Pseudomonas strains from the Livingston Island, Antarctica: a source of cold-active hydrolytic enzymes

Carolina Rubiano-Labrador
Facultad de Ciencias Básicas
Universidad Tecnológica de Bolívar
Cartagenas de Indias, Colombia
drubiano@utb.edu.co

Rosa Acevedo-Barrios
Facultad de Ciencias Básicas
Universidad Tecnológica de Bolívar
Cartagenas de Indias, Colombia
racevedo@utb.edu.co

Alba García Lazaro
Facultad de Ingenieria
Universidad Tecnológica de Bolívar
Cartagenas de Indias, Colombia
alazaro@utb.edu.co

Lilia Ward Bowie
Facultad de Ingenieria
Universidad Tecnológica de Bolívar
Cartagenas de Indias, Colombia
lward@utb.edu.co

Ana Karina Támara Acosta
Facultad de Ingeniería
Universidad Tecnológica de Bolívar
Cartagenas de Indias, Colombia
atamara@utb.edu.co

Blanca Mercado Molina
Facultad de Ingenieria
Universidad Tecnológica de Bolívar
Cartagenas de Indias, Colombia
bmercado@utb.edu.co

***Abstract* - *Pseudomonas* spp. is considered one of the most successful bacterial genera due to its plasticity and metabolic versatility, which has allowed it to colonize different ecosystems, including Antarctica. The ability of *Pseudomonas* to adapt and survive in the hostile conditions of the Antarctic makes them a reservoir of enzymes that can be used in different biotechnological applications; however, research on this genus in Antarctica is still in its infancy. Therefore, the aim of this study was to isolate and characterise cold-adapted *Pseudomonas* from Livingston Island, Antarctica, and explore their ability to produce cold-active hydrolytic enzymes. In the present study, we isolated seven cold-adapted bacteria related to the genus *Pseudomonas*. The isolated strains have the ability to produce hydrolytic enzymes. These results demonstrate that cold-adapted *Pseudomonas* from Antarctica are a promising source of cold-active enzymes with biotechnological potential.**

Keywords*-- *amylase, cellulose, extracellular enzymes, polar environments, protease.

I. INTRODUCTION

Pseudomonas spp. is a diverse and complex bacterial genus that includes more than 200 recognized species [1]. The species of this genus are characterized by their metabolic versatility, low nutritional requirements, rapid growth, and ability to adapt to fluctuating environmental conditions, which has allowed them to successfully colonize different ecosystems consisting of water and soil, as well as plants, animals, and human [2]. Based on these characteristics, *Pseudomonas* spp. represent a microbial group with potential biotechnological applications, which include the production of novel secondary metabolites, biocontrol, bioremediation, and plant growth promotion [3].

Digital Object Identifier (DOI):
http://dx.doi.org/10.18687/LACCEI2022.1.1.713
ISBN: 978-628-95207-0-5 **ISSN:** 2414-6390

Bacteria belonging to the genus *Pseudomonas* have been found in Antarctica [4], which is among the coldest environments on the planet due to its geographical isolation and extreme environmental conditions (low temperature, low humidity, high incidence of solar and UV radiation, and limited organic nutrients and water) [5]. Cold-adapted *Pseudomonas* spp. have developed different adaptation strategies to the extreme conditions of Antarctica which include regulation of membrane fluidity, decreased rates of transcription, translation and cell division, reduced enzyme activity, anti-freeze proteins, carotenoid pigments, cryoprotectants, cold-shock proteins and cold active enzymes [6].

The adaptability of these bacteria to the extreme conditions of the Antarctic makes them a reservoir of enzymes that can be used in different biotechnological applications. Cold-active enzymes are characterized by high catalytic efficiency at low temperatures compared to the enzymes produced by mesophilic microorganisms [7]. In addition, these enzymes display high flexibility in their structures, providing better access to the active site of substrates at low temperatures, as well as a high degree of thermolability, the ability to function in organic solvents and lower energy of activation [8]. The use of these enzymes can reduce the cost of consumption, minimize undesirable chemical reactions, reduce the risk of contamination, and allow for easy inactivation [9]. These characteristics are desirable in different industrial processes, and therefore, in recent years, the search for cold active hydrolytic enzymes has intensified. This group of enzymes includes proteases, lipases, amylases and cellulases, which have various applications in chemical synthesis, food processing, detergents, paper/pulp and textile industry, agriculture, mining, biofuels and energy production, waste

processing, bioremediation, and medicine and molecular diagnostics [10].

