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Identification of Antarctic culturable bacteria able to produce diverse enzymes of potential biotechnological interest

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Abstract It is estimated that more than three quarters of the Earth's biosphere is in perennially cold environments. Despite the extreme environmental conditions of desiccation and freezing, microbes can colonize these habitats through the adaptation of metabolic functions and the synthesis of structurally adapted enzymes. Enzymes within psychrophilic microbes exhibit high specific activity at low and moderate temperature, with low thermostability. In this study we used a classic microbiological approach to isolate Antarctic bacteria with cellulolytic, lipolytic, and ligninolytic activities. From 15 different environmental samples, we generated a collection of approximately 800 bacterial isolates that could grow on R2A or Marine medium at 4°C. This collection was then screened for the presence of the three types of activity at 4°C. We found that 47.7% of the isolates displayed lipolytic activity, 10.2% had cellulase/xylanase activity, and 7.7% showed guaiacol oxidase activity. Of these, 10% displayed two different types of activity, while 0.25% displayed all three types of activity. Our results indicate that cold environments represent outstanding resources for bioprospecting and the study of enzymatic adaptation.

Keywords **Antarctica, bioprospecting, bacterial collection, lipolytic activity, cellulolytic activity, guaiacol oxidase activity**

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1 Introduction

Antarctica is the coldest and driest continent on Earth, where conditions of desiccation, freezing, strong winds, and variable UV radiation flux is typical. Despite these conditions, microbes can colonize this extreme environment through specialized biological functions and the synthesis of adapted enzymes with activity in these harsh conditions. This is consistent with temperature being one of the main selective forces driving biological evolution, from molecules to whole organisms[1-2].

Psychrotolerant/psychrophilic bacteria have developed several mechanisms to endure extremely cold environments[3-5]. These include changes in protein composition, with a higher proportion of non-charged polar amino acids and a lower proportion of hydrophobic amino acids (particularly leucine), production of compatible solutes (glycine, betaine, and trehalose), increased membrane lipid fluidity, and changes in genome topology. Furthermore, these bacteria produce enzymes with high specific activity at low and moderate temperature, associated with low thermostability caused by lower proportions of proline and arginine residues, and higher proportions of glycine residues.

The unique properties exhibited by bacterial cold-adapted enzymes make them ideal models for fundamental research and biotechnology. The ability to perform enzymatic processes with high efficiency at low temperature, a characteristic not associated with their mesophilic counterparts, and the possibility of irreversibly inactivating these processes by heating at moderate temperatures, are some of the reasons why cold-adapted enzymes are exceptionally useful for various biotechnological and pharmaceutical processes,

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and for bioremediation[6]. Many enzymes of potential biotechnological interest (e.g., proteases, lipases, amylases, cellulases, xylanases, and agarases) are produced by culturable psychrotolerant/psychrophilic bacteria [7-11].

Despite the progress made in culture-independent techniques in the last few years, isolation of culturable bacteria is still a widely used approach, and is the primary strategy for bioprospecting[12-13]. To date, Antarctic culturable bacteria have been isolated from diverse environments, including soil, sediment, lakes, oceans, air, and rocks[14].

The goal of this study was to evaluate maritime Antarctica as a suitable environment for discovering novel cold-adapted functions of enzymes expressed by culturable bacteria. With this purpose, we gathered a collection of psychrophilic/psychrotolerant bacteria isolated from 15 environmental samples from King George Island and the Antarctic Peninsula. The collection was evaluated for expression of bacterial proteins with functions of potential biotechnological interest, mainly for biofuel production, such as lipolytic, cellulolytic, and ligninolytic activity.

2 Materials and methods

2.1 Sites and sampling

Eleven environmental samples were collected from different sites located on the Fildes Peninsula of King George Island, South Shetland archipelago, while further four samples were collected from the Antarctic Peninsula (Table 1). All samples were collected during the 29th ANTARKOS Expedition, organized by the Uruguayan Antarctic Institute, during the 2013 austral summer (January–March). Samples were aseptically collected, transported in sterile plastic bags or tubes, and maintained at ~0°C until processing in the lab (less than 3 weeks). To enrich one sample for cellulose-degrading bacteria, sample M18 was derived according to the following procedure. Small pieces of Whatman filter paper were placed in a 50-mL polypropylene tube with holes in the cap allowing water flow into the tube. Following sterilization, this "cellulolytic trap" was placed in a glacial meltwater stream in January and retrieved 2 months later. Bacteria present in the pieces of paper were further isolated.

