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Screening of Culturable Antarctic Soil Bacteria for Growth on Hydrocarbon-Supplemented Media at Low Temperature

Tamizaje de bacterias cultivables de suelos antárticos para evaluar el crecimiento en medios suplementados con hidrocarburos a baja temperatura.

Joselyne Silva-Cueva¹, Abigail Montero-Calderón², Mariela Pérez-Cárdenas¹, Daniela G. Navas-León³, Hortensia Rodríguez³, Alejandro Traverso-Ponce⁴, Esteban Pazmiño-Arias^{2*}.

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Highlights:

1. Antarctic soils harbor culturable bacterial groups with potential relevance for bioremediation in cold environments.
2. Bacterial groups from Antarctic soils showed growth on hydrocarbon-supplemented media at 9°C.
3. Diesel and toluene produced the highest frequencies of positive bacterial growth, at 100% and 50%, respectively.

¹ School of Biological Sciences and Engineering, University of Investigation and Experimental Technology Yachay, Ecuador.

² School of Agricultural and Agro-industrial Sciences, University of Investigation and Experimental Technology Yachay, Ecuador.

³ School of Chemical Sciences and Engineering, University of Investigation and Experimental Technology Yachay, Ecuador.

⁴ Faculty of Engineering in Agriculture and Environment Sciences, University of Investigation and Experimental Technology Yachay, Ecuador.

*Corresponding author: cpazmino@yachaytech.edu.ec

ABSTRACT

Introduction. Accidental hydrocarbon contamination is a global concern because of its adverse effects on human health, soils, water bodies, and natural ecosystems. Even remote and protected regions, such as Antarctica, have been affected by anthropogenic activities related to shipping and fuel supply. Bioremediation in these extreme environments faces limitations associated with low temperatures, reduced bacterial metabolite production, and decreased bacterial diversity. **Objectives.** To obtain mixed groups of bacterial isolates from Antarctic soils using hydrocarbon-supplemented media as a screening strategy for bioprospecting aimed at bioremediation. **Materials and Methods.** Soil samples were collected from six sites on King George Island, Antarctica. Initial bacterial isolation from Antarctic soils was performed at 9°C for 6 weeks using arginine–glycerol agar and yeast extract–malt agar. Subsequently, qualitative growth screening was performed on Bushnell–Haas agar supplemented with six different hydrocarbons—benzene, cyclohexane, diesel, dichloromethane, n-hexane, and toluene—using two exposure methods. **Results.** A total of 34 morphologically distinct mixed groups of isolates were obtained from the six sampling points. Groups recovered from sampling points closest to the Antarctic research station (Points 4–6, particularly Point 5) showed growth on hydrocarbon-supplemented media, particularly in the presence of diesel (growth in 100% of the assays) and toluene (growth in 50% of the assays). **Conclusions.** These findings confirm that Antarctic soils harbor native bacterial groups capable of growing on hydrocarbon-supplemented media at low temperatures. The highest growth frequencies were observed with diesel (100%) and toluene (50%). Therefore, although bacterial growth suggests potential for bioremediation strategies in polar environments and other cold regions, further studies are required to confirm their hydrocarbon-degrading capabilities.

Keywords: Antarctic microorganisms; bioremediation; hydrocarbons; cold-adapted bacteria.

RESUMEN

Introducción. La contaminación accidental por hidrocarburos constituye una preocupación mundial debido a sus efectos adversos sobre la salud humana y los ecosistemas. Incluso regiones remotas y protegidas, como la Antártida, se han visto afectadas por actividades antrópicas relacionadas con el transporte marítimo y el suministro de combustible. La biorremediación en estos ambientes extremos enfrenta limitaciones asociadas con las bajas temperaturas, la reducción en la producción de metabolitos bacterianos y la disminución de la diversidad bacteriana. **Objetivos.** Obtener grupos mixtos de aislados bacterianos de suelos antárticos mediante medios suplementados con hidrocarburos, como estrategia de tamizaje para la bioprospección con fines de biorremediación. **Materiales y métodos.** Se recolectaron muestras de suelo en seis sitios de la isla Rey Jorge, Antártida. El aislamiento bacteriano inicial se realizó a 9 °C durante 6 semanas en agar arginina–glicerol y agar extracto de levadura–malta. Posteriormente, se evaluó cualitativamente el crecimiento en agar Bushnell–Haas suplementado con benceno, ciclohexano, diésel, diclorometano, n-hexano y tolueno. **Resultados.** Se obtuvieron 34 grupos mixtos de aislados morfológicamente distintos. Los grupos recuperados de los puntos más cercanos a la estación de investigación antártica (P4–P6, particularmente P5) mostraron crecimiento en medios suplementados con hidrocarburos, especialmente con diésel (100% de los ensayos) y tolueno (50%). **Conclusiones.** Los suelos antárticos albergan grupos bacterianos nativos capaces de crecer en medios suplementados con hidrocarburos a bajas temperaturas. Aunque estos resultados sugieren potencial para estrategias de biorremediación en ambientes polares y otras regiones frías, se requieren estudios adicionales para confirmar su capacidad de degradación de hidrocarburos.

Palabras clave: microorganismos antárticos; biorremediación; hidrocarburos; bacterias adaptadas al frío.

INTRODUCTION

Antarctica, a fragile ecosystem of major importance for scientific research, is not immune to the environmental impacts associated with hydrocarbon use and spills. The introduction of petroleum hydrocarbons into this remote environment alters microbial communities and vegetation, restricts nutrient uptake, and modifies soil structure and composition ⁽¹⁾. Hydrocarbon contamination in Antarctic soils is caused by human activities such as tourism, oil transportation, oil platforms, and pipelines ⁽²⁾. Additional localized pollution results from fuel combustion, spills during refueling operations, and waste generation, as well as accidental releases during fuel distribution ^(3,4). Hydrocarbon spills pose a serious threat because of the limited natural recovery capacity of Antarctic ecosystems. A clear example is the sinking of the Bahía Paraíso vessel in 1989, which released approximately 600 m³ of diesel into waters near the Antarctic Peninsula, affecting seabirds and other aquatic organisms ⁽⁵⁾. This type of pollution not only has immediate effects on fauna but also alters soil microbial structure, reduces microbial diversity, and impairs natural biodegradation under low-temperature and other adverse environmental conditions ⁽⁶⁾.