Despite the metabolic potential of cold-adapted *Pseudomonas* spp., little is known about the biotechnological potential of their cold-active enzymes. Therefore, the aim of this study was to identify cold-adapted *Pseudomonas* from sediments of Livingston Island, Antarctica, and investigate their potential to produce cold-active hydrolytic enzymes.

II. Material and Methods

A. Sampling

This study was conducted in two geo-referenced areas on Livingston Island, South Shetland Islands, Antarctica: Spanish Antarctic research station Juan Carlos I and Byers Peninsula (Byers Camp). Marine sediment samples (0–10 cm depth) were collected from Byers Camp and Spanish base Juan Carlos I in February 2018 during the 4th Colombian Scientific Expedition to Antarctica (2017–2018) (Fig. 1). Approximately 50 g of each sample was taken using sterile spatulas, placed into sterile Falcon tubes and, frozen at -20°C until further processing in the laboratory.

B. Isolation of bacteria producing cold-active enzymes

For the isolation, 5 g of each sample was suspended in 45 mL of saline solution 0.85% (w/v) and agitated for 2 h. Subsequently, 1 mL of the homogenized sample was inoculated into tubes containing 5 mL of minimum basic sales medium (MBS) supplemented with different carbon sources [11]. The carbon sources evaluated in this study included: starch (10 g·L^{-1}), gelatin (10 g·L^{-1}), casein (10 g·L^{-1}), bovine serum albumin (BSA) (10 g·L^{-1}), cellulose (10 g·L^{-1}), Tween 80 (2 mL·L^{-1}), and olive oil (2 mL·L^{-1}) [12]. Cultures that showed growth were transferred to plates with MBS agar medium and incubated at 4°C for two weeks. Colonies with different morphologies were selected and repeatedly isolated until pure culture were obtained.

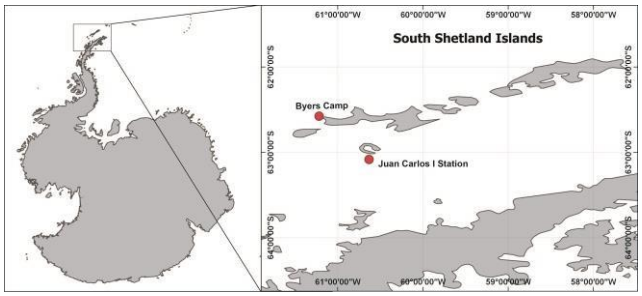

Fig 1: Geographic location of the sampling sites evaluated in this study

C. Physiological characterisation of bacterial isolates

Morphology and motility were determined using an optical microscope (Nikon E100). Gram-staining, catalase, and peroxidase activity assays were performed according to the protocol described by [12]. The biochemical profile was determined with BBL Crystal™ Kit. To determine the psychrophilic or psychrotolerant characteristics of the isolated bacteria cultures were set up in MBS medium and incubated for two weeks at 27°C [13]. The growth was measured directly by reading the optical density at 580 nm in the spectrophotometer. The test was performed in duplicate. The strains that showed growth at 27 °C after the incubation period were considered psychrotolerant.

D. Hydrolytic enzyme activity

The isolated bacteria were screened for the production of amylase, protease, cellulase and lipase. The qualitative detection of these extracellular hydrolytic enzymes was tested on MBS agar supplemented with a specific substrate. An aliquot (10 µL) of each isolate was spotted onto appropriate media and tested at 4 °C for two weeks; assays were performed in duplicate. Clearing zones around the colonies were used as an indication of positive enzyme activity (diameter of the zone of clearance) [14]. Amylase activity was evaluated on starch plates following the protocol described by [15]. Protease activity was determined on skim milk plates and lipase activity was screened on Tween 80 and olive oil plates according to the protocol described by [14]. Cellulase activity was tested on carboxymethylcellulose plates following the protocol described by [16].

III. Results

A. Isolation and characterisation of bacteria producing cold-active enzymes

In this study, seven heterotrophic, aerobic cold-adapted bacteria were isolated from marine sediments sampled from Livingston Island, Antarctica (Table 1). Most of the isolates (71%) were obtained from the culture media that contained protein-based substrates as a carbon source (casein, gelatin or BSA), which might be because of the characteristics of the natural habitat of the isolates. The different morphologies of the colonies were evident upon visual observation. Particularly, the UTB 118 isolate presented an orange pigmentation, which is probably related to a survival mechanism of this bacterium [17]. All isolates were gram negative, oxidase positive and non-sporoforming. After two weeks of incubation, three of the strains (UTB 108, UTB 115, and UTB 118) showed growth at 27°C, indicating that they were psychrotolerant, while the strains UTB 109, UTB 110. UTB 111, and UTB 117 did not show growth, suggesting that they were psychrophilic.

