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Perchlorate-reducing bacteria from Antarctic marine sediments

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Abstract Perchlorate is a contaminant that can persist in groundwater and soil, and is frequently detected in different ecosystems at concentrations relevant to human health. This study isolated and characterised halotolerant bacteria that can potentially perform perchlorate reduction. Bacterial microorganisms were isolated from marine sediments on Deception, Horseshoe and Half Moon Islands of Antarctica. The results of the 16S ribosomal RNA (rRNA) gene sequence analysis indicated that the isolates were phylogenetically related to *Psychrobacter cryohalolentis*, *Psychrobacter urativorans*, *Idiomarina loihiensis*, *Psychrobacter nivimaris*, *Sporosarcina aquimarina* and *Pseudomonas lactis*. The isolates grew at a sodium chloride concentration of up to 30% and a perchlorate concentration of up to 10,000 mg/L, which showed their ability to survive in saline conditions and high perchlorate concentrations. Between 21.6 and 40% of perchlorate was degraded by the isolated bacteria. *P. cryohalolentis* and *P. urativorans* degraded 30.3% and 32.6% of perchlorate, respectively. *I. loihiensis* degraded 40% of perchlorate, and *P. nivimaris*, *S. aquimarina* and *P. lactis* degraded 22%, 21.8% and 21.6% of perchlorate, respectively. *I. loihiensis* had the highest reduction in perchlorate, whereas *P. lactis* had the lowest reduction. This study is significant as it is the first finding of *P. cryohalolentis* and. *P. lactis* on the Antarctic continent. In conclusion, these bacteria isolated from marine sediments on Antarctica offer promising resources for the bioremediation of perchlorate contamination due to their ability to degrade perchlorate, showing their potential use as a biological system to reduce perchlorate in high-salinity ecosystems.

Keywords Extremophiles · Halotolerant bacteria · Psychrotolerant microorganism · Psychrophilic bacteria · Perchlorate biodegradation · Toxicity

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Introduction

Antarctica is the last pristine continent on Earth. However, human pressure in this region has increased with the growth of research, tourism and transport activities. These activities have led to the establishment of research stations and transfer systems, which represent potential sources of pollution (Marina-Montes et al., 2020; Pereira et al., 2017; Prabagaran et al., 2007; Xu et al., 2020). In addition to the direct and transient transport of pollutants, the Antarctic region experiences a variety of *in situ* chemical processes that have not yet been fully characterised (Chambers et al., 2014; Deng et al., 2020; Galbán-Malagón et al., 2019).

The Antarctic environment is affected by pollutants such as perchlorate, which can have ecotoxicological effects on the biota (Jackson et al., 2012; Jiang et al., 2020; Riddle & Chapman, 2005; Rose et al., 2012). Perchlorate is a toxic inorganic salt that affects iodide binding in the thyroid gland and acts as a potent endocrine disruptor; hence, it can negatively influence the normal development of living beings (Acevedo-Barrios et al., 2018, 2019b; Crawford et al., 2017; Eck, 2015; Jiang et al., 2013).

Perchlorate originates both naturally and anthropogenically (Brown & Gu, 2006; Isobe et al., 2013; Vega et al., 2018). This compound is used in military and firework industries for explosives and as a fertiliser. Their natural formation occurs during electrical storms and atmospheric reactions related to chlorine and ozone. Perchlorate has been detected in arid, hypersaline and cold environments, including Antarctica (Acevedo-Barrios et al., 2016, 2022; Ahn et al., 2009; Cao et al., 2019; Jackson et al., 2010, 2012; Kounaves et al., 2010; Parker, 2009) This contaminant is not efficiently reduced through physicochemical methods and the process is expensive and can generate residues that must be subsequently treated; thus, biological treatments are required for its degradation (Acevedo-Barrios & Olivero-Verbel, 2021; Kucharzyk et al., 2010; Kuppusamy et al., 2016; Sarria et al., 2019; Urbansky, 2002; Ye et al., 2012).

