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DETECTION OF MERCURIC REDUCTASE (*merA*) GENE FROM *Micrococcus* sp. ISOLATED FROM SIGNY ISLAND, ANTARCTICA

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Abstract

Mercury (Hg) is a heavy metal that can be released into the environment through natural activity such as volcanic eruptions and release from vegetation during bushfires, and anthropogenic activities such as the gold mining process and fossil fuel burning. Most atmospheric mercury is elemental Hg (Hg^0), which is less toxic than the Hg^{2+} ion. However, elemental Hg can be oxidised to a more toxic form through interaction with ozone in the presence of water. Certain microbes are capable of enzymic reduction of Hg^{2+} to Hg^0 using mercuric reductase, coded for by the *merA* gene. In this study, we screened the gene from bacterial strains isolated from soil collected at Signy Island, South Orkney Islands, maritime Antarctic, a gold mine in Sumbawa Island in Indonesia and a tin mine at Guar Perahu, Malaysia. A total of seven bacterial strains were isolated. One strain was isolated from the tin mine, and three each from the gold mine and Signy Island. The gene was absent from all the strains isolated from Sumbawa and Guar Perahu, but the gene was detected from an isolate from Signy Island. Based on analysis of the strain's 16S rRNA gene, it was assigned to the genus *Micrococcus* (similarity 97.22%) compared with the global recorded database at the National Centre for Biotechnology Information (NCBI) using the BLAST program. We thereby confirmed the presence of the gene in this bacterial strain, providing the first identification of this mercury-reducing gene in Signy Island microbiota.

INTRODUCTION

The toxic heavy metal mercury (Hg) occurs both naturally and as an anthropogenic pollutant. Sources such as gold mining industry wastewater produced from filtering or washing activities can result in surrounding water catchments and land being contaminated by high levels of mercury [1], decreasing soil fertility amongst other harmful effects.

Mercury can enter and be biomagnified in the food chain. Its contamination is damaging to natural ecosystems and human health [2]. Methyl mercury acts as a strong neurotoxin, causing pain in the lips, tongue, affecting movement, hallucinations, irritability, sleep disorders, ataxia, memory loss, difficulty in speaking, deterioration of

thinking capacity, emotional instability and even death [3]. For example, mercury derived from a paint solvent manufacturing plant in Minamata Bay, Japan, contaminated the local fish supply and resulted in an estimated 1,784 human casualties [3].

In the environment, the process of detoxifying mercury contamination, especially that of methyl mercury, begins with demethylation. Demethylation releases the mercury ion (Hg^{2+}), which can be reduced to elemental Hg^0 which is volatile and less toxic [4]. This conversion process involves an enzyme complex including organomercury lyase and mercury reductase [5]. One approach to remediation of mercury contamination is bioremediation, which utilises microorganisms with specific capabilities to remove the targeted pollutant from the environment [6]. Mercury is toxic to many microbes (and other organisms), but some bacteria can tolerate mercury exposure. Such bacteria have a mechanism of resistance to mercury contamination that is poorly understood [4].

Mercury-resistant bacterial cells can absorb the Hg^{2+} ion and reduce it to Hg^0 [7], which is released into the atmosphere. The ability of bacteria to remove mercury from the environment could potentially have application in lowering mercury concentrations in industrial waste. This ability is facilitated by the *merA*, a gene responsible for absorbing and reducing Hg^{2+} to Hg^0 [8].

Elemental mercury is used as an amalgam to extract gold from ore, and small-scale gold mining is the world's largest source of mercury pollution [9]. The discovery of gold (Au) in Sumbawa, Indonesia, since the beginning of 2000, has led to considerable illegal mining activities [10]. Illegal mining is difficult to stop for local people because mining is their current livelihood. If the mining activities continue, a higher level of Hg will be released into the environment and cause pollution. Elevated mercury concentrations in its air (>13 mg/kg) of illegal miners in the West Region of Sumbawa have been reported [11]. Although not used in the tin mining process, high mercury levels (0.171 ppm) were also detected from an abandoned tin mine in Tambon Bang-Rin, Thailand. Heavy metal-resistant bacteria were isolated from the same location [11]. Therefore, it is likely that mercury resistance bacteria can be isolated from either gold or tin mines.