A study conducted in the northwestern region of the Antarctic Peninsula correlated increased atmospheric concentrations of polycyclic aromatic hydrocarbons (PAHs) with proximity to Antarctic research stations, identifying these stations as possible primary sources of PAHs, probably because of the presence of waste incinerators ⁽⁷⁾. The presence of PAHs near scientific stations further highlights the localized risk to Antarctic ecosystems, emphasizing the need for stricter environmental monitoring and improved waste management to reduce impacts and preserve sensitive environments. Mitigating this problem is challenging because of environmental constraints such as low temperatures, pH variability, and limited nutrient availability. Within the Antarctic region, and in compliance with Annexes III and IV of the Antarctic Treaty, Antarctic ecosystems must be protected from oil and waste contamination ⁽⁸⁾. In conservation areas, invasive remediation is prohibited; therefore, bioremediation through biostimulation has been considered a less invasive yet effective strategy for hydrocarbon-contaminated soils ⁽⁹⁾.

For this reason, bioremediation depends on microorganisms adapted to specific ecosystems. For example, in contaminated Patagonian soil, a bacterial community composed of *Rhodococcus erythropolis*, *Achromobacter xylosoxidans*, and *Brevundimonas* was isolated and shown to have the capacity to primarily mineralize aliphatic petroleum fractions, such as diesel, kerosene, and oils ⁽¹⁰⁾. In tropical climates, the presence of soil actinobacteria with high metabolic capacity is notable. The strain *Gordonia amicalis* HS-11 degraded approximately 93% of diesel oil after 16 days of incubation ^(11,12). Because hydrocarbons are carbon-based molecules, bacteria can transform them into simpler

compounds and subsequently use them as a carbon source ⁽¹³⁾. Therefore, some bacteria can degrade different types of hydrocarbons—saturated, unsaturated, or aromatic—but their efficiency and capacity depend on the molecular structure of the compound ⁽¹¹⁾. From a theoretical perspective, the ability of these bacteria to degrade hydrocarbons is attributed to key physiological adaptations that enable them to use hydrocarbons as an energy source. In general, they express oxygenase enzymes that incorporate oxygen into the substrate, thereby initiating catabolic processes leading to central metabolites (e.g., intermediates of the Krebs cycle) ⁽¹⁴⁾.

Furthermore, many of these bacteria secrete biosurfactants or modify their cell surfaces to adhere to oily phases, thereby increasing the solubility of hydrophobic compounds and facilitating their uptake ⁽¹⁴⁾. For example, *Alcanivorax borkumensis* incorporates intermediate fatty acids derived from the degradation of alkanes (C₁₁–C₁₉) into its membrane, significantly increasing cell hydrophobicity when grown on oily substrates ⁽¹⁵⁾. Similarly, *Gordonia amicalis* can produce abundant extracellular biosurfactants that reduce surface tension and facilitate diesel degradation ⁽¹¹⁾. Altogether, the combination of enzymatic pathways, surfactant production, and membrane modifications explains, according to the literature, the efficiency of different bacteria in degrading both aliphatic and aromatic hydrocarbons across various environments ⁽¹⁴⁾.

Despite the characteristic conditions of Antarctica, such as low temperatures, high radiation, and nutrient deficiencies, the continent is home to diverse microorganisms that have developed unique metabolic adaptations. Several studies have found that Antarctic bacteria belonging to the genera *Psychrobacter*, *Leifsonia*, *Rhodococcus*, *Pseudomonas*, and *Streptomyces* possess remarkable capabilities to degrade pollutants (i.e., polychlorobiphenyls, heavy metals, and aliphatic and aromatic hydrocarbons), as well as to synthesize microbial compounds active against multidrug-resistant pathogens, biosynthesize extracellular polymeric substances, and produce enzymes of industrial interest, including amylases, proteases, cellulases, chitinases, and laccases ^(16–20). Therefore, Antarctica could represent a focal point for microbial bioprospecting.

The exploration of microorganisms capable of degrading hydrocarbons is necessary for the development of efficient and sustainable bioremediation strategies. Strains isolated from extreme environments, such as Antarctic soils or saline areas, exhibit metabolic adaptations that support survival under adverse conditions ^(17,21). Notably, *Corynebacterium variabile* HRJ4, degraded up to 78.9% of hydrocarbons when immobilized on biochar ⁽²²⁾. These findings highlight the importance of investigating microbial diversity in underexplored niches to optimize environmental remediation technologies ^(23,24). Furthermore, limited information is available regarding the functional diversity of native strains

from uncontaminated Antarctic soils—such as those examined in this study—as well as their potential to degrade specific fractions (e.g., polycyclic aromatic hydrocarbons) under conditions representative of Antarctic terrestrial ecosystems. Consequently, this study addresses this gap by screening culturable bacterial groups from pristine Antarctic soils and evaluating their growth capacity at low temperatures in media supplemented with various hydrocarbons as the sole carbon source. In this regard, the present research represents a preliminary approach to identifying the potential of these bacteria for future bioremediation strategies in Antarctic ecosystems or other cold regions. In this study, we aimed to evaluate the growth of culturable bacteria from Antarctic soils using hydrocarbon-supplemented media as the sole carbon source under low-temperature conditions.

MATERIALS AND METHODS

Experimental Design

The present study followed a completely randomized factorial design with two main factors: sampling point (six levels: P1–P6) and hydrocarbon type (six levels: benzene, toluene, n-hexane, cyclohexane, dichloromethane, and diesel). Two isolation media (arginine–glycerol agar and yeast extract–malt agar) were used for the initial recovery of mixed groups of bacterial isolates from Antarctic soils. Subsequently, two different methods of hydrocarbon exposure (filter-paper volatilization and direct mixing) were used to evaluate the presence or absence of bacterial growth under exposure to the different hydrocarbons. All incubations were carried out at 9°C for 6 weeks. The evaluation was qualitative, as the present study constitutes a preliminary qualitative screening analysis. For each combination, three biological replicates were performed for each culture medium. All experiments included their respective controls: negative sterility controls (uninoculated plates to verify sterility, plating of the sterile distilled water used for dilutions, and filtered hydrocarbons plated without bacteria) and carbon-free growth controls (Bushnell–Haas medium without hydrocarbons for each mixed group of isolates). Bacterial growth was recorded as positive when visible macroscopic colonies were observed on the surface of Bushnell–Haas agar supplemented with hydrocarbons after 6 weeks of incubation at 9°C. Conversely, growth was classified as negative when no visible development was detected. This qualitative criterion was applied systematically across all treatments, including the controls. Finally, hydrocarbon screening was performed using undefined mixed groups of isolates derived from each sampling point and initial isolation medium.