Biological perchlorate reduction is based on perchlorate-reducing microorganisms (PCRM), which use the enzyme chloride dismutase to reduce perchlorate to chlorate (ClO_3^-), and then to chlorite (ClO_2^-). Chlorite dismutase transforms ClO_2^- into molecular oxygen (O_2) and chloride (Cl^-) (Lv et al., 2020; Sarria et al., 2019; Vega et al., 2018). Perchlorate-reducing bacteria of the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* subclasses have been identified in different environments, including crystal clear water and Antarctic soil and lakes (Wang et al., 2020; Zhu et al., 2016). Antarctica has a high diversity of bacterial genera of various classes. These psychrotolerant and psychrophilic bacteria have been used for bioremediation because of their ability to maintain their activity under extreme Antarctic conditions (Abd-Elnaby et al., 2016).

The environmental conditions in Antarctica differ from those in other regions of the planet. Although the Antarctic climate is cold, it is not uniform. Thus, under these environmental conditions, extremophilic microorganisms have adaptive mechanisms, biochemical versatility and the ability to tolerate and reduce perchlorate. In addition, the natural origination of perchlorate has been reported in Antarctica (Jiang et al., 2013; Kounaves et al., 2010), increasing the possibility of the presence of perchlorate-reducing bacteria in this region. There has been interest in isolating perchlorate-reducing bacteria from halophilic environments because of their ability to tolerate perchlorate and potential reduction (Acevedo-Barrios et al., 2019a; Logan et al., 2001; Matsubara et al., 2017). In this work, we report the isolation of perchlorate-reducing bacteria from marine sediment samples from Antarctica. These bacteria present properties suitable for possible biotechnological applications and constitute the basis for expanding our knowledge of salt-tolerant bacteria that can reduce perchlorate.

Materials and methods

Study area and sample collection

Samples were obtained from marine sediments on Deception (62°58′35″ S, 60°40′31″ W), Half Moon (62°35′44″ S; 59°54′12″ W and 62°35′ 42.4″ S; 59°55′05.1″ W) and Horseshoe Islands (67°49′26.08″ S; 67°17′22.88″ W) (Fig. 1) during the III and V Colombian scientific expeditions to the Antarctic, the "Almirante Padilla" (January–March 2017) and "Almirante Campos" (January–March 2019). Approximately 40 samples were collected in triplicate for each experiment. A sterile spatula was used to collect approximately 100 g of sediment from the upper 1–10 cm of the sample profile. All samples were collected in 15-mL Falcon tubes and then refrigerated at 4 °C for transport to the laboratory for processing. Salinity and pH were recorded for each sample, according to the methods of Nozawa-Inoue et al. (2005). Jiang et al. (2016, 2021) previously reported that the presence of perchlorate in Antarctica is due to its natural formation in the atmosphere to later depositing in the snow, soil and sediments. $KClO_4^-$ was measured using a Thermo Scientific Orion-93 selective perchlorate electrode (Thermo Fisher Scientific Inc., Beverly, MA, USA) to determine the perchlorate concentration of each sample.