In contrast, Antarctica is generally considered a region that has suffered limited anthropogenic influences. However, heavy metal contamination has been reported in Antarctic soil from various locations there [5]. The presence of heavy metals, especially mercury, in soils of Antarctica is mainly a result of a natural bio-geochemical weathering of terrigenous sources [12]. Antarctic soil contains many metal-rich minerals, which are subject to natural bio-geochemical weathering [13]. Additionally, elemental mercury can remain suspended in the atmosphere for at least a year [14], transported long distances from other landmasses to Antarctica. The presence of microorganisms possessing the gene in the Station Nord in Northeastern Greenland (Arctic) and King George Island (Antarctica) has been reported [15, 12]. Signy Island is an Antarctic oceanic island in the southern Orkney Islands. The annual soil temperature is around -2°C, and the annual precipitation is estimated at 400 mm per year [16]. Hg-resistant (*merA*) bacteria from snow and sea ice brine in the High Arctic have been identified and characterized, showing that 0-31% of the culturable bacteria are resistant to Hg (10µM $HgCl_2$), and up to 6% of the total reduction in Hg^{2+} in snow may be explained by mercury-resistant bacteria [15]. There is mercury resistance in Bacteria and Archaea [25]. Mercury resistance in Bacteria is conferred by the *mer* operon. Several *mer* operon-encoded proteins are involved in transport of inorganic oxidized mercury into the cytosol, where the *merA*-encoded mercuric reductase protein, in a NAD(P)H-dependent manner, reduces Hg^{2+} to volatile, less reactive elemental mercury (Hg^0) (mercury in the environment and bacterial resistance has been thoroughly reviewed [25]). Mercury resistance has been reported in a wide range of bacteria, including Firmicutes, Actinobacteria, and Proteobacteria, both from clinical and environmental sources, and is considered an ancient mechanism [25]. The current study identified whether the mercuric reductase (*merA*) gene was present in bacteria isolated from a gold mine in Sumbawa, Indonesia, a tin mine in Malaysia and Signy Island (the South Orkney Islands, maritime Antarctic).

MATERIALS AND METHODS

Soil sample collection

Soil samples were collected from three locations: a gold mine in Sumbawa, Indonesia, a tin mine in Guar Perahu, Malaysia, in 2019 and Signy Island, South Orkney Islands (maritime Antarctic) in 2016. The coordinates of the sampling points are 8’32’30.4’S 117’00’45.0’E, 5’25’57’N 100’28’30’E and 42.629’S 36.568’W, respectively. Soil samples consisting of 10 g of topsoil were collected in a 50 ml screw-capped Falcon tube.

Isolation of Bacterial Strains

The standard spread plate method was used for screening bacterial isolates from the soil samples on Nutrient Agar (NA) plates. The plates were incubated at 37 °C for 24 h. The colonies were subcultured and purified into a single colony using the streak plate method. Visible single colonies growing on the plates were used for *merA* detection.

Detection of Mercuric Reductase *(merA)* Gene

Genomic DNA from colonies formed on plates was extracted using the GF-1 Bacterial DNA Extraction Kit based on the manufacturer's instructions. Polymerase Chain Reaction (PCR) was conducted using the Vivantis Master Mix with 12.5 µl *Taq polymerase,* 1 µl primer Forward, 1 µl primer Reverse, 8.5 µl autoclaved H_2O and 2 µl of each DNA base

as a template. The total reaction mix was 25 µl. For mercury reductase gene identification, the combination of primers Forward F1 *merA* – 5' TCGTGATGTTCGACCGCT 3' and Reverse F2 *merA* – 5' TACTCCCGCCGTTTCCAAT 3' was used to amplify a 443 bp *merA* fragment with an optimised PCR protocol [8]. Amplification conditions included an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and an extension step at 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products were analysed using gel electrophoresis using 1.5% agarose gel run at 70 V, 400 A for 40 min. Then, the gel was dyed with SYBR® Safe DNA Gel Stain in TAE buffer and visualised with a UV gel doc.