We observed different biochemical profiles in the isolated strains, which indicate metabolic versatility amongst them. On the other hand, all the isolated strains, except for the UTB 109 strain, degraded urea. Furthermore, the biochemical profiles were useful for the preliminary detection of hydrolytic enzyme activity, as we identified that strains UTB 111, UTB 115, and UTB 118 produced β-galactosidase, while strains UTB 108, UTB 115, and UTB 118 produced β-glucosidase.

Based on the analysis of the 16S rRNA gene, we identified that the isolated strains belonged to the Gammaproteobacteria subclass and were related to the genus

TABLE I
PSYCHROPHILIC AND PSYCHROTOLERANT STRAINS ISOLATED FROM LIVINGSTON ISLAND, ANTARCTICA

Sample point	Strain	Closest relative (% 16S rRNA sequence similarity)	Relation to temperature
Byers Peninsula	UTB 108	*Pseudomonas fluorescens* (100)	Psychrotolerant
	UTB 109	*Pseudomonas antarctica* (100)	Psychrophilic
	UTB 110	*Pseudomonas fluorescens* (99)	Psychrophilic
	UTB 111	*Pseudomonas fluorescens* (99)	Psychrophilic
Station Juan Carlos I	UTB 115	*Pseudomonas mandelii* (99.28)	Psychrotolerant
	UTB 117	*Pseudomonas mandelii* (98.28)	Psychrophilic
	UTB 118	*Pseudomonas mandelii* (99.35)	Psychrotolerant

Pseudomonas. All the sequences obtained shared a high similarity (98-100%) to their nearest-neighbour sequences deposited in database. Most of the strains isolated from Byers Peninsula were related to the species *Pseudomonas fluorescens*, followed by *P. antarctica*, while all the isolates from Station Juan Carlos I were related to the species *P. mandelii* (Table 1).

B. *Isolation of bacteria producing cold-active enzymes*

Hydrolytic enzymatic activities were determined by observing for substrate degradation or formation of hydrolysis halos around colonies, after growing at 4°C. The hydrolytic enzymes produced by the isolated strains are presented in Table 2. Distribution of enzymatic activity of the isolate strains, according to the isolation site, is shown in Fig. 2. All the isolates presented hydrolytic enzymatic activity. Urease degradation was detected in all the isolates, except in strain UTB 109. Proteolytic activity was also frequently detected, represented by the five isolates related to *P. fluorescens* (UTB 108 and UTB 111 isolated from Byers Peninsula) and *P. mandelii* (UTB 115, UTB 117, and UTB 118 isolated from Juan Carlos I Station). These strains exhibited hydrolysis halos that ranged from 3 to 5.5 mm in diameter. Hydrolysis of starch was detected in strains UTB 108, UTB 110 and UTB 111 (related to *P. fluorescens*); UTB 109 (related to *P. antarctica*); UTB 115 and UTB 118 (related to *P. mandelii*); which showed hydrolysis halos ranging between 1 to 4 mm in diameter.

The production of β-galactosidase and β-glucosidase was detected in the strains UTB 108, UTB 111, UTB 115, and UTB 118, respectively. Cellulolytic activity was detected less frequently than the other hydrolytic activities. These strains were isolated from the Byers Peninsula and presented a hydrolysis halo between 1-1.5 cm in diameter. No hydrolysis was observed on media containing Tween 80 and olive oil. Remarkably, most of the isolated strains showed multiple

TABLE II
COLD-ACTIVE HYDROLYTIC ENZYMES PRODUCED BY STRAINS ISOLATED FROM LIVINGSTON ISLAND, ANTARCTICA

Sample point	Strain	Taxonomic affiliation	Hydrolysed substrates
Byers Peninsula	UTB 108	*Pseudomonas fluorescens*	Starch, Skin Milk , Celullose, β-glucosidase, Urease
	UTB 109	*Pseudomonas antarctica*	Starch and Cellulose
	UTB 110	*Pseudomonas fluorescens*	Urea
	UTB 111	*Pseudomonas fluorescens*	Starch, Skin Milk, β-galactosidase, Urease
Station Juan Carlos I	UTB 115	*Pseudomonas mandelii*	Skin Milk, Urease, β-galactosidase, β-glucosidase
	UTB 117	*Pseudomonas mandelii*	Skin Milk, Urease
	UTB 118	*Pseudomonas mandelii*	Starch, Skin Milk, Urea, β-galactosidase, β-glucosidase

hydrolytic enzyme activities; strains UTB 108, UTB 111, and UTB 118 hydrolyzed five of the evaluated substrates, and strain UTB 115 produced four of the tested enzymes, while strains UTB 109 and UTB 117 tested positive for two of them.