Sample Collection

Soil samples were collected during the XVI Ecuadorian Antarctic Expedition in the summer of 2023 in Admiralty Bay, King George Island, South Shetland Islands, Antarctica. Sampling was conducted at six locations, with coordinates expressed in decimal degrees: P1 (−62.07381, −58.41817), P2 (−62.07425, −58.41967), P3 (−62.07433, −58.41717), P4 (−62.08319, −58.39192), P5 (−62.08175, −58.39231), and P6 (−62.08597, −58.39281). Points 1–3 were located northwest of Keller Peninsula, approximately 3 km from Comandante Ferraz Research Station, whereas Points 4–6 were located near the station, within an approximate radius of 0.25 km (**Figure 1**). At each location, a composite sample was obtained by combining five subsamples collected at a depth of 20 cm to ensure representativeness. All samples were stored at −20°C until processing. Points 1–3 were located northwest of Keller Peninsula, approximately 3 km from Comandante Ferraz Research Station. Points 4–6 were located near the station, within an approximate radius of 0.25 km (**Figure 1**). At each location, a composite sample was obtained by combining five subsamples collected at a depth of 20 cm to ensure representativeness. All samples were stored at −20°C until processing.

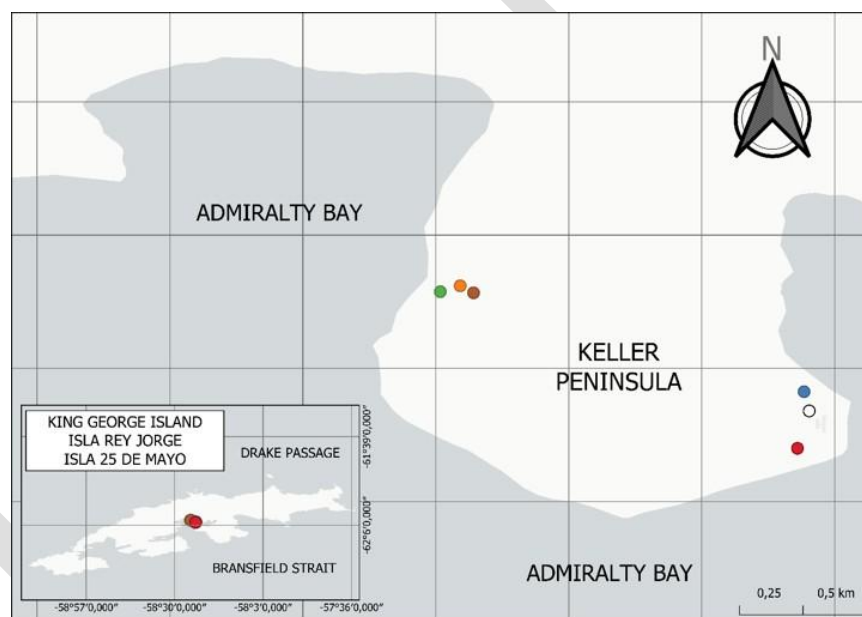


Figure 1. Soil sampling locations on King George Island, South Shetland Islands, Antarctica. **Points 1** (orange), **2** (green), and **3** (brown) are located northwest of Keller Peninsula, Admiralty Bay, whereas **Points 4** (white), **5** (blue), and **6** (red) are located near the Brazilian Antarctic Research Station “Comandante Ferraz.”

Initial Processing of Antarctic Soils

Antarctic bacteria were isolated using two culture media: arginine–glycerol agar and yeast extract–malt agar, with a final volume of 25 mL per plate. Arginine–glycerol agar was prepared with 1 g·L⁻¹ arginine monohydrochloride, 12.5 g·L⁻¹ glycerol, 1 g·L⁻¹ K₂HPO₄, 1 g·L⁻¹ NaCl, 0.5 g·L⁻¹ MgSO₄·7H₂O, 0.01 g·L⁻¹ FeCl₃, 0.001 g·L⁻¹ CuSO₄·5H₂O, 0.001 g·L⁻¹ ZnSO₄·7H₂O, 0.001 g·L⁻¹ MnSO₄·H₂O, and 20 g·L⁻¹ agar, with the pH adjusted to 6.9–7.1. Yeast extract–malt agar was prepared with 4 g·L⁻¹ yeast extract, 10 g·L⁻¹ malt extract, 4 g·L⁻¹ dextrose, and 20 g·L⁻¹ agar, with a final pH of 7.3. The media were prepared following a standard protocol by boiling until complete dissolution of the components, followed by sterilization in an autoclave at 121°C for 15 min. These general formulations were selected to provide adequate nutritional conditions to support bacterial growth from Antarctic soil samples ⁽²⁵⁾. To improve isolation, an antifungal treatment was added to each sterilized medium: 2 µL of nystatin (100,000 IU·mL⁻¹) per liter of medium was used. A 1:10 dilution of soil from each sampling point was prepared in sterile distilled water, and 0.1 mL of each dilution was inoculated by surface spreading. The inoculated plates were incubated at 9°C for 6 weeks ⁽²⁶⁾. For this procedure, the following controls were included: a negative control to verify the sterility of the process and a water control to verify that the water used was sterile. Three replicates were performed.

Macroscopic Characterization

Macroscopic characterization of the mixed groups of isolates obtained from each Antarctic soil sampling point was performed. After incubation, photographs were taken using a stereomicroscope (Leica M205C, Leica Microsystems, Wetzlar, Germany) equipped with a 0.63× objective lens, a 0.78× zoom factor, and 10×/23 eyepieces. LAS-X software was used to evaluate colony size, brightness, and structure, whereas colony color was assessed by direct visual observation under the stereomicroscope ⁽²⁷⁾.

Screening of Bacteria on Hydrocarbon-Supplemented Media

Bacterial growth obtained from Antarctic soils was transferred and inoculated onto Bushnell–Haas agar (HiMedia Laboratories, Maharashtra, India), and growth was evaluated in the presence of six different hydrocarbons: benzene (99%), toluene (99.5%), n-hexane (99%), cyclohexane (99%), dichloromethane (99.8%), and diesel (250 ppm). The hydrocarbons were previously filtered through 0.20-µm filters to remove particles and microorganisms that could interfere with the results. For each assay, 0.2 mL of filtered hydrocarbon was used.