Table 1 Location, relevant characteristics, and procedure for sample preparation prior to serial dilution

ID	Relevant characteristic	pH	Temp./°C	Dilution 0	CFU[a]
M1	Iceberg ice (63°07′20.0″S, 57°12′12.0″W)	6.5	1.1	Concentrated 50X	3.2×10^3
M2	Moraine iceberg (63°06′56.0″S, 57°12′50.0″W)	6.5	1.1	1.98 g·mL^{-1}	7.5×10^4
M4	Rock with lichens (63°13′14.7″S, 57°13′07.5″W)	Nd[b]	Nd	Suspension of scraped surfaces	Nd
M5	Small pond of glacier meltwater (63°13′12.8″S, 57°13′05.4″W)	9.0	Nd	Without treatment	1.6×10^3
M12	Red seaweed (62°11′00.1″S, 58°53′01.1″W)	Nd	Nd	Suspension of scraped surfaces	Nd
M13	Brown seaweed (62°11′06.9″S, 58°52′50.0″W)	8.17	1.7	Suspension of scraped surfaces	Nd
M14	Sediment (62°11′34.3″S, 58°55′56.8″W)	Nd	Nd	0.25 g·mL^{-1}	Nd
M15	Sediment, Ardley Island (62°12′36.1″S, 58°55′45.1″W)	Nd	Nd	0.13 g·mL^{-1}	1×10^8
M16	Red mat, Ardely Island (62°12′43.1″S, 58°55′52.3″W)	Nd	Nd	Without treatment	Nd
M17	Seawater pond with algae (62°09′02.9″S, 58°56′27.7″W)	8.2	1.3	Without treatment	1.4×10^8
M18	Cellulolytic trap (62°11′01.0″S, 58°54′24.8″W)	7.0	0.8	Suspension of scraped surfaces	Nd
M19	Sediment (62°11′27.5″S, 58°55′07.0″W)	Nd	Nd	0.16 g·mL^{-1}	Nd
M20	Sediment attached to the underside of a piece of wood (62°11′29.0″S, 58°55′02.7″W)	Nd	Nd	0.04 g·mL^{-1}	Nd
M21	Seawater pond with green algae (62°11′30.2″S, 58°54′58.9″W)	8.5	3	Without treatment	Nd
M22	Red mat (62°11′1.4″S, 58°55′16.5″W)	Nd	Nd	Without treatment	6×10^5

Note: CFU[a]: colony forming units in R2A solid medium per g (solid samples) or per mL (liquid samples); Nd[b]: Not determined.

2.2 Bacterial isolation and culture conditions

Sample processing is summarized in Table 1. Soil, sediment, and superficial rock scrapings were suspended by shaking in 0.9% (w/v) NaCl (designated dilution 0). A bacterial pellet from sample M1 was obtained by centrifugation at 3 000 × g for 10 min at 4°C, and the pellet was resuspended in 0.9% (w/v) NaCl in a volume sufficient to obtain a 50× concentrated bacterial sample. Original samples from mats, glacial meltwater, and seawater ponds were considered to be dilution 0. Dilutions of 1:10 and 1:100 were further prepared in sterile 0.9% (w/v) NaCl. One-hundred microliters of each dilution (0, 10^{-1}, 10^{-2}) were spread on R2A[15] and Marine medium (MM)[16] containing 18 $g \cdot L^{-1}$ agar and 100 $\mu g \cdot mL^{-1}$ cycloheximide. R2A and MM were purchased from Difco (Becton, Dickinson and Company, MD, USA). Inoculated plates were incubated at 4°C for 30 d.