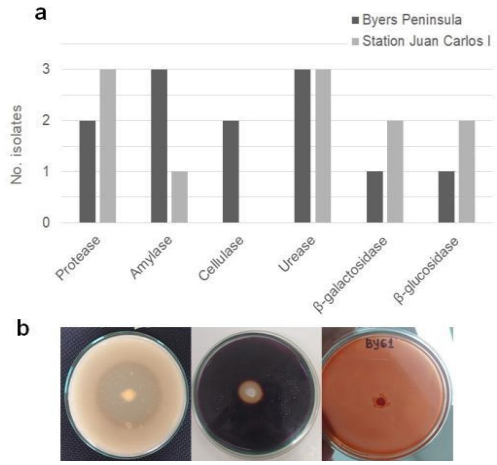

Fig 2: Evaluation of the hydrolytic enzymatic activity of the isolated strains. **(a)** Distribution of the hydrolytic enzymatic activity of the isolated strains according to the sampling site. **(b)** Hydrolysis halo of the extracellular activities observed

IV. DISCUSSION

In our work, seven hydrolytic enzyme-producing strains were isolated from the sediments of Byers Peninsula and the Juan Carlos I Station, which suggests that the habitat of these strains probably has a reservoir of organic matter that stimulates the production of extracellular enzymes [18]. In addition, it also demonstrates the versatility of the bacterial communities present in the Livingston Island sediments in

using high molecular weight compounds, which can be a limiting step in the recycling of organic matter in the Antarctic [4]. On the other hand, 57% of the isolates were psychrophilic, which suggests that these isolates are polar microorganisms and are well adapted to low temperatures [15]; 43% of the isolates were psychrotolerant, which can be considered an advantageous survival strategy in polar ecosystems [19].

The cold-adapted bacteria isolated from Livingston Island were taxonomically characterized based on analysis of 16S rRNA gene. Results showed that all the isolates were assigned to the genus *Pseudomonas*, which coincides with previous studies that have reported an abundance of this genus in sediments and soils from cold environments [20]-[22] and [11]. Different studies have shown that *Pseudomonas* spp. is the dominant genus among Arctic and Antarctic cultivable bacteria [23], owing to its metabolic versatility, low nutritional requirements, and ability to resist extreme environmental conditions [9] y [20]. These characteristics allow the species of this genus to colonize different ecological niches, as they have an r-strategy that allows them to grow rapidly on enriched media and successfully compete in heterotrophic conditions [24]. In addition, the abundance of this genus in the sediments of Livingston Island may suggest that it plays a key role within its ecosystem, as this genus produces extracellular enzymes that may be involved in the degradation of organic matter [4].

The strains isolated from Byers Peninsula were related to *P. fluorescens* and *P. antarctica*. These results are consistent with the data reported by [24] and [25], where a dominance of the genus *Pseudomonas* was observed in marine and lacustrine sediments of Byers Peninsula. *P. fluorescens* is an obligate aerobe, although some strains can use nitrate as the final electron acceptor during cellular respiration. It has an extremely versatile metabolism and can be found in soil and water [26]. The presence of *P. fluorescens* has been previously reported in seawaters [27], marine sediments [22] and [28], soils [29-30], and freshwaters [22] from the Antarctic, as well as in other cold environments around the world [19-20] and [31]. *P. antarctica* is a gram-negative, motile, rod-shaped, and psychrophilic [32]. This bacterium has previously been isolated from samples of freshwater [27] and [32], glacier sediments [17], and deep-sea sediments [28] from King George Island, Antarctica.

On the other hand, species isolated from Juan Carlos I Station were related to *P. mandelii*. *P. mandelii* is a psychrotolerant bacterium characterized by its metabolic versatility, plasticity, and biotechnological potential [33]. This bacterium was first isolated from mineral water in France [34] and it was also identified in mineral water samples from South Korea, and soil samples from China, United States, and Canada [33]. Previous studies have reported the isolation of *P. mandelii* from Antarctic soils in King George Island [22] and Deception Island [20].