Selection of Hydrocarbons

Six hydrocarbons were selected for this study. Selection was based on two main criteria: their use in various industries and products and their structural diversity, which allowed the inclusion of different types of hydrocarbons. Accordingly, benzene and toluene were selected as aromatic hydrocarbons present in fuels and industrial solvents; n-hexane and cyclohexane as alkanes used in the chemical industry and as solvents; and dichloromethane as a halogenated hydrocarbon used in industrial formulations, primarily for cleaning. Finally, diesel was included because of its complex hydrocarbon mixture and because it is a frequent pollutant of ecosystems, particularly in Antarctica, where the use and transport of diesel are of major concern in the South Shetland Islands and around scientific stations.

Hydrocarbon-Supplemented Media

For each plate showing growth during the initial processing of Antarctic soil on arginine–glycerol agar or yeast extract–malt agar, the total microbial biomass developed on the plate was collected. It should be noted that the sample corresponded to the total biomass recovered from each Petri dish and was resuspended without prior cell quantification. Consequently, the initial bacterial density was not standardized. Therefore, the observed results reflect the growth of mixed groups of isolates rather than a cellular-level response. The recovered biomass was resuspended in 1.5 mL of sterile saline solution (0.9% NaCl). The resulting suspensions thus corresponded to mixed groups of isolates derived from each sampling point and initial isolation medium. The suspensions were subsequently inoculated onto Bushnell–Haas medium supplemented with hydrocarbons using two methods: *i) Filter-Paper Method with Hydrocarbon*. A 0.2-mL aliquot of the resuspended sample was spread over the agar surface of the prepared Bushnell–Haas medium. The hydrocarbon (0.2 mL) was applied to sterilized filter paper, which was then placed on the lid of the Petri dish. During the 6-week incubation at 9°C, the plates were maintained in an inverted position. The hydrocarbons were supplied to the mixed groups of isolates by volatilization from the filter paper ⁽²⁸⁾ *ii) Hydrocarbon Inoculation Method*. A 1:1 ratio of microorganisms to hydrocarbon was applied to Bushnell–Haas agar plates. Accordingly, 0.2 mL of the resuspended mixed-isolate sample was mixed with 0.2 mL of hydrocarbon by pipetting, and the resulting mixture was applied to the agar by surface spreading.

In both methods, to ensure that bacterial growth was not a consequence of carbon carryover from the initial isolation media, a growth control was used. This control consisted of Petri dishes containing Bushnell–Haas medium and the initial mixed groups of isolates without carbon supplementation, i.e., without the addition of hydrocarbons.

Data Processing

Bacterial growth was determined qualitatively based on the presence (+) or absence (−) of growth. To determine the frequency of isolation for each hydrocarbon or sampling point, a percentage was calculated based on N , the number of plates showing growth, and T , the total number of plates tested (Eq. 1):

$$\text{Frequency of isolation (\%)} = (N/T) \times 100 \quad (1)$$

Furthermore, to evaluate the effects of hydrocarbons and sampling points on microorganism growth, penalized logistic regression was used. This approach allows the probability of a binary event to be predicted using variables that influence the probability of the outcome. Hydrocarbon type and sampling point were considered predictors, whereas growth was treated as a binary response (1 = growth/presence; 0 = absence). Observations obtained from the two initial culture media (arginine–glycerol agar and yeast extract–malt agar) were pooled and treated as repeated observations (replicates) in the penalized logistic regression model rather than as an independent explanatory factor. For this study, an effect was considered significant when the p value was < 0.05 and nonsignificant when the p value was ≥ 0.05 . Analyses were performed using RStudio and the `logistf` package.

RESULTS

Bacterial Isolation from Antarctic Samples

Bacterial growth was recorded at all six sampling points on both culture media, arginine–glycerol agar and yeast extract–malt agar, as shown in **Figure 2**. At Sampling Point 1, eight groups of isolates were obtained. Six groups of isolates were identified at each of Sampling Points 2 and 3. Sampling Point 4 yielded ten groups of isolates, whereas two groups of isolates were identified at each of Sampling Points 5 and 6.

Bacterial Macroscopic Characterization

The groups of isolates from Point 1 exhibited distinct morphologies, including irregular (A-P1, F-P1), punctiform (B-P1, D-P1, H-P1), filamentous (C-P1, G-P1), and circular (E-P1) shapes; flat (A-P1, F-P1), convex (B-P1, H-P1), and pulvinate (D-P1, E-P1) elevations; and undulate (A-P1, F-P1), entire (B-P1, D-P1, E-P1, H-P1), and filamentous (C-P1, G-P1) margins.

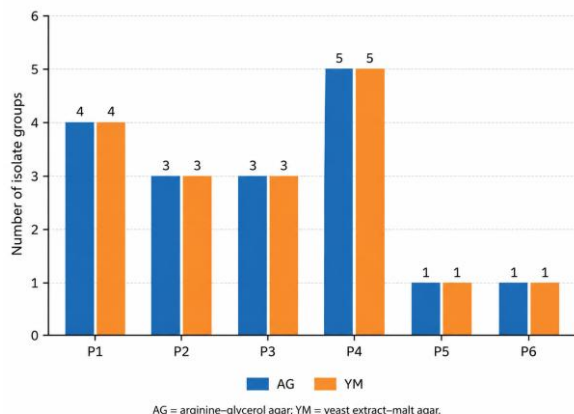


Figure 2. Number of mixed groups of isolates obtained from Antarctic soil samples at six sampling points (P1–P6) using arginine–glycerol agar (AG) and yeast extract–malt agar (YM).

Colony colors ranged from brown (A-P1) and milky (B-P1, F-P1, H-P1) to white (C-P1, G-P1) and yellow (D-P1, E-P1).