When possible, 32 distinct isolates from each sample were selected from R2A and MM solid media based on differences in colony morphology (e.g., color, size, and shape). Colonies were picked with sterile toothpicks and transferred to a sterile 96-well plate containing 100 µL of either R2A or MM broth per well. Cultures were incubated at 4°C for 20 d. The collection was stored in 25% (v/v) glycerol at −80°C in 96-well plates.

2.3 Functional screening

With the aim of evaluating the ability to degrade lignocellulose, we assayed for the presence of cellulases (EC 3.2.1.4), xylanases (EC 3.2.1.8), guaiacol oxidases, peroxidases (EC 1.11.1.14), and manganese peroxidases (EC 1.11.1.13). To identify bacterial lipolytic enzymes, we screened for the presence of esterase (EC 3.1.1.1) and lipase (EC 3.1.1.3) activities. For functional screening, cultures grown in 96-well plates were transferred to Petri dishes filled with appropriate media using a 48-pin replica plater. Inoculated media containing thesubstrates outlined below were incubated at 4°C, and enzymatic activity was periodically measured for at least 20 d. Otherwise, inoculated plates were incubated for 20 d at 4°C and then overlaid with the specific reagent. All substrates were purchased from Sigma-Aldrich.

Lipolytic activity

Lipase/esterase-producing isolates were screened for on solid media containing 1% (v/v) emulsified tributyril, tricaprylin, or triolein. A clear halo around the colony indicated lipase/esterase activity[17-18].

Cellulolytic activity

Cellulolytic bacteria were identified on plates containing 0.5% (w/v) of either carboxymethyl cellulose (CMC) or microcrystalline cellulose (Avicel PH-101). Cellulase activity was detected using Congo Red staining[19]. Colonies were overlaid with 0.05% (w/v) Congo Red, incubated for 10 min at room temperature, and then washed with 1 M NaCl. Production of a clear orange halo around colonies grown on CMC- or Avicel-containing media indicated cellulose-hydrolyzing activity.

Xylanase activity

Xylanase-producing isolates were detected on plates containing 0.5% (w/v) beech wood xylan as a substrate, followed by Congo Red staining [20-21]. Positive isolates gave a clear orange halo around the colony.

Ligninolytic activity

Bacteria able to oxidize guaiacol were screened on medium containing 2.4 mM guaiacol and 0.16 mM $CuSO_4$. Isolates able to oxidize guaiacol produced a brownish color[22]. Peroxidase activity was assessed on medium containing 0.025% (w/v) 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) whereas manganese peroxidase activity was assessed on medium containing 0.025% (w/v) ABTS and 0.5 mM $MnCl_2$. Well-grown colonies were covered with 1.25% (v/v) H_2O_2, and peroxidase-producing bacteria were identified by production of a blue color, caused by ABTS oxidation [23].

2.4 Bacterial identification based on 16S rDNA sequence

Colony PCR was performed using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′)[24]. Each 50-µL PCR contained 0.2 mM dNTPs, 0.2 µM of each primer, 1× Dream*Taq* buffer (Thermo Scientific), and 1 U of Dream*Taq* polymerase (Thermo Scientific). Amplification conditions were: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 90 s, then a final extension at 72°C for 7 min. Amplicons were sequenced at Macrogen Inc. (Korea) using the 1492R primer. Sequences were manually curated to eliminate ambiguous 5′- and 3′-sequences, and the 800-bp frame that displayed the highest quality was selected for further analysis. Nucleotide sequence identities were determined using the Ribosomal Database Project (RDP) Sequence Match tool[25]. Partial 16S rRNA nucleotide sequences were deposited in the GenBank database under the accession numbers indicated in Table 2.