In this study, all the isolates showed hydrolytic enzyme activity at low temperatures, which demonstrates the enzymatic versatility of the genus *Pseudomonas*. Also, this suggests that these bacteria probably contribute to the hydrolysis of polymeric compounds (esters, proteins, α- and β-linked polysaccharides) present in the Antarctic sediments, and therefore, are involved in the cycling of organic matter in this ecosystem [18]. Our results coincide with the results of studies by [7], [35], and [18], which reported the presence of bacteria that produce hydrolytic enzymes in sediments present in South Shetland Islands; this demonstrates the potential of this location as a source of biotechnological products, such as extracellular hydrolytic enzymes. On the other hand, we found that most of the strains isolated in this study are producers of multiple enzymes, which may be an adaptation strategy of the strains to the changing conditions of availability of substrates in their ecosystem, allowing them to take advantage of a broad spectrum of substrates for growth [18].

Among the enzymes evaluated in this study, most of the isolates were able to produce proteases. Cold-active proteases have been previously reported in psychrophilic and psychrotolerant bacteria of the genus *Pseudomonas* isolated from Antarctica [4], [15] and [35]. Remarkably, proteolytic activity has been the most frequently detected enzyme activity in marine sediments of cold environments, as it possibly has an important function in the organic matter cycle and in the regulation of algal blooms [7], [15] and [36]. Another important group of hydrolytic enzymes detected in this study was amylases. These results are in agreement with results of previous studies, which report the isolation of cold-adapted *Pseudomonas* with the ability to hydrolyze starch from Antarctic sediments [4], [15], [28] and [35]. Notably, strain UTB 109 related to *P. antarctica* displayed high amilolytic activity, although [32] reported their inability to hydrolyze starch. Most isolated bacteria were ureolytic, which is a typical characteristic of the genus *Pseudomonas* [22]; however, there have been only a few studies on cold-active ureases and their possible applications. The production of β-galactosidase, β-glucosidase and cellulases in the isolated strains was detected in a smaller proportion. Various studies have detected the production of β-galactosidase and β-glucosidase by psychrophilic and psychrotolerant bacteria of the genus *Pseudomonas* [32] and [37], but little has been explored about their biotechnological potential. In terms of cellulolytic activity, it was only detected in the isolates from Byers Peninsula, which is in agreement with the study by [25], that reported a high cellulose degradative capacity of the microbial communities in Byers sediments. This could be due to the high availability of organic matter in these sediments. In recent years, the search for psychrophilic and psychrotolerant enzymes has intensified: however, there are only a few reports on cold-active cellulases. [35] reported cellulolytic activity at different sites in the Antarctic, while [38] demonstrated the presence of cellulolytic bacteria in Antarctic soils. However, this is the first report of cold-active cellulases from *Pseudomonas* sp.

The enzymes produced by the psychrophilic and psychrotolerant bacteria isolated in this study display great potential for various biotechnological applications due to their high specific activity at low and moderate temperatures and greater structural flexibility [6]. Cold-active proteases are widely used in various industrial applications, including food manufacture for peptide synthesis [39], detergent production as an additive [40], in the textile industry to improve the production and finish of fabrics [40], and in the pharmaceutical industry for the treatment of cardiovascular diseases and digestive disorders [6]. Cold-active amylases have especially been used in the fermentation process for the production of beer and wine [41], in the detergent industry as an additive, and in the textile industry for the desizing of woven [6]. β-glucosidase from cold-adapted bacteria has been applied to produce biofuels via bioconversion of lignocellulosic materials [42]. Cold-active β-galactosidase has been used in the food industry to eliminate lactose from dairy products, in order to improve the digestibility of these products for the lactose intolerant population [6]. Urease from psychrophilic or psychrotolerant bacteria have not been extensively studied, but different industrial applications have been reported for these enzymes which include usage in diagnostic kits for measuring urea, production of alcoholic beverages [43], bioremediation of heavy metals [44], and biocementation [45]. Finally, the use of cellulases has been reported in the detergent industry to remove cellulose microfibrils from cotton-based cloth and as colour brightening and softening agents [46].