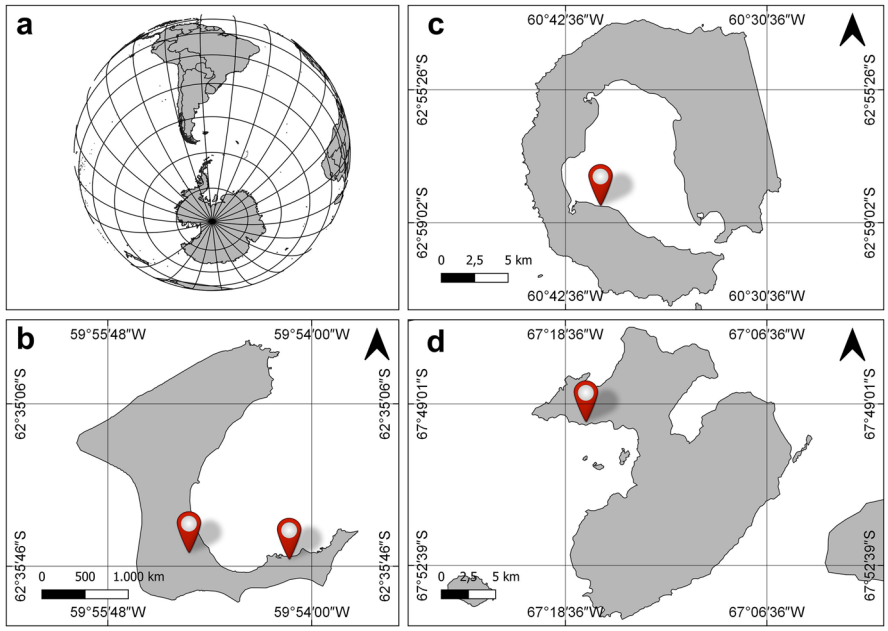

Fig. 1 Geographic location of Antarctica (**a**), Half Moon Island (**b**), Deception Island (**c**), and Horseshoe Island (**d**)

Isolation and culture conditions

Isolation, purification and conservation were performed as described by Acevedo-Barrios et al. (2019a). The samples were treated with amphotericin B prior to isolation. Cultivation was performed in four different media with the following compositions: Luria–Bertani (LB) medium: 10 g of NaCl, 10 g of tryptone and 5 g of yeast extract; AAD12 medium: 5 g of yeast extract, 3 g of sodium citrate, 20 g of $MgSO_4 \cdot 7H_2O$, 2 g of KCl, 250 g of NaCl and 20 g of agar; R2A medium: 0.5 g of yeast extract, 0.5 g of Proteose Peptone no. 3, 0.5 g of Casamino acids, 0.5 g of glucose, 0.5 g of soluble starch, 0.3 g of K_2HPO_4, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of sodium pyruvate and 15 g of agar per litre of laboratory quality water and M63 medium: 2.0 g of $(NH_4)_2SO_4$, 13.6 g of KH_2PO_4 and 0.0005 g of $MgSO_4 \cdot 7H_2O$. These media were modified using seawater with a $KClO_4^-$ concentration of 750 mg/L. Subsequently, the isolates were incubated at 4 °C for 14 d under aerobic conditions. Bacterial growth was monitored by observing the colonies. For the conservation of the bacteria, a colony was transferred to a cryovial with 720 µL of culture and 80 µL of glycerol and stored at −80 °C, as described by Acevedo-Barrios et al. (2019a).

Morphological characterisation

The morphology was observed using a light microscope (Olympus BX41). Gram staining was conducted according to Bergey's Manual Taxonomic Key (Boone et al., 2005) and Koneman's Microbiologic Atlas (Koneman et al., 2006). The isolates were incubated on LB, AAD12, R2A and M63 agar.

Biochemical characterisation

Biochemical characteristics were determined using the BBL Crystal™ Kit (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA), as described by the manufacturer. Catalase and oxidase tests were performed according to previously reported methods (Boone et al., 2005).

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of the isolated bacteria was extracted using the DNAzol Kit (Invitrogen), according to the manufacturer's instructions. The 16S rRNA gene was sequenced using the universal bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Rubiano-Labrador et al., 2019). Amplification of 16S rRNA was performed

following the protocol described by Rubiano-Labrador et al. (2019). The polymerase chain reaction (PCR) products were sequenced using an ABI PRISM ® 3500 system (Laboratorio de secuenciación de ADN, Universidad de Los Andes, Bogotá, Colombia). The resultant 16S rRNA sequences were assembled using the sequence editor BioEdit (version 7.2.5) (Hall, 1999) and then compared with data from the Ribosomal Database Project II (RDPII). The GenBank accession numbers for the 16S rRNA gene sequences of the isolates are MW130840, MW130841, MZ420735, MZ420736, MZ420737, MZ420738 and MZ420739.

Chloride susceptibility assay

All isolates were assayed for perchlorate susceptibility in LB broth in the presence of NaCl (3.5%, 5.0%, 7.5% and 30% w/v) (Bahamdain et al., 2015). The experiments were initiated by adding 20 µL of cell suspension (optical density (OD) = 0.6) to 5 mL of LB broth (Acevedo-Barrios et al., 2019a).