Identification of Bacteria using 16S rRNA

Strains positive with *merA* from the section above were streaked onto a new plate and used for species identification. Bacterial identification was carried out by sequencing the 16S rRNA gene, using the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') to generate a 1465 bp fragment of the 16S rDNA [17]. The thermocycling conditions included an initial denaturation step at 94°C for 5 min, 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C and extension for 1 min at 72°C [8], with final extension for 5 min at 72°C [8]. The PCR products were analysed using gel electrophoresis (1.5% agarose gel, 70 V, 400 A for 40 min), followed by staining with SYBR® Safe DNA Gel Stain in TAE buffer and visualisation using a UV gel doc. The PCR products from both 16S and *merA* were purified and sent to NHK Bioscience Solution SDN. BHD (Malaysia) for sequencing. The sequence was aligned using Molecular Evolutionary Genetics Analysis (MEGA) Version 6.0 software and BLAST search against the National Centre for Biotechnology Information (NCBI) Nucleotide collection (nt) database for bacterial identification. The tree was produced using BLAST pairwise alignments using neighbour-joining tree methods.

Morphology of *merA* Positive Strains

Isolates positive for *merA* were analysed for morphology, shape, and colours of the colonies on the media. Isolates negative with *merA* were not analysed further.

RESULTS AND DISCUSSION

A total of seven bacterial strains were isolated. One strain was isolated from Guar Perahu, Malaysia, and three strains were isolated from each of Sumbawa Island, Indonesia, and Signy Island, Antarctica. 16S rRNA genes were detected from all the isolated strains, confirming successful extraction of genomic DNA and absence of PCR inhibition at extracted DNA from all isolates. This conformation is important to verify that the absence of *merA* in the subsequent PCR was not due to PCR inhibition. The inhibitors may originate from samples or reagents used to process and purify DNA such as KCl, NaCl, other salts, ionic detergents such as sodium deoxycholate, sarkosyl and SDS, ethanol and isopropanol, phenol, bile salts, complex polysaccharides, collagen, humic acid, melanin, eumelanin, myoglobin, proteinases, calcium ions and urea [18]. Figure shows the presence of the 443 bp *merA*, detected from a strain isolated from Signy Island. The other six strains were negative for *merA* detection.

Comparison between the sequences from the sample with the available database (NCBI) showed that these strains were closest to uncultured *bacterium* clone SS3-14 mercuric reductase (*merA*) gene (Figure 2). The highest sequence similarity with mercuric reductase bacteria was 90.15%, partial sequence ID: JQ228797.1.

The basic local alignment search tool result showed that the strain positive for *merA* was *Micrococcus* sp. 3309, with a 16 ribosomal RNA gene similarity of 97.22% with query cover 63%, and partial sequence ID: KP345923.1. The phylogenetic tree analysis of the isolate from this study compared to other bacteria is shown in Figure 3, and the morphology of *merA* positive strains is shown in Figure 4 below. The other *merA* negative strains were not identified.

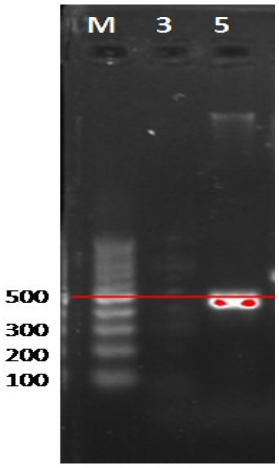

Figure 1. Positive detection of the 443 bp *merA* from a strain isolated from Signy Island, Antarctica.