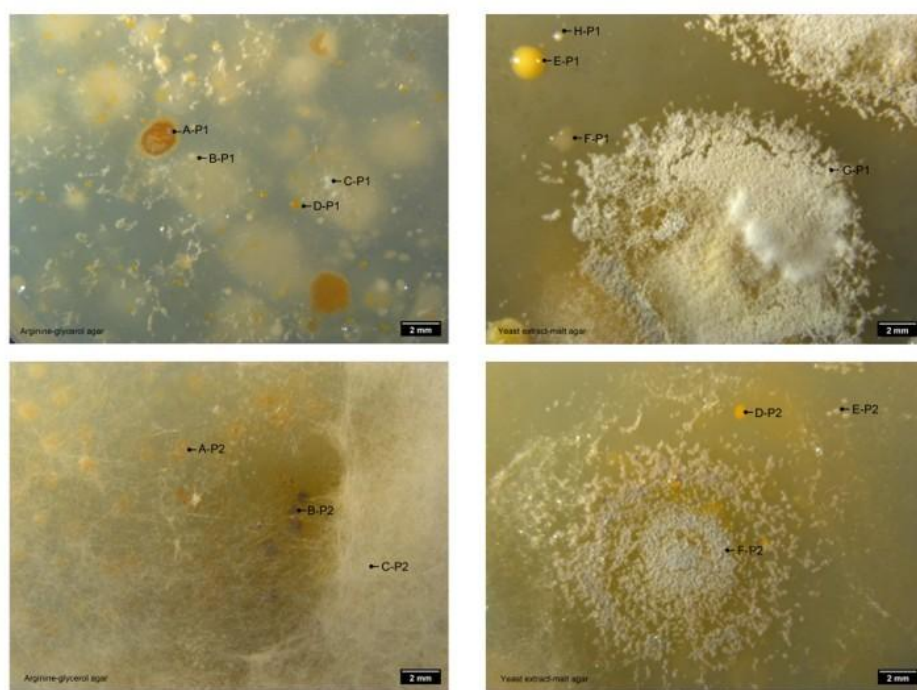


Figure 3. Photographs of bacterial growth from Antarctic soils at Sampling Points 1 (top) and 2 (bottom), using two bacterial culture media: arginine–glycerol agar (left) and yeast extract–malt agar (right). Each group of isolates was labeled with a letter of the alphabet followed by the corresponding sampling point (P1 or P2).

Similarly, the groups of isolates from Point 2 showed irregular (A-P2), filamentous (B-P2, C-P2, F-P2), circular (D-P2), and punctiform (E-P2) shapes; flat (A-P2, B-P2, F-P2), umbonate (C-P2), and convex (D-P2, E-P2) elevations; and undulate (A-P2), filamentous (B-P2, C-P2, F-P2), curled (D-P2), and entire (E-P2) margins. Pigmentation included brown (A-P2), dark brown (B-P2), white (C-P2), yellow (D-P2), milky (E-P2), and gray (F-P2). The groups of isolates from Point 3 (**Figure 4**) exhibited punctiform (A-P3, D-P3), filamentous (B-P3), irregular (C-P3, F-P3), and circular (E-P3) shapes; convex (A-P3, D-P3, E-P3), umbonate (B-P3), and flat (C-P3, F-P3) elevations; and entire (A-P3, D-P3), filamentous (B-P3), undulate (C-P3, F-P3), and curled (E-P3) margins. Colors ranged from milky (A-P3, D-P3) and white (B-P3) to yellow (C-P3, E-P3) and brown (F-P3).

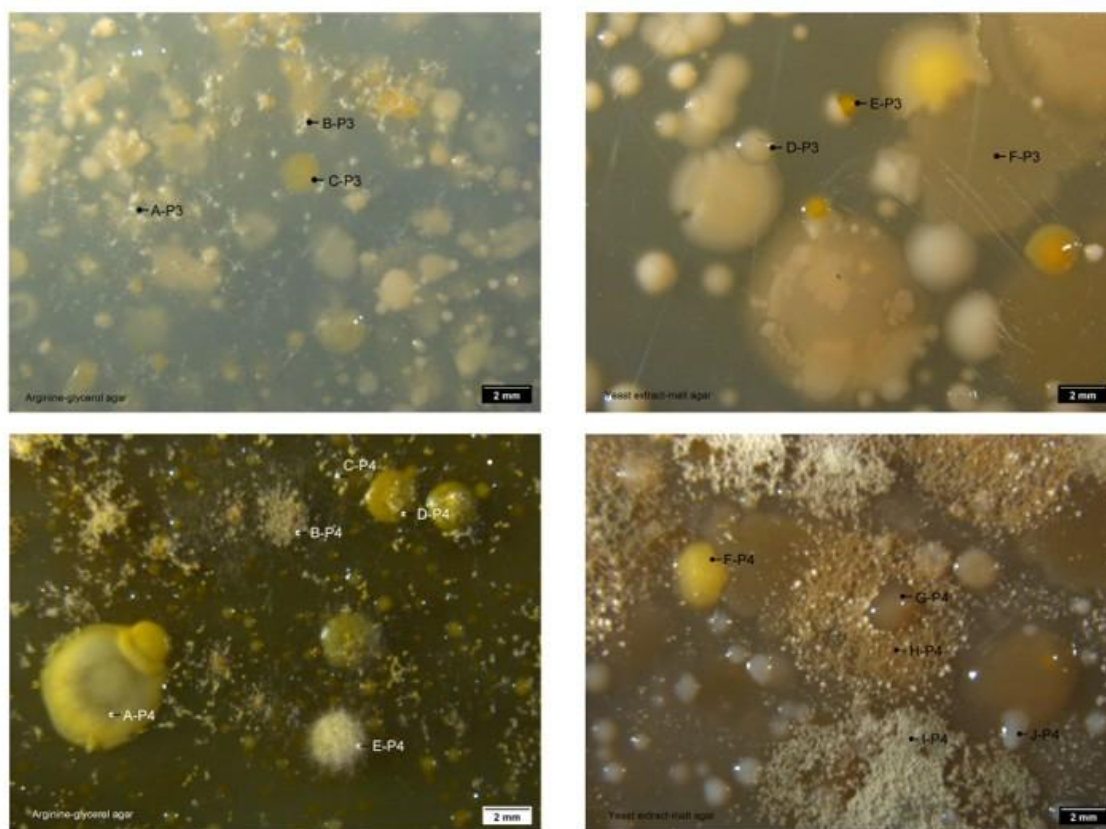


Figure 4. Photographs of growth from Antarctic soil-derived mixed groups of isolates at Sampling Points 3 (top) and 4 (bottom), using two culture media: arginine–glycerol agar (left) and yeast extract–malt agar (right). Each group of isolates was labeled with a letter of the alphabet followed by the corresponding sampling point (P3 or P4).