Perchlorate susceptibility assay

All isolates were assayed for perchlorate susceptibility in LB broth in the presence of perchlorate at concentrations of 500 mg/L, 750 mg/L, 1000 mg/L, 2500 mg/L, 5000 mg/L, 7500 mg/L and 10,000 mg/L (Fernández et al., 2005; Gholamian et al., 2011). Experiments were performed as described for the chloride susceptibility assay. After incubation for 14 d at 4 °C, the culture of each isolate was analysed on LB agar at their corresponding $KClO_4^-$ concentrations to confirm the viability of each bacterial isolate (Acevedo-Barrios et al., 2019a).

Evaluation of perchlorate reduction by isolates

The experiments were performed using a $KClO_4^-$ concentration of 10000 mg/L in LB medium containing 3.5% NaCl. Inoculation of the isolates was as described for the chloride susceptibility assay and incubation was for 14 d at 4 °C. After incubation, the final $KClO_4^-$ concentration was measured using a Thermo Scientific Orion-93 perchlorate electrode (Thermo Fisher Scientific Inc., Beverly, MA, USA), according to the manufacturer's instructions. The difference between the concentrations before and after incubation was used to calculate the perchlorate reduction percentage elicited by each isolate (Acevedo-Barrios et al., 2019a).

Results

Morphological and biochemical identification

In this study, six isolations from Antarctica were used. UTB-113 and UTB-114 were isolated from sediment samples of Deception Island under aerobic heterotrophic conditions at 4 °C. Both isolates were gram negative and mobile. UTB-113 was coccobacilli shaped, whereas UTB-114 was rod shaped. Biochemical characteristics (for example, lactose fermentation) varied. They both presented positive catalase and negative oxidase activity and showed positive reactions for N-acetylglucosaminidase and nitrate reduction activity; however, they were both negative for H_2S. UTB-154 and UTB-156 were isolated from Horseshoe Island and were gram negative, rod shaped and both presented motility. Catalase and oxidase reactions were positive. In addition, UTB-154 and UTB-156 exhibited nitrate reduction and negative H_2S production. On Half Moon Island, the isolates were identified as UTB-160, UTB-161 and UTB-162. UTB-160 was coccobacilli shaped and gram negative, UTB-161 was rod shaped and gram positive and UTB-162 was rod shaped and gram negative. All strains isolated from Half Moon Island presented positive catalase and oxidase reactions, nitrate reduction and negative reactions and were negative for H_2S. The characteristics of these isolates are listed in Table 1.

Phylogenetic analysis of the isolates

The results of the phylogenetic analysis showed that isolates UTB-113 and UTB-114 isolated from Deception Island belong to the genus *Psychrobacter*. UTB-113 shares 100% sequence identity with *P. cryohalolentis*, whereas UTB-114 shares 99.9% sequence identity with *P. urativorans*. UTB-154 and UTB-156, isolated from Horseshoe Island, belong to *I. loihiensis* and share 100% similarity. The isolates from Half Moon Island, UTB-160 and UTB-162, share 99.9% sequence identity with *P. nivimaris* and *P. lactis*, respectively. Additionally, UTB-161 shares 98.8% sequence identity with *S. aquimarina.*

Table 1 Morphological and biochemical characteristics of isolates from Antarctic marine sediment samples

