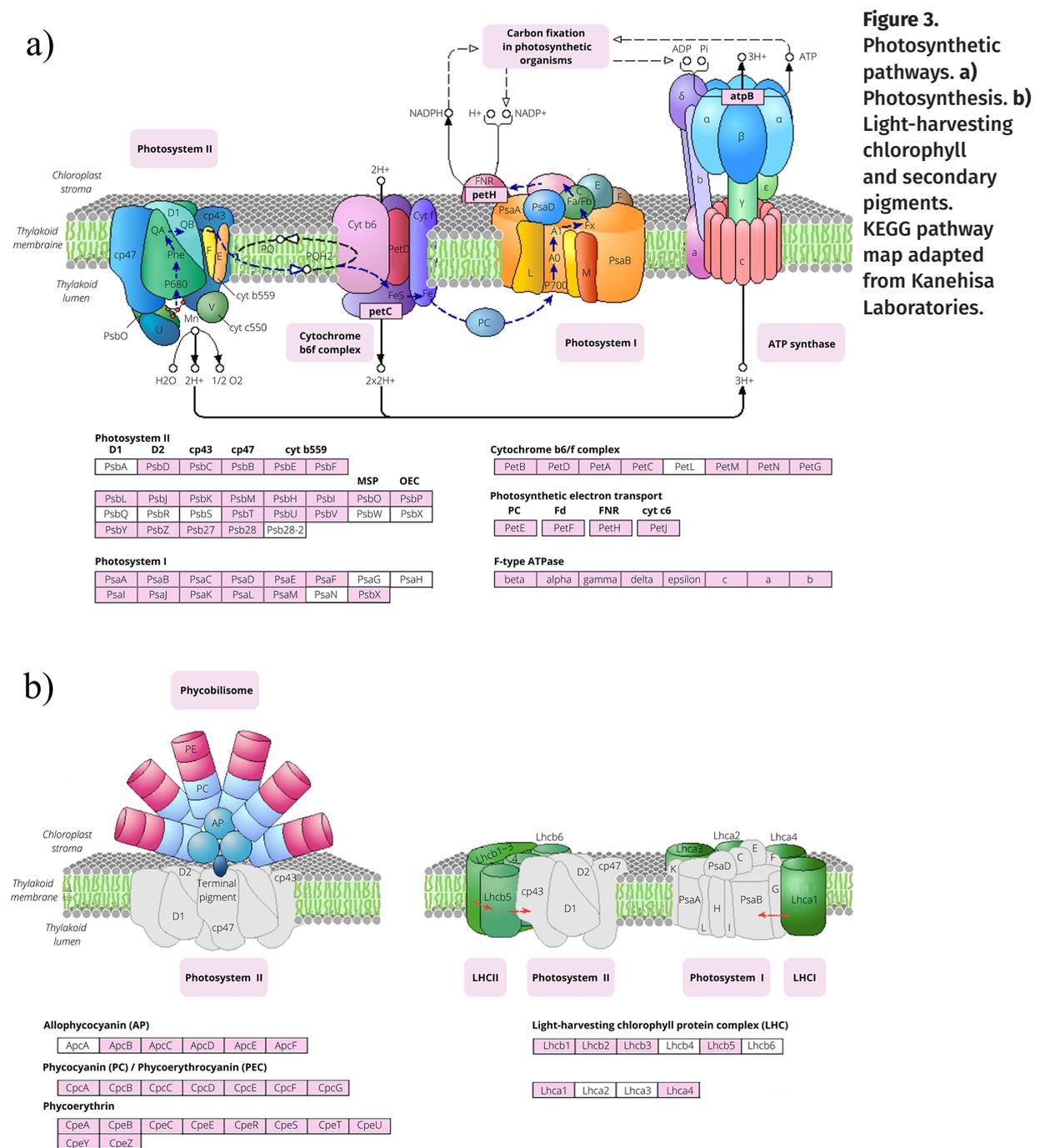


Figure 3. Photosynthetic pathways. a) Photosynthesis. b) Light-harvesting chlorophyll and secondary pigments. KEGG pathway map adapted from Kanehisa Laboratories.

ice-tephra communities. The possession of antenna complexes can be largely accountable for such important functions, with secondary pigments allophycocyanin, phycocyanin, and phycoerythrin absorbing different wavelengths of the sunlight spectrum and, thus, contributing for an extended range of

photosynthetically-active radiation that can be absorbed for photosynthesis (Campbell et al. 1998).