V. Conclusions

The present study allowed the isolation of seven cold-adapted *Pseudomonas* strains with hydrolytic enzyme activity, demonstrating the metabolic versatility of this genus and its ability to survive in the extreme conditions of Antarctica. Furthermore, our results showed that the Livingston Island sediments are a promising source in the search for cold-active enzymes with biotechnological potential. Future enzymatic characterisation studies are required to determine the potential of the enzymes produced by the strains isolated during this study.

Acknowledgment

This work was supported by a grant from Universidad Tecnológica de Bolívar. Logistical support for displacement and sampling at the different points evaluated in Antarctica was provided by the Colombian Ocean Commission (CCO) coordinator of the Colombian Antarctic Program - PAC, Spanish Polar Committee - CPE, the Spain Navy, and the BIO Hespérides Oceanographic Research Vessel (A-33). The authors also thank Sandra Baena of the Pontificia Universidad Javeriana and Carolina Díaz Cardenas for their support in the molecular analysis of the isolated strains.

References

[1] J. Lalucat, M. Mulet, M. Gomila, and E. García-Valdés, "Genomics in Bacterial Taxonomy: Impact on the Genus Pseudomonas," *Genes*, vol 11, no. 2, pp. 139, 2020.

[2] S.-R. Jun et al., "Diversity of *Pseudomonas* genomes, including populus-associated isolates, as revealed by comparative genome analysis", *Appl. Environ. Microbiol.*, vol. 82, no. 1, pp. 375–383, 2015.

[3] M. W. Silby, C. Winstanley, S. A. C. Godfrey, S. B. Levy, y R. W. Jackson, "*Pseudomonas* genomes: diverse and adaptable", FEMS Microbiol. Rev., vol. 35, no. 4, pp. 652–680, 2011.

[4] S. C. Vazquez, S. H. Coria, y W. P. MacCormack, "Extracellular proteases from eight psychrotolerant Antarctic strains", *Microbiol. Res.*, vol. 159, no. 2, pp. 157–166, 2004.

[5] S. Lambrechts, A. Willems, y G. Tahon, "Uncovering the uncultivated majority in Antarctic soils: Toward a synergistic approach", *Front. Microbiol.*, vol. 10, p. 242, 2019.

[6] S. Bruno, D. Coppola, G. di Prisco, D. Giordano, y C. Verde, "Enzymes from marine polar regions and their biotechnological applications", *Mar. Drugs*, vol. 17, no. 10, p. 544, 2019.

[7] C. Lamilla, M. Pavez, A. Santos, A. Hermosilla, V. Llanquinao, y L. Barrientos, "Bioprospecting for extracellular enzymes from culturable Actinobacteria from the South Shetland Islands, Antarctica", *Polar Biol.*, vol. 40, no. 3, pp. 719–726, 2017.

[8] J. Maiangwa, M. S. M. Ali, A. B. Salleh, R. N. Z. R. A. Rahman, F. M. Shariff, y T. C. Leow, "Adaptational properties and applications of cold-active lipases from psychrophilic bacteria", *Extremophiles*, vol. 19, núm. 2, pp. 235–247, 2015.

[9] J. K. Vester, M. A. Glaring, y P. Stougaard, "Improved cultivation and metagenomics as new tools for bioprospecting in cold environments", *Extremophiles*, vol. 19, no. 1, pp. 17–29, 2015.

[10] S. Thapa *et al.*, "Biochemical characteristics of microbial enzymes and their significance from industrial perspectives", *Mol. Biotechnol.*, vol. 61, no. 8, pp. 579–601, 2019.

[11] S. Venkatachalam, V. Gowdaman, y S. R. Prabagaran, "Culturable and culture-independent bacterial diversity and the prevalence of cold-adapted enzymes from the Himalayan mountain ranges of India and Nepal", *Microb. Ecol.*, vol. 69, no. 3, pp. 472–491, 2015.

[12] C. Rubiano-Labrador, C. Díaz-Cárdenas, G. López, J. Gómez, y S. Baena, "Colombian Andean thermal springs: reservoir of thermophilic anaerobic bacteria producing hydrolytic enzymes", *Extremophiles*, vol. 23, no. 6, pp. 793–808, 2019

[13]L. Selbmann, L. Zucconi, S. Ruisi, M. Grube, M. Cardinale, y S. Onofri, "Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance", *Polar Biol.*, vol. 33, no. 1, pp. 71–83, 2010.

[14]T. Menasria *et al.*, "Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes", *Microbiol. Res.*, vol. 207, pp. 289–298, 2018.