Characteristic	UTB-113	UTB-114	UTB-154	UTB-156	UTB-160	UTB-161	UTB-162
Molecular identification	*P. cryohalolentis*	*P. urativorans*	*I. loihiensis*	*I. loihiensis*	*P. nivimaris*	*S. aquimarina*	*P. lactis*
Source	Deception Island	Deception Island	Horseshoe Island	Horseshoe Island	Half Moon Island	Half Moon Island	Half Moon Island
Morphology	Coccobacilli shaped	Rod shaped	Rod shaped	Rod shaped	Coccobacilli shaped	Rod shaped	Rod shaped
Colour of colony	Orange	White	Cream	Cream	White	Cream	Light yellow
Motility	+	+	+	+	-	+	+
Gram straining	-	-	-	-	-	+	-
Oxidase	-	-	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-
Nitrate reduction	+	+	-	-	+	+	+
Arabinose	-	-	-	-	-	-	-
Galactose	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-
Lactose	v	v	-	-	v	-	v
Mannitol	-	-	-	-	-	-	-
Mannose	-	-	-	-	-	-	-
Melibiose	-	-	-	-	+	-	-
Rhamnose	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-
p-n-p-Phosphate	-	+	-	-	-	-	-
p-n-p a-ß-Glucoside	-	+	-	-	-	-	-
p-n-p-ß-Galactoside	-	-	-	-	-	-	-
Proline nitroanilide	-	-	-	-	-	-	-
p-n-p bis-Phosphate	-	-	-	-	-	-	-
p-n-p-Xyiloside	-	-	-	-	-	-	-
p-n-p-a-Arabinoside	-	-	-	-	-	-	-
p-n-p-Phosphorylcholine	-	-	-	-	-	-	-
p-n-p-ß-Glucuronide	-	-	-	-	-	-	-
p-n-p-N-Acetylglucosamine	+	-	-	-	-	-	-
γ-L-Glutamyl p-nitroanilide	-	-	-	-	-	-	-
p-nitro-DL-phenylalanine	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-
Glycine	-	-	-	-	-	-	-

Table 1 (continued)

Characteristic	UTB-113	UTB-114	UTB-154	UTB-156	UTB-160	UTB-161	UTB-162
Citrate	-	-	-	-	-	-	-
Malonic acid	-	-	-	-	-	-	-
Triphenyltetrazolium chloride	-	-	-	-	-	-	-

+: positive reaction, −: negative reaction, v: variable reaction.

Sodium chloride and perchlorate susceptibility assay

All the isolates grew in a culture medium with a high NaCl concentration, reaching a tolerance of up to 30% NaCl, except for UTB-161, which presented a lower tolerance of up to 30% NaCl. In the case of perchlorate, the concentration measured on Deception Island ranged from 450–480 mg/L; therefore, *P. cryohalolentis* and *P. urativorans* can tolerate this concentration range of perchlorate in the environment. When these isolates were exposed to higher concentrations of $KClO_4^-$, they grew at between 500 mg/L $KClO_4^-$ and 10,000 mg/L $KClO_4^-$; however, they formed a biofilm at the highest concentration. The concentration of perchlorate measured in situ on Horseshoe Island ranged from 70 to 110 mg/L, and the concentration measured in situ on Half Moon Island ranged from 180 to 220 mg/L. When *I. loihiensis*, *P. nivimaris*, *S. aquimarina* and *P. lactis* were exposed to higher concentrations of $KClO_4^-$, between 500 and 10,000 mg/L, they grew effectively. Thus, these isolates showed the ability to survive at higher concentrations than those recorded in their environmental habitats.

Evaluation of perchlorate reduction by the isolates

In this study, the bacterial isolates exhibited the biological capacity to reduce $KClO_4^-$ (Fig. 2). They were initially exposed to 10,000 mg/L and after 15 d, they reduced perchlorate between 21.6 and 40%. The bacteria from Deception Island, UTB-113 and UTB-114, reduced 30.3% and 32.6% of perchlorate, respectively. UTB-154 and UTB-156 isolated from Horseshoe Island reduced 40% of perchlorate, and bacteria isolated from Half Moon Island, UTB-160, UTB-161 and UTB-162, reduced 22%, 21.8% and 21.6% of perchlorate, respectively.