Due to the increased incidence of UV radiation, the Antarctic continent represents a hotspot habitat for UV-resistant microorganisms (Marizcurrena et al. 2017, Monsalves et al. 2020),

with Antarctic pigmented strains generally presenting higher resistance than their non-pigmented counterparts (Dieser et al. 2010). Besides chlorophyll and the aforementioned secondary pigments, carotenoids also play a major role for photosynthetic microorganisms living in Antarctica. Carotenoids are liposoluble tetraterpenoid pigments that serve as photoprotective compounds (Stahl & Sies 2003), reducing the deleterious effects of radiation either directly, absorbing light especially in the spectrum between 400-550 nm, or indirectly, acting as strong antioxidants through the quenching and scavenging of reactive oxygen species (Dieser et al. 2010). Carotenoids may also act in the regulation of membrane fluidity and stability under low temperatures (Mohammadi et al. 2012, Reis-Mansur et al. 2019). In our metagenomic analysis, the pathway for carotenoid biosynthesis is almost complete

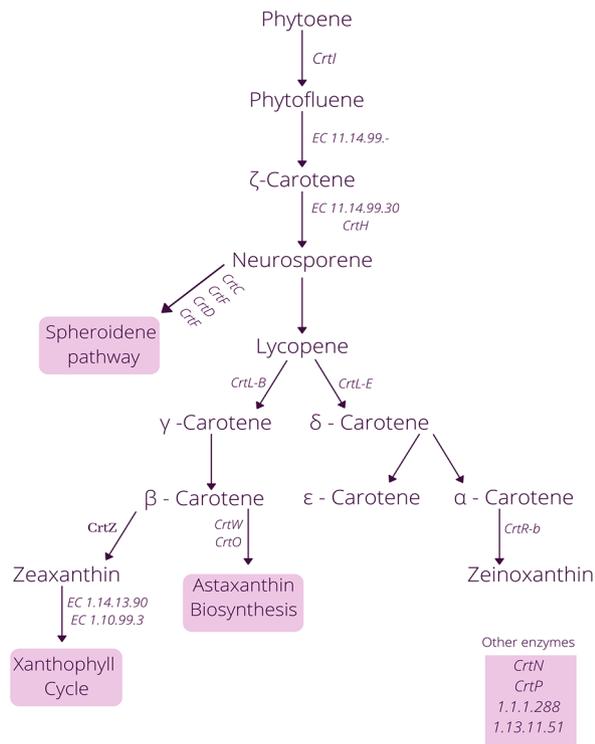


Figure 4. Carotenoid biosynthesis pathway based on KEGG map for the ice-tephra metagenome.

(Figure 4), except for specific enzymes such as 15-cis-phytoene synthase.

Extremophilic microorganisms have been drawing attention in the last few years as novel sources of bioproducts with distinct properties, and extremophiles-derived carotenoids represent promising environmental-friendly alternatives for the biotechnological industry (Órdenes-Aenishanslins et al. 2016, Reis-Mansur et al. 2019). Carotenoids can be used in a vast range of applications, from cosmetology (Núñez-Montero & Barrientos 2018) to food industry (Singh et al. 2019). Moreover, the light-harvesting properties of carotenoids provide for the increasing interest in their application for Dye-Sensitized Solar Cells (Órdenes-Aenishanslins et al. 2016). Recent research demonstrating the antimicrobial potential of pigmented Antarctic microorganisms (Mojib et al. 2010, Leiva et al. 2015, Ramesh et al. 2019) has also claimed attention towards their use for pharmacological purposes. Future bioprospection studies shall continue revealing promising bioactive molecules from Antarctic pigmented microorganisms (Silva et al. 2020), and the ice-tephra communities represent a potential source for the discovery of new metabolites.

Biotechnology Potential

Life in the cold Antarctic environments and volcano surroundings may also select for enzymatic pathways with further biotechnological potential (Zaikova et al. 2019). For example, our functional profiling analysis revealed enzymes with potential for bioremediation techniques, with annotations for aerobic and anaerobic degradation of aromatic compounds (e.g., toluene, xylene, methyl-naphthalene, and styrene) found in 14,615 reads. Degradation of styrene, an aromatic hydrocarbon present in industrial effluents (Mooney et al. 2006, Tan et al. 2015), revealed almost complete pathways, with