The groups of isolates from Point 4 showed circular (A-P4, C-P4, D-P4), filamentous (B-P4, E-P4, H-P4, I-P4), irregular (F-P4, G-P4), and punctiform (J-P4) shapes; raised (A-P4, F-P4, G-P4), flat (B-P4, H-P4, I-P4), and convex (C-P4, D-P4, J-P4) elevations; and curled (A-P4), filamentous (B-P4, E-P4, H-P4, I-P4), entire (C-P4, D-P4, J-P4), and undulate (F-P4, G-P4) margins. Pigmentation included light green (A-P4), gray (B-P4, I-P4), dark green (C-P4), green (D-P4), white (E-P4, J-P4), yellow (F-P4), and brown (G-P4, H-P4). Finally, at Sampling Points 5 and 6 (**Figure 5**), bacterial growth exhibited a filamentous shape, flat elevation, filamentous margin, and gray color (A-P5, B-P5, A-P6, B-P6).

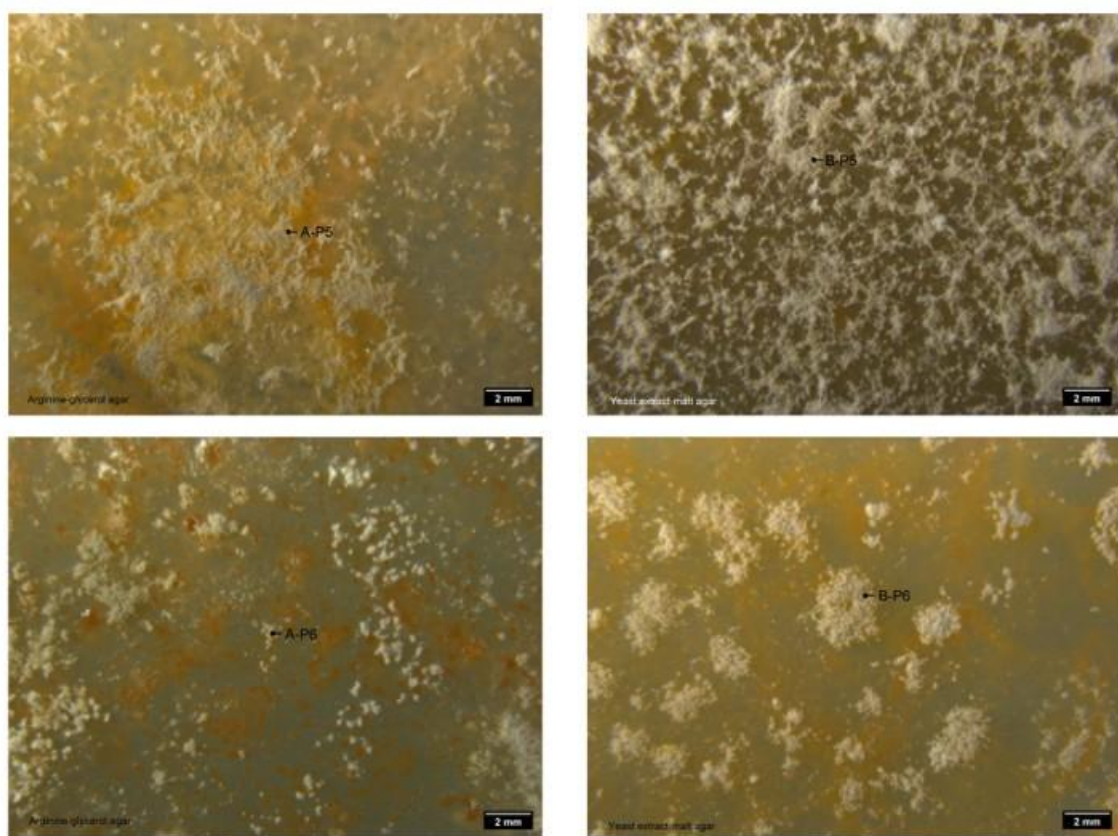


Figure 5. Photographs of growth from Antarctic soil-derived mixed groups of isolates at Sampling Points 5 (top) and 6 (bottom), using two bacterial culture media: arginine–glycerol agar (left) and yeast extract–malt agar (right). Each group of isolates was labeled with a letter of the alphabet followed by the corresponding sampling point (P5 or P6).

Figure 6 summarizes the morphological characteristics observed in the mixed groups of isolates according to sampling point and culture medium. Analysis of the 34 mixed groups of isolates revealed several predominant characteristics: filamentous shape, flat elevation, filamentous margin, and variable colony pigmentation.

Hydrocarbon Assays

To evaluate bacterial growth in the presence of hydrocarbons, groups of isolates from Antarctic soils were tested using two methods. With the filter-paper method, no microbial growth was observed after 6 weeks of incubation at 9°C in any of the plates corresponding to the six sampling points or any of the six hydrocarbons evaluated. In contrast, bacterial growth was observed on Bushnell–Haas agar supplemented with hydrocarbons, as shown in **Table 3**. Hydrocarbon-associated bacterial growth was evaluated using groups of isolates initially obtained on the two different culture media: arginine–glycerol agar and yeast extract–malt agar.



Figure 6. Morphological characterization matrix of mixed groups of bacterial isolates obtained from Antarctic soil samples. The heatmap displays colony shape, elevation, margin, and color across groups of isolates grown on arginine–glycerol agar and yeast extract–

malt agar. Individual groups are identified on the y-axis by isolate code and sampling site (P1–P6). Grid tiles display two-letter attribute codes and are color-coded according to the adjacent code legend.

Table 3. Antarctic bacterial growth on Bushnell–Haas agar supplemented with hydrocarbons. The table shows the frequency of isolation for each hydrocarbon tested and for each sampling point

Hydrocarbon	Agar	P1	P2	P3	P4	P5	P6	Frequency per hydrocarbon (%)
Benzene	AG	–	–	–	–	–	–	8
	YM	–	–	–	–	+	–	
Cyclohexane	AG	–	–	–	–	–	–	8
	YM	–	–	–	–	+	–	
Diesel	AG	+	+	+	+	+	+	100
	YM	+	+	+	+	+	+	
Dichloromethane	AG	–	–	–	–	+	–	17
	YM	–	–	–	–	+	–	
n-Hexane	AG	–	–	–	–	+	–	17
	YM	–	–	–	–	+	–	
Toluene	AG	+	–	–	+	+	+	50
	YM	–	–	–	+	+	–	
Frequency per point		25%	17%	17%	33%	83%	25%	

AG= arginine–glycerol agar; **YM** = yeast extract–malt agar; **P1** = Point 1; **P2** = Point 2; **P3** = Point 3; **P4** = Point 4; **P5** = Point 5; **P6** = Point 6; (+) = presence; (–) = absence.