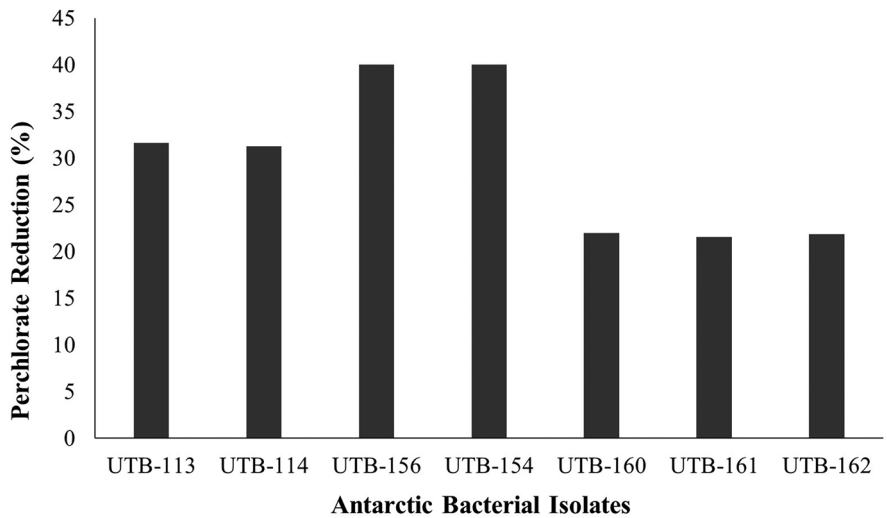

Fig. 2 Percentage reduction of $KClO_4^-$ concentration from marine sediment samples taken from Deception, Horseshoe and Half Moon Islands. Effect after 15 d of contact at an optical density (OD) of 600 and optimal pH of 7.0 ± 0.5

Discussion

Deception Island

The isolates were taxonomically characterised based on 16S rRNA gene sequencing and phylogenetic analyses. The results showed that the three isolates belong to the genus *Psychrobacter*. They were characterised as aerobic, osmotolerant and oxidase-positive bacteria and were either psychrophilic or psychrotolerant. *Psychrobacter* bacteria are found in a wide range of wet, saline and cold habitats as well as in warm and slightly salty habitats (Bowman, 2006; Lasek et al., 2017; Silva et al., 2018; Smith et al., 2009). Our results are consistent with those of previous studies on microbial communities in various Antarctic habitats, where this genus was also isolated from Antarctic soils (Bendia et al., 2018a; Bowman et al., 1996; Bozal et al., 2003; Centurion et al., 2019; Che et al., 2013; Lasek et al., 2017; Muñoz-Villagrán et al., 2018). However, the present study represents the first analysis of species of *Psychrobacter* that are associated with marine sediments from Deception Island (Bendia et al., 2018a; Flores et al., 2018). The environmental gradients of temperature and salinity, along with the geochemical processes on Deception Island, relate to the isolation of this genus. *Psychrobacter* has been previously isolated from various environmental settings owing to its diverse metabolic characteristics (Bendia et al., 2018b; Bowman et al., 1996; Lasek et al., 2017; Silva et al., 2018; Smith et al., 2009).

To date, *Psychrobacter* has been known to include 41 valid species that have been isolated from different sources (Kokoulin et al., 2020; LPSN, n.d.). UTB-113 and UTB-114 were identified as *P. cryohalolentis* and *P. urativorans*, respectively. These species were previously isolated under similar low-temperature, hypersaline conditions (Amato & Christner, 2009; Bakermans et al., 2006; Bowman et al., 1996), which is a common environment for their growth. UTB113 represents the first isolation of *P. cryohalolentis* from the Antarctic continent (Amato & Christner, 2009; Bakermans et al., 2006; Goordial et al., 2013; Smith et al., 2009). Bacterial diversity can occur in this region, and there is a need to extend research to areas with extreme environmental conditions.

The isolates were negative for oxidase and positive for catalase and showed a positive reaction to nitrate reduction. The latter promotes the analysis of isolated bacteria based on nitrate acting as an electron acceptor, which is characteristic of the reduction of perchlorate (Bardiya & Bae, 2011; Sevda et al., 2018). The genes responsible for nitrate reduction generally coexist with those that reduce perchlorate because of their similar potential to reduce nitrate to molecular nitrogen and perchlorate to chloride (Ucar et al., 2017; Wan et al., 2017; Zhao et al., 2011).