3 Results

3.1 Isolation of culturable bacteria

After 30 d of incubation, 1.6×10^3–1.4×10^8 colony-forming units (CFU) per mL or per g of sample were obtained in R2A medium (Table 1). Samples obtained from iceberg ice (M5) or from glacier meltwater (M1) showed the lowest culturable cell counts, whereas samples consisting of sediment from the penguin reserve at Ardley Island, or from a seawater pond containing microalgae and, located near animal colonies (Weddell seals, sea elephants, and penguins), showed the

Table 2 Taxonomic classification of selected bacterial isolates

Sample ID	Strain	16S rDNA, partial seq- Accession number[a]	Closest relative (Accession number)[b]	Similarity score	S_ab score	Ref.	Activity displayed		
							L[c]	G[d]	C[e]
M1	UYIF20	KP744588	*Pseudomonas collierea* PR212 (AM421016)	1.000	1.000	[37]	+	+	-
M1	UYIF28, UYIF29	KP744596 KP744597	*Pseudomonas* sp. Nj-55 (AM409368)	1.000	1.000	n/p[f]	+	+	-
M1	UYIF42	KP744609	*Psychrobacter luti* NF11T (AJ430828)	1.000	1.000	[38]	+	-	+
M2	UYIF55	KP744621	*Loktanella salsilacus* LMG 21507T (AJ440997)	1.000	1.000	[39]	-	+	+
M4	UYIF44	KP744611	*Psychrobacter glacincola* NF1 (AJ430829)	0.999	0.992	[38]	+	-	+
M5	UYIF45	KP744612	*Pseudoalteromonas* sp. BSi20312 (DQ492735)	0.997	0.977	[40]	+	+	+
M5	UYIF46	KP744613	*Pseudoalteromonas* sp. BSi20631 (EU330370)	0.997	0.976	[40]	+	+	-
M5	UYIF47	KP744614	*Pseudoalteromonas* sp. LD710 (EF210333)	1.000	1.000	n/p	+	+	-
M5	UYIF49	KP744616	*Psychrobacter* sp. AR-5 (FJ888375)	1.000	1.000	n/p	+	+	+
M5	UYIF53	KP744619	uncultured bacterium IWB057 (FR744477)	0.999	0.987	[41]	+	+	-
M12	UYIF16	KP744586	*Flavobacterium* sp. BF03_Thol (DQ677871)	1.000	1.000	[42]	+	-	-
M12	UYIF38	KP744605	*Psychrobacter* sp. CH56 (AJ582399)	1.000	0.991	n/p	+	-	+
M12	UYIF34	KP744601	*Psychrobacter* sp. CH56 (AJ582399)	0.999	0.991	n/p	+	-	+
M12	UYIF50	KP744617	*Psychrobacter* sp. FI 8A (KF365479)	1.000	1.000	n/p	+	+	-
M14	UYIF9	KP744583	*Arthrobacter psychrolactophilus* D2 (AF134181)	1.000	1.000	[43]	-	-	-
M14	UYIF11	KP744584	*Polaromonas* sp. LI9.9 (KC433619)	1.000	0.992	n/p	-	-	-
M14	UYIF19	KP744587	*Arthrobacter* sp. N12 (EF423370)	0.996	0.963	n/p	+	-	-
M15	UYIF48	KP744615	*Rhodococcus fascians* 7Y (AJ576249)	0.999	1.000	n/p	+	-	+
M16	UYIF1	KP744578	uncultured bacterium clone SW-Jan-47 (HQ203863)	1.000	1.000	n/p	+	-	-
M16	UYIF56	KP744622	*Cryobacterium* sp. DR9 (FJ464984)	0.997	1.000	n/p	-	-	-
M17	UYIF2	KP744579	*Chryseobacterium* sp. A17U (JQ995770)	0.997	0.995	[44]	-	+	-
M17	UYIF23	KP744591	*Psychrobacter glacincola* NF1 (AJ430829)	1.000	1.000	[38]	+	-	+
M17	UYIF32	KP744600	uncultured bacterium clone 32c02 (EF515686)	0.999	0.994	n/p	+	+	-
M18	UYIF21	KP744589	*Pseudomonas* sp. Pi 3-58 (AB365069)	1.000	1.000	[45]	+	-	+

(To be continued)

(Continued)