[15]Y. Yu, H.-R. Li, Y.-X. Zeng, y B. Chen, "Bacterial diversity and bioprospecting for cold-active hydrolytic enzymes from culturable bacteria associated with sediment from Nella Fjord, Eastern Antarctica", *Mar. Drugs*, vol. 9, no. 2, pp. 184–195, 2011.

[16]M. E. Danilovich, L. A. Sánchez, F. Acosta, y O. D. Delgado, "Antarctic bioprospecting: in pursuit of microorganisms producing new antimicrobials and enzymes", *Polar Biol.*, vol. 41, no. 7, pp. 1417–1433, 2018.

[17]S. A. García-Echauri, M. Gidekel, A. Gutiérrez-Moraga, L. Santos, y A. De León-Rodríguez, "Isolation and phylogenetic classification of culturable psychrophilic prokaryotes from the Collins glacier in the Antarctica", *Folia Microbiol. (Praha)*, vol. 56, no. 3, pp. 209–214, 2011.

[18]M. Tropeano *et al.*, "Culturable heterotrophic bacteria from Potter Cove, Antarctica, and their hydrolytic enzymes production", *Polar Res.*, vol. 31, no. 1, p. 18507, 2012.

[19]T. Groudieva *et al.*, "Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen", *Extremophiles*, vol. 8, no. 6, pp. 475–488, 2004.

[20]M. Orellana-Saez *et al.*, "In-depth genomic and phenotypic characterization of the Antarctic psychrotolerant strain Pseudomonas sp. MPC6 reveals unique metabolic features, plasticity, and biotechnological potential", *Front. Microbiol.*, vol. 10, p. 1154, 2019.

[21]T. R. Silva *et al.*, "Bacteria from Antarctic environments: diversity and detection of antimicrobial, antiproliferative, and antiparasitic activities", *Polar Biol.*, vol. 41, no. 7, pp. 1505–1519, 2018.

[22]F. Vásquez-Ponce, S. Higuera-Llantén, M. S. Pavlov, S. H. Marshall, y J. Olivares-Pacheco, "Phylogenetic MLSA and phenotypic analysis identification of three probable novel Pseudomonas species isolated on King George Island, South Shetland, Antarctica", *Braz. J. Microbiol.*, vol. 49, no. 4, pp. 695–702, 2018.

[23]Romaniuk et al., "Diversity and Horizontal Transfer of Antarctic Pseudomonas spp. Plasmids", Genes (Basel), vol. 10, no. 11, p. 850, 2019.

[24]M. Papale et al., "Prokaryotic assemblages in the maritime Antarctic Lake Limnopolar (Byers Peninsula, South Shetland Islands)", Extremophiles, vol. 21, no. 6, pp. 947–961, 2017.

[25]C. Gugliandolo et al., "Prokaryotic community in lacustrine sediments of Byers Peninsula (Livingston Island, Maritime Antarctica)", Microb. Ecol., vol. 71, no. 2, pp. 387–400, 2016.

[26]I. Dickinson, W. Goodall-Copestake, M. A. S. Thorne, T. Schlitt, M. L. Ávila-Jiménez, y D. A. Pearce, "Extremophiles in an antarctic marine ecosystem", Microorganisms, vol. 4, no. 1, p. 8, 2016.

[27]S. Ciesielski, D. Górniak, J. Możejko, A. Świątecki, J. Grzesiak, y M. Zdanowski, "The diversity of bacteria isolated from antarctic freshwater reservoirs possessing the ability to produce polyhydroxyalkanoates", *Curr. Microbiol.*, vol. 69, no. 5, pp. 594–603, 2014.

[28]J. W. Zhang y R. Y. Zeng, "Psychrotrophic amylolytic bacteria from deep sea sediment of Prydz Bay, Antarctic: diversity and characterization of amylases", *World J. Microbiol. Biotechnol.*, vol. 23, no. 11, pp. 1551–1557, 2007.

[29]H. Koo, M. K. Basu, M. Crowley, J. Aislabie, y A. K. Bej, "Draft genome sequence of Pseudomonas sp. Strain Ant30-3, a psychrotolerant bacterium with biodegradative attribute isolated from Antarctica", *Genome Announc.*, vol. 2, no. 3, 2014.

[30]N. Pacheco *et al.*, "Exploiting the natural poly(3-hydroxyalkanoates) production capacity of Antarctic Pseudomonas strains: from unique phenotypes to novel biopolymers", *J. Ind. Microbiol. Biotechnol.*, vol. 46, no. 8, pp. 1139–1153, 2019.