Horseshoe Island

Bacteria isolated from Horseshoe Island were related to *I. loihiensis*. This species was isolated for the first time from a hydrothermal source of the submarine Loihi volcano in Hawaii, at a depth of 1300 m. *Idiomarina loihiensis* is a halophilic bacterium with an optimum grow at temperatures of 4–50 °C (Donachie, 2003). Thus, the marine ecosystem of Antarctica may be one of their habitats. This genus was reported by Malavenda et al. (2015), who isolated *I. loihiensis* from Arctic and Antarctic sediments. Specifically, this psychrotolerant bacterium was isolated from hydrocarbon-amended microcosms containing crude or diesel oil. Malavenda et al. (2015) demonstrated the biosurfactant-production capability of *Idiomarina* sp. 185 in cold environments. In addition, *I. loihiensis* was isolated from the Peruvian Andes under halophilic conditions (Castelán-Sánchez et al., 2019).

Half Moon Island

Bacteria isolated from Half Moon Island were related to *P. nivimaris*, *S. aquimarina* and *P. lactis.* The genus *Psychrobacter* was also isolated from Deception Island, indicating the psychrophilic or psychrotolerant behaviour of this genus. In addition, *P. nivimaris* has been isolated from hypersaline environments and low temperatures in the Antarctic Ocean (Yumoto et al., 2010). *S. aquimarina* is a facultative anaerobic bacterium isolated for the first time in South Korea. In addition, this species has been isolated from Antarctica and is resistant to extreme conditions and low temperatures (Reddy et al., 2003; Santos et al., 2015). *Pseudomonas* genera have been isolated from different environments, even under extreme conditions, owing to their versatile metabolic mechanisms (Peix et al., 2018). However, to the best of our knowledge, *P. lactis* is the first isolated from this species in Antarctica.

Sodium chloride and perchlorate susceptibility of the bacterial isolates

Environmental factors such as salinity can influence bacterial growth and inhibit metabolic activity as a result of (i) increased osmotic stress on microorganisms and (ii) altered solubility or sorption of toxic/essential ions (Yan et al., 2015). However, some bacteria can adapt to low osmotic potential through salt-in-cytoplasm and osmolyte accumulation mechanisms (Long et al., 2018). The low availability of freshwater in the Antarctic continent has led to the development of salt tolerance mechanisms as a microbial survival strategy (Aguila-Müller, 2015; Cowan et al., 2014; Zhang et al., 2013). Therefore, the tolerance of the isolates in our experiments to a NaCl concentration of 30% can be attributed to the low availability of freshwater and high salinity of the sampled ecosystem. Isolates related to *Psychrobacter*, *Idiomarina*, *Sporosarcina* and *Pseudomonas* genera have been reported to be tolerant to salinity, particularly those isolated in this study; therefore, they have an adaptive advantage (Azevedo et al., 2013; Bakermans et al., 2006; Bowman et al., 1996). The ability of the isolated species to tolerate an NaCl concentration of 30% is promising for their use as a biological system to reduce perchlorate in high salinity ecosystems, where the presence of perchlorate has been reported in numerous studies (Cang et al., 2004; Chung et al., 2009; Martin et al., 2009; Ryu et al., 2011; Singh & Jha, 2016).

Perchlorate contamination of marine sediment and seawater, as well as other environmental matrices (for example, Antarctic soil), has resulted in the stimulation of bacterial growth and an increase in the number of bacteria that can resist and degrade these pollutants (Achenbach & Coates, 2004; Calderón et al., 2014, 2017; Jiang et al., 2016, 2020; Kounaves et al., 2010; Nam et al., 2016). The dominant members of bacterial communities that have been found to resist and degrade perchlorate belong to the Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria subclasses. The genera isolated in this study are part of the Gammaproteobacteria subclass (Achenbach et al., 2001; Carlström et al., 2016; Sevda et al., 2018; Zhu et al., 2016). However, there are no previous reports on the evaluation of perchlorate resistance in these psychrophilic and psychrotolerant genera and species. Our results confirmed that native Antarctic bacteria in the sediment samples were tolerant to environmental concentrations of perchlorate and can tolerate higher concentrations up to 10,000 mg/L, even at low temperatures. This factor can limit the degradation process, given that perchlorate-reducing bacteria are more efficient under mesophilic or thermophilic conditions (Liebensteiner et al., 2015; Song et al., 2019).