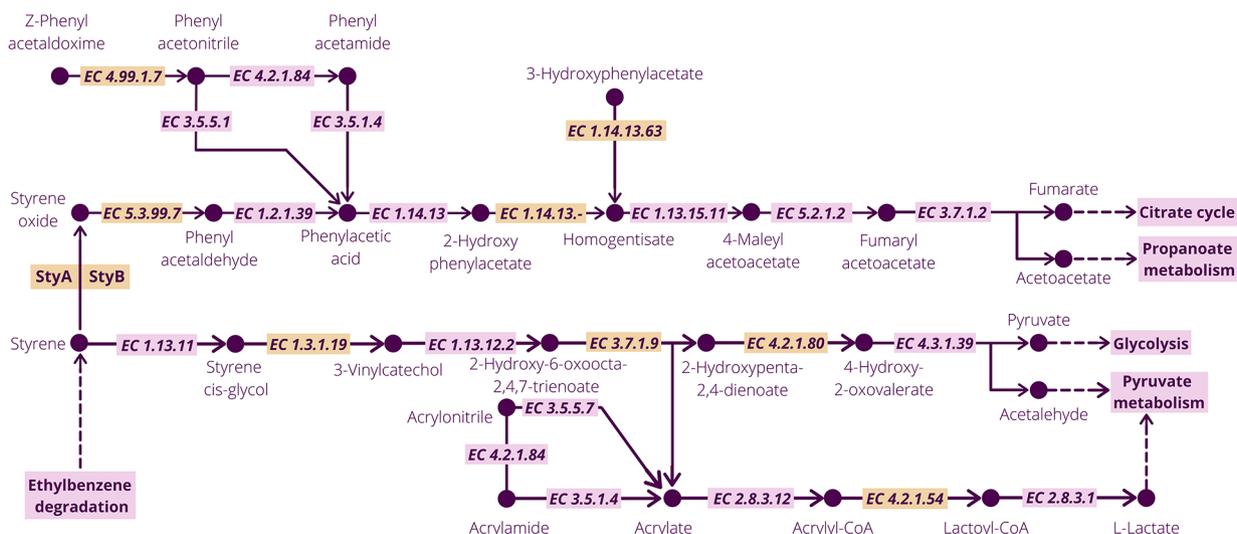


Figure 5. Styrene degradation pathway. Purple boxes represent the enzymes found in the ice-tephra metagenome.

enzymes such as homogentisate 1,2-dioxygenase (250 reads) (Figure 5). Styrene biodegradation relies especially on Proteobacteria species which oxidize this toxic compound into styrene oxide that is further isomerized into phenylacetaldehyde (Mooney et al. 2006, Runye et al. 2015). Final enzymatic reactions lead the converted compounds into the Citrate Cycle (Mooney et al. 2006), thus participating in carbon metabolism. Styrene bioremediation processes have attracted attention in the last few years especially for the conceivable bioconversion of toxic wastes into market-valuable compounds such as polyhydroxyalkanoates (PHA), which offer high commercial interest for the pharmaceutical industry (Rai et al. 2011). *Xanthobacter* and *Pseudomonas* strains have been widely studied for their potential in styrene bioremediation (Mooney et al. 2006, Tan et al. 2015). Nonetheless, the isolation and characterization of novel strains with enzymatic capacity for degradation of styrene – as for other aromatic hydrocarbons – is highly appreciated (Tan et al. 2015), and the discovery of enzymes for degradation of these toxic compounds in ice-tephra samples could represent one of the many potentials for biotechnological applications of such exquisite

and unknown environment. Further analysis of glacial ice with volcanic sediments using both culture-dependent and independent approaches should reveal more details on such perspectives.

CONCLUSIONS

To our knowledge, this is the first description of the taxonomic and functional diversity of an ice-tephra (volcanic) community using metagenomics. Our results showed this unique site presenting distinct features of its own, probably related to the influence of volcanic material. Since the glacial ice-tephra ecosystem is now better understood, future studies could explore other questions on these habitats. For example, the use of RNA-sequencing (metatranscriptomics) could shed light on the active microbial community of these glacial ecosystems, revealing their strategies for nutrition, survival, and reproduction in the extreme cold.

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SUPPLEMENTARY MATERIAL

Figure S1

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Author contributions

RTDD and VHP conceived the project idea and obtained the financial support from public funding agencies. RTDD collected the glacier ice samples. RTDD and AGB performed the DNA extraction, library preparation and sequencing submission. RTDD, CTK and MGK performed the bioinformatic analysis, drafted the manuscript and designed the figures.