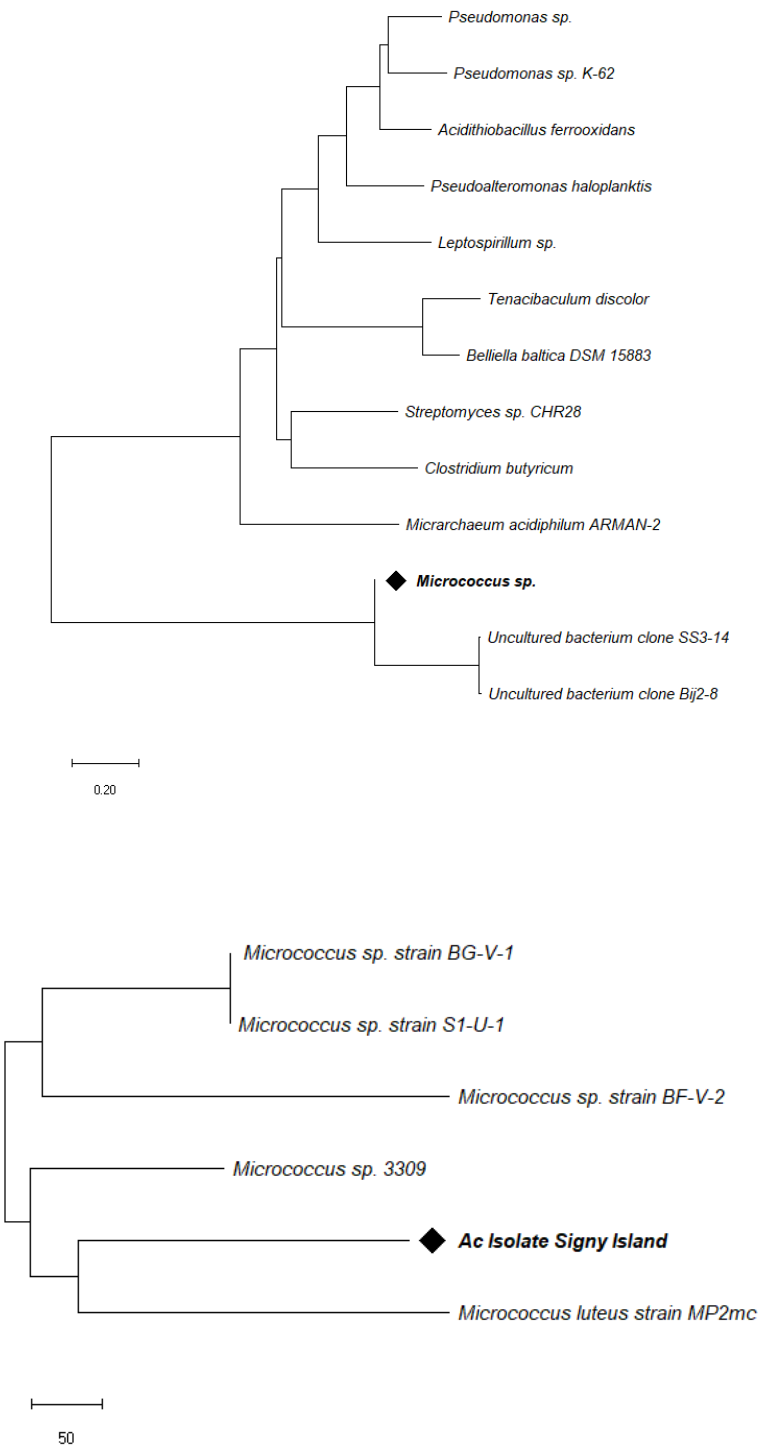

Figure 3. Phylogenetic trees analysis for 16S rRNA gene of Ac isolate produced using the BLAST pairwise alignments.

Figure 4. The red circle shows the *merA* positive strains.

This study included samples taken from a gold mine in Sumbawa, Indonesia and a tin mine in Guar Perahu, Malaysia. Mercury-resistant bacteria have been isolated from gold mines in Indonesia, such as from Pongkor Village, Bogor [19], Kabupaten Sijunjung, West Sumatera [20] and Lombok [21]. In addition, mercuric-resistant bacteria have also previously been isolated from Malaysia [22]. Therefore, we initially hypothesized that bacterial strains isolated from these sites might possess the mercuric reductase gene. However, none of the previous studies in Malaysia and Indonesia reported the presence of *merA*. Similarly, in this study, the gene was not present in samples collected from both the tropical sites of Malaysia and Indonesia. Instead, the results showed that strains with positive *merA* were isolated from Signy Island, Antarctica.