Most perchlorate-reducing bacteria are anaerobic and facultative, and molecular oxygen is produced as an intermediate for microbial perchlorate reduction in a process that exudes nitrate (Bruce et al., 1999; Sevda et al., 2018). Although these bacteria undergo degradation processes in a wide range of environmental conditions, for the low temperature, high salinity environments of Antarctica, *H. lacusprofundi* has been shown to tolerate elevated concentrations of highly oxidative perchlorate salts. In addition, its enzyme (β-galactosidase) was able to maintain its activity under these conditions, which is a possible key mechanism for the stable activity of this microorganism (Correa & Abreu, 2020; Laye & DasSarma, 2018). The isolates in this study were negative for β-galactosidase. Further research is required to understand the enzymatic activity of the mechanisms related to the resistance and reduction of perchlorate. It is possible that some critical anaerobic isolates were missed during the aerobic treatment in this study; hence, additional experiments should be carried out under anaerobic conditions to enrich for active microorganisms that may improve perchlorate degradation. These may include the species isolated in this study, which are found in a wide range of low-temperature habitats and marine environments, including Antarctic ice, soil and orogenic sediments (Bozal et al., 2003; Che et al., 2013).

Evaluation of perchlorate reduction by isolates

A variety of perchlorate-reducing bacterial species can reduce this contaminant; however, the percentage of reduction varies according to genus and the period of exposure to the pollutant. The rates of perchlorate reduction determined in this study were comparable to those reported by Acevedo-Barrios et al. (2019a), where *Nesiotobacter*, *Salinivibrio*, *Vibrio*, *Bacillus* and *Staphylococcus* genera were isolated from Caribbean hypersaline soils, and reduced between 10 and 25% of 10,000 mg/L of $KClO_4^-$. In the present study, perchlorate reduction was performed by *P. cryohalolentis*, *P.*

urativorans, *I. loihiensis*, *P. nivimaris*, *S. aquimarina* and *P. lactis*, thus demonstrating their ability to reduce this contaminant. However, further investigation is required to determine the optimum reduction under different environmental conditions (e.g. temperature and the presence/absence of electron acceptors/donors) (Chaudhuri et al., 2002).

Additionally, the bacteria isolated in this study can be used together or mixed with other cultures to enhance the bioremediation process through symbiotic interactions. For example, Kucharzyk et al. (2013) reported that the rate of perchlorate reduction increased 2.06-fold and 4.08-fold when using consortia in comparison to the use of isolated bacteria. In addition, Nor et al. (2011) found that perchlorate-reducing cultures could treat high perchlorate concentrations.

Conclusion

This study confirmed that native Antarctic bacteria isolated from sediment samples were tolerant to environmental concentrations of perchlorate and can tolerate higher concentrations of up to 10 000 mg/L. *P. cryohalolentis* and *P. urativorans* were isolated from Deception Island; *I. loihiensis* from Horseshoe Island and *P. nivimaris*, *P. lactis* and *S. aquimarina* from Half Moon Island. Only a few studies have reported on the reduction of perchlorate by Antarctic microorganisms, our findings demonstrated that these isolated bacteria can reduce $KClO_4^-$, with reduction between 21.6 and 40%, thus providing a possibility for biotechnology and the treatment of areas polluted by perchlorate. *I. loihiensis* was the bacterium with the highest reduction in perchlorate, while *P. lactis* presented the lowest reduction. In addition, the isolates are capable of growing in a culture medium with a high NaCl concentration; therefore, they are halophilic or halotolerant. This salinity tolerance is promising for use as a biological system to reduce perchlorate in high-salinity ecosystems. It should be noted that there are no previous reports on the isolation of *P. cryohalolentis* and *P. lactis* from the Antarctic continent. Therefore, this study expands the existing knowledge regarding the presence of perchlorate-reducing bacteria in Antarctica.

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Availability of data and materials The datasets generated and/or analysed during the current study are available in the GenBank repository with code numbers MW130840, MW130841, MZ420735, MZ420736, MZ420737, MZ420738 and MZ420739.

Declarations

Conflict of interests The authors declare no competing interests.

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