The bacterial group of isolates from Point 5 obtained on yeast extract–malt agar showed growth on all six hydrocarbons tested. In contrast, the group from Point 5 obtained on arginine–glycerol agar grew on four of the six hydrocarbons (diesel, dichloromethane, n-hexane, and toluene), representing 66.7% of the hydrocarbons evaluated. Regarding the overall frequency of growth for each hydrocarbon, diesel supported growth in 100% of the assays, followed by toluene at 50%.

Dichloromethane and n-hexane each supported growth in 17% of the assays, whereas benzene and cyclohexane showed the lowest frequencies, at 8% each. Finally, Table 4 shows the effects of the six hydrocarbons tested—benzene, cyclohexane, diesel, dichloromethane, n-hexane, and toluene—and the six sampling points on bacterial growth. Penalized logistic regression was used to estimate the probability of bacterial growth, with a *p* value < 0.05 considered statistically significant and a *p* value ≥ 0.05 considered nonsignificant.

Table 4. Penalized logistic regression analysis of the effects of sampling point and hydrocarbon type on bacterial growth. Positive coefficients indicate a higher probability of growth relative to the reference categories (benzene and Sampling Point 1), whereas negative coefficients indicate a lower probability. p values < 0.05 were considered statistically significant.

Predictors	p -value	Coefficients
Microorganisms_P2	0.3	-1.7
Microorganisms_P3	0.3	-1.7
Microorganisms_P4	0.4	1.2
Microorganisms_P5	0.0004	4.7
Microorganisms_P6	1.0	0.0
Hydrocarbon_Cyclohexane	1.0	0.0
Hydrocarbon_Dichloromethane	0.3	1.7
Hydrocarbon_Diesel	1×10^{-7}	8.1
Hydrocarbon_Hexane	0.3	1.7
Hydrocarbon_Toluene	0.002	4.6

*Likelihood ratio test: $\chi^2 = 58.0$; $p < 0.001$. **P1** = Point 1; **P2** = Point 2; **P3** = Point 3; **P4** = Point 4; **P5** = Point 5; **P6** = Point 6.

DISCUSSION

To ensure bacterial growth in culture media, previous studies have suggested different sample pretreatment approaches, including air-drying or drying soil at 120°C for 1 h, using antibiotics such as penicillin and antifungal agents, and inoculating samples into media containing inorganic salts, arginine, and glucose ⁽²⁹⁾. In the present study, given the nature of the Antarctic soil samples, heat treatment was not applied to avoid disrupting the growth of native microorganisms adapted to extreme cold conditions. However, nystatin was incorporated into all culture media to inhibit fungal growth during the 6-week incubation period used for the isolation of Antarctic microorganisms ⁽³⁰⁾. For the initial isolation of native microorganisms, general culture media containing essential compounds were used, including arginine in arginine–glycerol agar and dextrose as a glucose source in yeast extract–malt agar. Both media successfully supported bacterial isolation from Antarctic soils, with relatively similar numbers of groups of isolates recovered on each medium (**Figure 2**). Considerable bacterial morphological diversity was observed among the different sampling points on both arginine–glycerol agar and yeast extract–malt agar (**Figure 3-5**). Therefore, both media supported the recovery of bacterial groups under the conditions evaluated; however, equal effectiveness of the two media cannot be concluded without a specific statistical comparison.

The mixed groups of bacterial isolates evaluated in this study showed positive growth responses on hydrocarbon-supplemented media, providing preliminary screening evidence of microorganisms that could be of interest for subsequent studies of hydrocarbon biodegradation. However, growth under these conditions does not, by itself, demonstrate hydrocarbon consumption, transformation, or mineralization. The groups of isolates that grew in the presence of hydrocarbons showed an apparent relationship with sampling location, with Point 5 exhibiting the strongest growth response and being located near the Antarctic research station (**Figure 1**). Nevertheless, hydrocarbon concentrations in the sampled soils were not measured; therefore, proximity to the station cannot be established as the cause of bacterial adaptation or of the observed growth response. In this context, the penalized logistic regression analysis showed that Point 5 was associated with a higher probability of bacterial growth (**Table 4**), whereas diesel and toluene were also significantly associated with a higher probability of positive growth in the screening assay (p value < 0.05). One possible explanation for this observation could be previous exposure to hydrocarbon-contaminated soils or organic-enriched sediments, although this hypothesis was not evaluated in the present study. A study conducted on King George Island found that alkane-degrading bacteria were more prevalent in hydrocarbon-contaminated soils than in pristine areas ⁽³¹⁾.

Sampling Points 4 and 6, which were also located near the scientific station, exhibited growth in the presence of hydrocarbons, although the corresponding associations were not statistically significant. In contrast, Points 1, 2, and 3, located farther from the station in areas with lower human activity northwest of Keller Peninsula (**Figure 1**), exhibited bacterial growth in the presence of at least one hydrocarbon, but showed lower overall growth frequencies than Points 4 and 5; accordingly, the statistical results were not significant (**Table 3**). Thus, the spatial patterns observed in this study should be interpreted as a possible association that requires confirmation through direct measurements of hydrocarbon contamination at the sampling sites.

Antarctic research stations are sites exposed to potential fuel spills and other human activities, and previous exposure to hydrocarbons may impose selective pressures on surrounding microbial communities. However, such adaptation was not directly assessed in the present study. This premise is further supported by a previous study ⁽³²⁾, in which *Pseudomonas* and *Sphingomonas* species were isolated directly from contaminated soils surrounding Scott Base, Antarctica, and were shown to degrade various aromatic hydrocarbons, including toluene and naphthalene, as well as jet fuel. Considering the similar environmental context, proximity to a scientific station could potentially be associated with microbial communities previously exposed to hydrocarbons, but this relationship cannot be confirmed in the

present study because contamination levels and mechanisms of microbial adaptation were not measured ⁽³³⁾. Nevertheless, although the presence of hydrocarbons in soil can reduce overall microbial diversity, it can also promote the growth of bacteria with specific capacities to degrade these compounds ⁽³⁾. In this context, the bacterial culture recovered from Point 5 after initial isolation on yeast extract–malt agar showed positive growth on media supplemented with all six hydrocarbons tested: benzene, toluene, n-hexane, cyclohexane, dichloromethane, and diesel. This response identifies Point 5 as particularly interesting for subsequent purification and characterization studies but should not be considered evidence of confirmed hydrocarbon degradation.