The isolate had a similarity of 97.22% with *Micrococcus* sp. 3309 using the 16S rRNA gene. The presence of *Micrococcus* sp. in Signy Island was reported in 1973 [23]. Rego *et al*. [24] also reported the presence of *Micrococcus* sp. from sampling points in McMurdo Dry Valleys in Antarctica. The displayed gene of *merA* has a size of 443 bp [7]. The *merA* sequences detected in this study had a similarity of 90.15% with uncultured bacterium clone SS3-14 mercuric reductase (*merA*). Previous research conducted by Romaniuk *et al.* [12] found *merA* from isolated strains such as *Arthrobacter*, *Pseudomonas*, and *Psychrobacter* genera from King George Island in Maritime Antarctica. Moller *et al.* [15] also reported seven *merA* determinants were identified among bacterial isolates from High Arctic snow, freshwater, and sea-ice brine. Although *Micrococcus* sp. has been reported in Signy Island previously [23], this study is the first record of detecting *merA* from any species isolated from the island.

Mercury-resistant microorganisms are often enriched to even higher abundances in Hg contaminated environments, where their activities enhance the conversion of CH_3Hg^+ and Hg^{2+} to Hg^0. The presence of bacterial strains possessing *merA* in Antarctica may be due to the environmental conditions of the continent, such as extreme temperature, acidic pH, deficient levels of light, oxygen, and soil with high heavy metal content. Mercury naturally occurs in the Earth's crust and typically enters the atmosphere through volcanic eruptions. Thus, the element cycles between the atmosphere and ocean quickly. However, mercury deposited on land from the atmosphere binds with organic matter in plants. After the plants die, soil microbes consume the dead organic matter, releasing mercury into the atmosphere or water. Therefore, mercury removal is a challenge for environmental management. The present study showed that an isolate from Signy Island poses a mercuric reductase (*merA*) gene with a similarity of 97.22% to *Micrococcus* sp. Thus, the bacterial strain may be useful as an agent for

bioremediation technology to remove Hg^{2+} from the environment. The data presented here confirm that the *Micrococcus* sp. has the gene but does not confirm the enzyme is produced or active. Additional enzymatic studies are needed to confirm the presence and activity of the enzyme.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