Sample ID	Strain	16S rDNA, partial seq- Accession number[a]	Closest relative (Accession number)[b]	Similarity score	S_ab score	Ref.	Activity displayed		
							L[c]	G[d]	C[e]
M19	UYIF22	KP744590	*Janthinobacterium* sp. 339 (GU213339	1.000	0.984	n/p	+	-	+
M19	UYIF37, UYIF41, UYIF43, UYIF25	KP744604 KP744608 KP744610 KP744593	*Pseudomonas* sp. Ant5 (AF184220)	1.000	1.000	n/p	+	+	-
M19	UYIF51	KP744618	*Janthinobacterium* sp. P39 (KF301586)	0.992	0.974	n/p	+	-	+
M20	UYIF35	KP744602	Gamma proteobacterium NH14 (AB232025)	1.000	1.000	[47]	+	+	-
M20	UYIF36	KP744603	*Pseudomonas* sp. Ant5 (AF184220)	1.000	1.000	n/p	+	+	-
	UYIF30	KP744598	*Pseudomonas* sp. BG2dil (AY263468)	1.000	1.000	[48]	+	+	-
M21	UYIF4	KP744580	*Pseudomonas syringae* Lz4W (AJ576247)	0.999	0.992	n/p	+	-	-
M21	UYIF27	KP744595	*Pseudomonas* sp. Nj-10 (AJ842212)	1.000	1.000	n/p	+	+	-
M22	UYIF6	KP744581	uncultured bacterium clone SWB09 (AB294320)	0.999	0.993	[49]	-	+	-
M22	UYIF7	KP744582	*Janthinobacterium* sp. RHLS18 (JX949459)	1.000	1.000	n/p	-	-	-
M22	UYIF15	KP744585	*Pseudomonas* sp. Nj-27 (AJ842224)	1.000	1.000	n/p	+	-	-
M22	UYIF24	KP744592	*Pseudomonas congelans* SS157 (EU545156)	1.000	1.000	n/p	+	+	-
M22	UYIF26	KP744594	*Pseudomonas* sp. Nj-10 (AJ842212)	1.000	1.000	n/p	-	+	+
M22	UYIF31	KP744599	*Pseudomonas* sp. BG2dil (AY263468)	1.000	1.000	[48]	+	+	-
M22	UYIF39	KP744606	*Pseudomonas* sp. An22 (AJ551160)	1.000	1.000	n/p	+	+	-
M22	UYIF40	KP744607	*Pseudomonas syringae* Lz4W (AJ576247)	1.000	1.000	n/p	+	+	-

Note: [a]: Nucleotide accession number of partial 16S ribosomal RNA gene; [b]: Closest relative according to the RDP data project. In parenthesis, nucleotide accession number of partial 16S ribosomal RNA gene; L[c]: lipolytic; G[d]: guaiacol oxidase; C[e]: cellulolytic; n/p: not published.

highest number of culturable bacteria.

As has been previously observed for polar bacteria, most colonies were pigmented, with strong reddish, salmon, yellow, brown, and even purple colors (data not shown).

3.2 Construction of a collection of isolates and functional screening

With the aim of creating a collection harboring diverse groups of bacteria, we selected colonies with different morphologies. Up to 32 isolates per sample per media were picked, for a total collection containing 805 bacterial isolates able to grow at 4°C.

We then assessed the ability of the bacteria within the culture collection to perform different enzymatic functions at low temperature. We focused the screening on lignocellulolytic and/or lipolytic activity because of their potential uses in bioethanol and biodiesel production, and in cellulose pulp industries.

We found that 8% of the isolates hydrolyzed CMC, 2.5% hydrolyzed xylan, and almost 8% of the isolates showed guaiacol oxidase activity (Table 3). It is noteworthy that a

Table 3 Number of bacterial isolates per sample showing specific functional capabilities

Sample	Lypolitic activity				Cellulolytic activity				Guaiacol oxidase
	Total	Tbut[a]	Tcap[b]	Tole[c]	Total	CMC[d]	Avicel	Xylan	Total
M1	10	10	Nd[e]	Nd	4	3	0	1	3
M2	3	3	Nd	Nd	8	4	3	2	3
M4	5	5	Nd	Nd	11	11	0	1	0
M5	19	19	Nd	Nd	9	8	1	2	11
M12	56	54	6	0	14	14	3	0	1
M13	23	21	16	0	15	14	0	2	0
M14	23	18	8	0	2	0	0	2	1
M15	23	16	13	6	2	2	0	0	0
M16	10	5	5	4	2	2	0	0	0
M17	13	6	11	0	1	0	1	0	5
M18	48	46	35	2	6	0	0	6	0
M19	63	62	37	10	2	2	1	0	19
M20	40	30	28	0	3	2	0	2	5
M21	45	34	36	2	0	0	0	0	2
M22	40	32	22	6	3	1	0	2	12