[31]I. Tapia-Vázquez et al., "Isolation and characterization of psychrophilic and psychrotolerant plant-growth promoting microorganisms from a high-altitude volcano crater in Mexico", Microbiol. Res., vol. 232, no. 126394, p. 126394, 2020.

[32]G. S. N. Reddy, G. I. Matsumoto, P. Schumann, E. Stackebrandt, y S. Shivaji, "Psychrophilic pseudomonads from Antarctica: *Pseudomonas antarctica* sp. nov., *Pseudomonas meridiana* sp. nov. and *Pseudomonas proteolytica* sp. nov", *Int. J. Syst. Evol. Microbiol.*, vol. 54, no. Pt 3, pp. 713–719, 2004.

[33]P. A. Formusa, T. Hsiang, M. B. Habash, H. Lee, y J. T. Trevors, "Genome sequence of Pseudomonas mandelii PD30", *Genome Announc.*, vol. 2, no. 4, 2014.

[34]S.-H. Jang, J. Kim, J. Kim, S. Hong, y C. Lee, "Genome sequence of cold-adapted *Pseudomonas mandelii* strain JR-1", *J. Bacteriol.*, vol. 194, no. 12, p. 3263, 2012.

[35]L. Loperena *et al.*, "Extracellular enzymes produced by microorganisms isolated from maritime Antarctica", *World J. Microbiol. Biotechnol.*, vol. 28, no. 5, pp. 2249–2256, 2012.

[36]H. A. Cristóbal, J. Benito, G. A. Lovrich, y C. M. Abate, "Phylogenentic and enzymatic characterization of psychrophilic and psychrotolerant marine bacteria belong to γ-Proteobacteria group isolated from the sub-Antarctic Beagle Channel, Argentina", *Folia Microbiol. (Praha)*, vol. 60, no. 3, pp. 183–198, 2015.

[37]N. Bozal, M. J. Montes, y E. Mercadé, "*Pseudomonas guineae* sp. nov., a novel psychrotolerant bacterium from an Antarctic environment", *Int. J. Syst. Evol. Microbiol.*, vol. 57, no. Pt 11, pp. 2609–2612, 2007.

[38]F. L. Soares Jr, I. S. Melo, A. C. F. Dias, y F. D. Andreote, "Cellulolytic bacteria from soils in harsh environments", *World J. Microbiol. Biotechnol.*, vol. 28, no. 5, pp. 2195–2203, 2012.

[39]S. Singh y B. K. Bajaj, "Potential application spectrum of microbial proteases for clean and green industrial production", *Energy Ecol. Environ.*, vol. 2, no. 6, pp. 370–386, 2017.

[40]M. Kuddus y P. W. Ramteke, "Recent developments in production and biotechnological applications of cold-active microbial proteases", *Crit. Rev. Microbiol.*, vol. 38, no. 4, pp. 330–338, 2012.

[41]S. C. B. Gopinath *et al.*, "Biotechnological processes in microbial amylase production", *Biomed Res. Int.*, vol. 2017, p. 1272193, 2017.

[42]J. Wu, A. Geng, R. Xie, H. Wang, y J. Sun, "Characterization of cold adapted and ethanol tolerant β-glucosidase from Bacillus cellulosilyticus and its application for directed hydrolysis of cellobiose to ethanol", *Int. J. Biol. Macromol.*, vol. 109, pp. 872–879, 2018.

[43]R. Rajendran *et al.*, "Extracellular urease from Arthrobacter creatinolyticus MTCC 5604: scale up, purification and its cytotoxic effect thereof", *Mol. Biol. Rep.*, vol. 46, no. 1, pp. 133–141, 2019.

[44]T. Wang *et al.*, "Isolation of urease-producing bacteria and their effects on reducing Cd and Pb accumulation in lettuce (*Lactuca sativa L.*)", *Environ Sci Pollut Res*, vol. 27, pp. 8707-8718, 2020.

[45]V. Stabnikov, J. Chu, V. Ivanov, y Y. Li, "Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand", *World J. Microbiol. Biotechnol.*, vol. 29, no. 8, pp. 1453–1460, 2013.

[46]R. C. Kasana y A. Gulati, "Cellulases from psychrophilic microorganisms: a review", *J. Basic Microbiol.*, vol. 51, no. 6, pp. 572–579, 2011.