1. Arbestain, M. C., Rodriguez-Lado, L., Bao, M., & Macias, F. (2009). Assessment of mercury-polluted soils adjacent to an old mercury-fulminate production plant. *Applied and Environmental Soil Science*, *2009*.
2. Von Canstein, H., Kelly, S., Li, Y., & Wagner-Döbler, I. (2002). Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions. *Applied and Environmental Microbiology*, *68*(6), 2829-2837.
3. Barkay, T. (1992). Mercury Cycle. Encyclopedia of Microbiology, Vol. 3.
4. Retnowati, Y. (2011). Isolasi dan identifikasi bakteri pengguna merkuri dari sedimen sungai yang terkontaminasi limbah tambang emas. *Saintek*, *6*(1), 1-9.
5. Benison, G. C., Di Lello, P., Shokes, J. E., Cosper, N. J., Scott, R. A., Legault, P., & Omichinski, J. G. (2004). A stable mercury-containing complex of the organomercurial lyase MerB: catalysis, product release, and direct transfer to *MerA*. *Biochemistry*, *43*(26), 8333-8345.
6. Dash, H. R., & Das, S. (2012). Bioremediation of mercury and the importance of bacterial mer genes. *International Biodeterioration & Biodegradation*, *75*, 207-213.
7. Nascimento, A. M., & Chartone-Souza, E. (2003). Operon mer: bacterial resistance to mercury and potential for bioremediation of contaminated environments. *Genetics and Molecular Research*, *2*(1), 92-101.
8. Sotero-Martins, A., Jesus, M. S. D., Lacerda, M., Moreira, J. C., Filgueiras, A. L. L., & Barrocas, P. R. G. (2008). A conservative region of the mercuric reductase gene (*merA*) as a molecular marker of bacterial mercury resistance. *Brazilian Journal of Microbiology*, *39*, 307-310.
9. Esdaile, L. J., & Chalker, J. M. (2018). The mercury problem in artisanal and small-scale gold mining. *Chemistry (Weinheim an Der Bergstrasse, Germany)*, *24*(27), 6905.
10. Muhammad, M. (2019). Peran Pemerintah dalam Upaya Penanggulangan Tambang Emas Ilegal di Desa Lamungan Kecamatan Taliwang Kabupaten Sumbawa Barat. (*Doctoral dissertation*, Universitas MuhammadiyahMataram).
11. Krisnayanti, B. D., Vassura, I., Asmara, M. D., Ekawanti, A., & Suheri, H. (2016). Analysis of artisanal small-scale gold mining sector in West Sumbawa Regency, Indonesia. *Journal of Health and Pollution*, *6*(12), 26-33.
12. Romaniuk, K., Ciok, A., Decewicz, P., Uhrynowski, W., Budzik, K., Nieckarz, M., ... & Dziewit, L. (2018). Insight into heavy metal resistome of soil psychrotolerant bacteria originating from King George Island (Antarctica). *Polar Biology*, *41*(7), 1319-1333.
13. Dold, B., Gonzalez-Toril, E., Aguilera, A., Lopez-Pamo, E., Cisternas, M. E., Bucchi, F., & Amils, R. (2013). Acid rock drainage and rock weathering in Antarctica: Important sources for iron cycling in the Southern Ocean. *Environmental Science & Technology*, *47*(12), 6129-6136.
14. Gionfriddo, C. M., Tate, M. T., Wick, R. R., Schultz, M. B., Zemla, A., Thelen, M. P., ... & Moreau, J. W. (2016). Microbial mercury methylation in Antarctic Sea ice. *Nature Microbiology*, *1*(10), 1-12.
15. Møller, A. K., Barkay, T., Hansen, M. A., Norman, A., Hansen, L. H., Sørensen, S. J., ... & Kroer, N. (2014). Mercuric reductase genes (*merA*) and mercury resistance plasmids in High Arctic snow, freshwater and sea-ice brine. *FEMS Microbiology Ecology*, *87*(1), 52-63.
16. Bokhorst, S., Bjerke, J. W., Bowles, F. W., Melillo, J., Callaghan, T. V., & Phoenix, G. K. (2008). Impacts of extreme winter warming in the sub-Arctic: growing season responses of dwarf shrub heathland. *Global Change Biology*, *14*(11), 2603-2612.
17. Heuer, H., Krsek, M., Baker, P., Smalla, K., & Wellington, E. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Applied and Environmental Microbiology*, *63*(8), 3233-3241.
18. Bessetti, J. (2007). An introduction to PCR inhibitors. *Journal of Microbiological Methods*, *28*, 159-67.
19. Sanjaya, W. T. A., Khoirunhisa, N. S., Ismiani, S., Hazra, F., & Santosa, D. A. (2021). Isolation and characterisation of mercury-resistant microbes from gold mine area in Mount Pongkor, Bogor District, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(7).
20. Febria, F. A., Zakaria, I. J., Syukriani, L., Rahayu, S. P., & Fajri, M. A. (2016). The highest mercury resistant bacteria as a mercury remediator from gold mining soil in West Sumatera, Indonesia. *Journal of Chemical and Pharmaceutical Research*, *8*(1), 394-397.
21. Nurfitriani, S., Arisoesilaningsih, E., Nuraini, Y., & Handayanto, E. (2020). Bioaccumulation of Mercury by Bacteria Isolated from Small Scale Gold Mining Tailings in Lombok, Indonesia. *Journal of Ecological Engineering*, *21*(6).
22. Abbas, S. Z., Yee, C. J., Hossain, K., Ahmad, A., & Rafatullah, M. (2019). Isolation and characterisation of mercury-resistant bacteria from industrial wastewater. *Desalination and Water Treatment*, *138*, 128-133.
23. Herbert, R. A., & Bell, C. R. (1973). Nutrient recycling in freshwater lakes on Signy Island, South Orkney Islands. *British Antarctic Survey Bulletin*, *37*, 15-20.

24. Rego, A., Raio, F., Martins, T. P., Ribeiro, H., Sousa, A. G., Séneca, J., ... & Magalhães, C. (2019). Actinobacteria and cyanobacteria diversity in terrestrial antarctic microenvironments evaluated by culture-dependent and independent methods. *Frontiers in Microbiology*, *10*, 1018.

25. Oregaard, G., & Sørensen, S. J. (2007). Ore High diversity of bacterial mercuric reductase genes from surface and sub-surface floodplain soil (Oak Ridge, USA). *The ISME journal*, *1*(5), 453-467.