Note: Tbut[a]: tributyrin; Tcap[b]: tricaprylin; Tole[c]: triolein; CMC[d]: carboxymethyl cellulose; Nd[e]: Not determined.

high number of isolates displaying cellulolytic activity were obtained from seaweeds (M12 and M13) (Table 3). None of the isolates produced detectable peroxidase or manganese peroxidase activities.

As shown in Figure 1a and Table 3, hydrolysis of tributyrin (C4) was the most widely observed activity, and was displayed by almost 45% of the isolates. The number of isolates with lipolytic activity decreased as the complexity of the fatty acid chains increased, with tricaprylin (C8) and triolein (C18:1) hydrolyzed by 27% and 4% of the isolates, respectively. Almost 4% (15 out of 384) of the lipolytic isolates were active against all three substrates (Figure 1a). Not all of the triolein-hydrolyzing isolates were able to degrade less-complex substrates, suggesting a chain-length preference. Several isolates displayed more than one type of activity. As shown in Figure 1b, two isolates exhibited lipolytic, guaiacol oxidase, and cellulolytic activities, and 78 isolates displayed two different types of activity (Figure 1b).

No cellulase activity was detected in isolates from the cellulose-enrichment trap (M18). Nonetheless, six isolates hydrolyzed xylan (Table 3). Unexpectedly, this sample contained a high number of isolates with lipolytic activity.

Although not intentionally assayed for, some isolates from sample M13 (brown seaweed) displayed agarolytic activity (data not shown), as shown by soft pits on the agar.

Forty-five isolates were selected for bacterial identification. The selected isolates included the two isolates that displayed lipolytic, cellulolytic and guaiacol oxidase activity; the two isolates that showed cellulolytic and guaiacol oxidase activity; 20 out of the 42 isolates that presented both guaiacol oxidase and lipolytic activity; 10 out of the 34 isolates that displayed both cellulolytic and lipolytic activities; four isolates that only displayed guaiacol oxidase activity; and three isolates that only displayed lipolytic activity. Four isolates that presented no detectable activity in any of the assays were also selected (Table 2). The closest relatives in the GenBank database for almost all (39 out of 45) of the selected isolates were also isolated from cold environments. In contrast, the closest relatives of the remaining six isolates were uncultured bacteria from diverse origins (data not shown), suggesting the possible novelty of these strains.

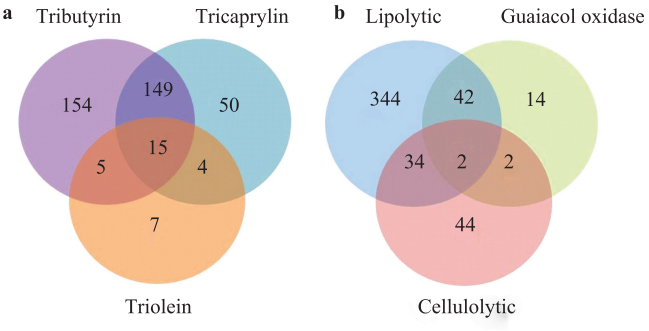

Figure 1 Venn diagram showing the relationship between the different types of enzymatic activities tested in the collection. **a**, The diagram shows the total number of isolates with lipolytic activity and the substrate they hydrolyzed. **b**, This set shows the number of isolates that displayed lipolytic, guaiacol oxidase, and/or cellulolytic activity.

Pseudomonas was the most represented genus, accounting for 18 out of 46 isolates. According to our data, 17 out of the 18 *Pseudomonas* isolates had lipolytic activity and 15 had guaiacol oxidase activity. The second-most represented genus was *Psychrobacter* (seven isolates). Interestingly, six out of the seven *Psychrobacter* isolates displayed both lipolytic and cellulotytic activity. The two isolates that demonstrated all three types of activity were identified as *Pseudoalteromonas* sp. UYIF45 and *Psychrobacter* sp. UYIF49.