The absence of growth observed with the filter-paper method may be related to the limited availability of volatile hydrocarbon compounds at 9°C. Under the conditions evaluated, this result suggests that the method may be unsuitable for low-temperature incubation studies. Regarding the hydrocarbons evaluated, positive growth responses differed among compounds (**Table 3**). These differences may be influenced by the structural and physicochemical characteristics of each hydrocarbon, including bioavailability and toxicity, as well as by the physiological characteristics of the microorganisms. These mechanisms were not experimentally evaluated in this study and are therefore discussed only as possible explanations based on previous literature. For instance, previous studies have described monooxygenase-mediated pathways involved in alkane degradation by hydrocarbon-degrading bacteria ⁽³⁴⁾.

Aromatic hydrocarbons such as benzene and toluene may require different enzymatic pathways because of their aromatic ring structure. Previous studies have reported bacterial degradation of toluene through monooxygenase- and dioxygenase-dependent pathways ⁽³⁵⁾. Similarly, studies of microorganisms capable of utilizing dichloromethane have demonstrated specific metabolic adaptations to this substrate ⁽³⁶⁾. Therefore, the different growth responses observed among hydrocarbons in the present study may be consistent with differences in substrate properties and microbial physiology, but the underlying degradation pathways cannot be established from the qualitative growth assay performed here.

On the other hand, hydrocarbons such as toluene and diesel supported bacterial growth from multiple sampling points. Growth on media supplemented with these hydrocarbons, regardless of geographical differences among sampling points, could be influenced by substrate characteristics or previous environmental exposure; however, neither hydrocarbon availability in the sampled soils nor degradation activity was measured in this study. Soils on King George Island have been reported to contain aromatic hydrocarbons and to be continuously exposed to diesel contamination ⁽⁶⁾. According to the results of the present study, diesel supplementation was significantly associated

with positive bacterial growth (p value < 0.05), indicating that this substrate produced the most consistent growth response under the conditions evaluated. This finding should not, however, be interpreted as direct evidence that diesel was consumed or degraded. After diesel, toluene was also significantly associated with bacterial growth (**Table 3**), indicating a positive growth response in its presence.

A study conducted outside Antarctica, along the Mediterranean coast, evaluated the growth of actinomycetes and their hydrocarbon degradation capacity using media supplemented with mixed fuel, benzene, toluene, diesel, dichloromethane, n-hexane, n-octane, n-dodecane, n-pentadecane, and n-hexadecane. Positive growth was observed on six of the ten supplemented media: mixed fuel, diesel, n-octane, n-dodecane, n-pentadecane, and n-hexadecane⁽²⁸⁾. These findings provide a useful comparison with the broad positive growth response observed for Point 5, although degradation was not quantified in the present experiment. In another study conducted on two Antarctic islands, Deception Island and Livingston Island, the majority of bacterial groups possessing hydrocarbon-degrading genes belonged to *Proteobacteria* and *Actinobacteria* and exhibited catabolic capacities related to compounds such as toluene, xylene, and both saturated and unsaturated hydrocarbons⁽³⁷⁾. These previous studies demonstrate that Antarctic microbial communities can contain bacteria with hydrocarbon-degradation pathways. In the present study, however, the results should be interpreted as preliminary qualitative screening evidence of growth on hydrocarbon-supplemented media at 9°C.

Future studies should focus on the purification and taxonomic identification of the bacterial isolates, including molecular identification by 16S rRNA gene sequencing. The most promising cultures, particularly those showing positive growth in the presence of diesel and toluene and those recovered from Point 5, should subsequently be evaluated for the presence of genes associated with hydrocarbon degradation and subjected to quantitative degradation assays capable of measuring substrate removal or transformation. Growth profiling across different temperatures would also be necessary to determine their psychrophilic characteristics and establish the temperature conditions under which hydrocarbon degradation could potentially occur.

CONCLUSIONS

In this study, 34 morphologically distinct mixed bacterial groups were identified during preliminary screening of Antarctic soils using arginine–glycerol agar and yeast extract–malt agar under low-temperature incubation conditions (9°C). Direct growth assays on hydrocarbon-supplemented Bushnell–Haas medium revealed positive growth

responses across soil samples, most notably among those associated with sites of human activity, with growth observed most frequently in the presence of diesel (100%) and toluene (50%). Although these qualitative observations indicate bacterial tolerance and potential hydrocarbon utilization at low temperatures, they do not confirm active biodegradation. To definitively evaluate the bioremediation potential of these bacteria in extreme environments, future investigations should include bacterial isolation and purification, molecular identification, temperature-dependent growth profiling, and quantitative hydrocarbon degradation assays.

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ETHICAL CONSIDERATIONS

This study has the necessary permits for collecting Antarctic scientific samples within the Antarctic Program of the Republic of Ecuador.

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DECLARATION OF COMPETING INTEREST

The authors declare that they are not aware of any financial conflicts of interest or personal relationships that could have influenced the work presented in this article.

AUTHOR CONTRIBUTIONS

Silva-Cueva J: Software, Formal analysis, Investigation, Data curation, Writing – original draft preparation, and experimental execution (sample processing, initial microbial isolation, hydrocarbon growth assays). **Montero-**

Calderón A: Conceptualization, Methodology, Writing – review & editing, and experimental execution (sample processing, initial microbial isolation, hydrocarbon growth assays). **Pérez-Cárdenas M:** Visualization, Supervision. **Navas-León D :** Conceptualization, Methodology, Validation, and field experimental activities (soil sample collection during the Antarctic expedition). **Rodríguez H:** Visualization, Funding acquisition. **Traverso-Ponce A:** Validation, Resources. **Pazmiño-Arias E:** Conceptualization, Visualization, Supervision, Project administration, Funding acquisition, and field experimental activities (soil sample collection during the Antarctic expedition).

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