4 Discussion

In this work, we generated a culture collection containing over 800 bacterial isolates capable of growth at 4°C. The original samples were collected from different environments on King George Antarctic Island and the Antarctic Peninsula. The valuable role of *ex situ* collection of Antarctic bacteria was highlighted at the XXXIII Antarctic Treaty Consultative Meeting held in Uruguay in 2010, which emphasized their contribution to the study, use, and conservation of Antarctic biodiversity[28].

To isolate culturable bacteria inhabiting different Antarctic environments, we used two different culture media, R2A and MM, which allow the growth of microorganisms with different requirements. R2A medium was selected because it allows recovery of heterotrophic slow-growing bacteria that would be easily outcompeted by fast-growing bacteria in a rich medium. Because some samples were obtained from marine environments, we also chose Marine medium, which contains a high salt concentration and numerous minerals, as it was developed for growing marine bacteria.

To include different phyla and genera in the collection, isolates were not randomly selected, but rather were gathered according to differences in features such as colour, shape, and size of colonies. As has been previously reported for cold-adapted bacteria, several isolates were pigmented[27, 29-30]. Pigmentation is one of the strategies employed by bacteria to endure extreme environments. In particular, carotenoid pigments enhance bacterial resistance to UV radiation, and contribute to membrane stability at low temperatures[26-27].

Analysis of 16S rDNA sequences revealed that 39 out of 45 selected isolates shared high sequence identity with strains related to polar or very cold environments (data not shown). Although there are few studies on the biogeography of psychrophilic bacteria, there is general agreement that the sea ice microbial communities at both poles share common genera suggesting a cosmopolitan distribution of these genera in cold environments, but harbor distinct microbial species[31-34]. The poles are geographically distant but share common extreme environments; therefore, microbial communities at both poles represent a unique model for understanding the factors shaping biogeography. This is certainly a fertile area for future studies.

With the aim of identifying and characterizing some cold-adapted enzymatic functions, the culture collection was screened for the presence of lignocellulolytic and/or lipolytic activities. Most notably, we found that lipase/esterase-producing isolates were common in the collection. Hydrolysis of tributyrin was the most common activity detected, whereas few isolates could hydrolyze more complex triacylglycerides such as tricaprylin and triolein (Table 3, Figure 1a). Overall, these results reinforce previous observations indicating that lipase/esterase activity is widely distributed among microorganisms[17]. This characteristic could be more pronounced in microorganisms inhabiting oligotrophic environments, such as the Antarctic, where carbohydrates are scarce, and therefore not readily available as a potential energy source. Consistent with this, phytoplankton mainly fix carbon into lipids or proteins rather than into polysaccharides[35].

Although our aim was to generate a collection harboring diverse groups of bacteria, and colonies showing different morphologies were selected, we cannot discard the possibility that the same strains were selected more than once. Therefore, we cannot conclude that the observed distribution of bacterial activities in this study reflects the real distribution of all culturable bacteria.

Isolates producing lignocellulolytic enzymes were also detected, with cellulase activity more common than xylanase activity. Not surprisingly, many of the isolates showing cellulolytic activity were obtained from seaweeds or lichen samples (Table 3). Although it was not intentionally assessed, some bacteria isolated from seaweeds displayed agarolytic activity. Given that agar and cellulose are components of the algal cell wall, these compounds represent an abundant source of energy for bacteria. Moreover, agarolytic bacteria are involved in putrefaction of algae[36]. Unexpectedly, the cellulose trap was not a good enrichment method for obtaining culturable cellulolytic bacteria, and only xylanase-producing isolates were detected in this sample.

In conclusion, the data obtained in this study reinforce the opinion that Antarctic islands and the Antarctic Peninsula are a relevant reservoir for bioprospecting for bacterial cold-adapted enzymes. According to our results, algae and soil seem to hold the greatest bacterial enzymatic diversity, although diverse functions were detected in all the samples collected.

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