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BIOPROSPECTING IN ANTARCTICA: A NEW FRONTIER OR A NOVEL THREAT?

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ABSTRACTS SUBMITTED TO THE (CANCELLED) SCAR 2020 OSC IN HOBART

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Characterization of perchlorate reducing cultivable halophilic bacteria from Deception Island, Antarctica.

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Perchlorate (ClO4-) has several industrial applications and is frequently detected in environmental matrices at relevant concentrations to human health. Currently, perchlorate-degrading bacteria are promising strategies for bioremediation in polluted sites. The aim of this study was to isolate and characterize halophilic bacteria with the potential for perchlorate reduction. Two bacterial strains were isolated marine sediments on Deception Island, Antarctica. Isolates grew at concentrations up to 30% sodium chloride. The isolates tolerated pH variations ranging from 6.5 to 9.5, and perchlorate concentrations up to 10000 mg/kg. Perchlorate was degraded by these bacteria on percentages between 30 and 40 16S rRNA gene sequence analysis indicated that the strains were phylogenetically related to Psychrobacter cryohalolentis and Psychrobacter fozii specie. In conclusion, halophilic isolated bacteria the genus Psychrobacter from the marine sediments on Deception Island, Antarctica are promising resources for the bioremediation of perchlorate contamination.

Keywords: Antarctic, environmental face, halophilic bacteria, marine soil, perchlorate, toxicity

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Morphological characterization and composition of the tardigrades from the Media Luna island, Antarctic Peninsula

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The tardigrades are microscopic animals of at least 1 mm in length, better known as "water bears". Famous for its ability to withstand extreme conditions, when entering a dormant state, this resistance ability is called cryptobiosis. This ability allows them to conquer places as far away as Antarctica. Antarctica is a continent with a high interest in its extreme climatic conditions, getting the organisms that live there to develop strategies to withstand extreme conditions. For this reason, Media Luna Island was chosen as the study area. The study of Antarctic tardigrades was carried out by collecting samples of bryophytes on Half Moon Island on the Capa Negra Hill. The analysis of 10 samples allowed the identification of 4 main families of Hypsibiidae, Macrobiotidae, Echiniscidae and Calohypsibiidae tardigrades. Among these, we have identified the genera Diphascon, Hexapodibius, Hypsibius, Macrobiotus and Echiniscus. Some of the confirmed species have been previously reported in the Antarctic continent, but this is the first report on the Crescent Island for the species Diphascon victoriae, Diphascon rudnicki, Hypsibius conwentzii, Hypsibius dujardini and Macrobiotus aradasi.

Keywords: Antarctic, Crescent Island, identification, tardigrade

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Natural products from Antarctic marine benthic invertebrates

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Recent research in our lab is focusing at new approaches to study the natural products from Antarctic marine benthic invertebrates and their chemical ecology. A part of the description of the new compounds found and their bioactivities, we are looking at the production of these metabolites under situations of stress, trying to see changes in gene expression related to natural compounds synthesis. On the other hand, we are using CADD (Computer-Aided Drug Design) techniques to help us in the search for bioactivities for these compounds, and experimentally validating these results. For these, we are studying selected tunicate and gastropod molluscs compounds, but also sponges, bryozoans, and others. In this talk, a summary of these recent developments will be provided.

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Detection of antimicrobial activity and multiple resistance to antibiotics of bacterial isolates from pristine Antarctic soil samples and from rhizosphere of Deschampsia antarctica Desv.

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In this study, 50 bacterial isolates from pristine Antarctic soil samples from King George and Greenwich Islands (South Shetland Islands) and 25 bacterial isolates from the rhizosphere of Deschampsia antarctica Desv, were characterized for their antimicrobial activity and response to 21 antibiotics.
Antibiotic susceptibility was assayed following the disc diffusion method using different groups of antibiotics: penicillins, cephalosporins, carbapenems, aminoglycosides, quinolones, tetracycline, phenicols, sulfonamides, and trimethoprim. Antimicrobial compounds production by the Antarctic bacterial isolates were determined using the agar diffusion method, using Escherichia coli and Staphylococcus aureus as indicators of human pathogenic bacteria. The bacterial isolates were characterized by optical microscopy assays, biochemical batteries and fingerprinting using PCR with arbitrary primers.
The bacterial isolates studied showed resistance to 14 (67%) out of the 21 antibiotics tested. Three bacterial isolates (4%) were resistant to at least one antibiotic and 47 (63%) were multidrug-resistant. Additionally, 25 (33%) bacterial isolates do not show antimicrobial activity and were susceptible to all antibiotics studied. Finally, 27 (36%) bacterial isolates combining antimicrobial activity and multiple resistance to antibiotics.
The bacterial isolates combining antimicrobial activity and multiple resistance to antibiotics are especially interesting, suggesting that these Antarctic bacteria are potential sources of genes encoding for antimicrobial compounds and antibiotic resistance. These two capabilities probably provide a competitive advantage to Antarctic bacteria to enable them to survive in a harsh environment. These microbial isolates are potential new sources of active compounds for the control of pathogenic microorganisms.

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Bioprospection of lipase producing microorganisms of biotechnological interest.

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Antarctic environments are promising locations for the search of novel bio-productos of biotechnological interest, because they are still little explored. In this study, the bacterial diversity and their cold-active lipolytic activity was investigated in different soil samples from Fildes Peninsula, King George Island. Microbial lipases and esterases are very prominent biocatalysts, which can carry out the hydrolysis and synthesis of ester compounds in aqueous and non-aqueous media. They have wide applications in industry such as detergent, environmental bioremediation and plant waste degradation, among others.
In this work, two primers random amplified polymorphic DNA (TP-RAPD) was used as a fingerprinting method to study the diversity of 74 isolates. Identification of those isolates that presented different pattern bands profiles was performed by sequencing of the 16S rRNA gene. The isolates were evaluated together for their lipolytic and/or esterase capacity according to the presence of halos around their colonies in tributyrin agar and tween esterase agar media respectively. The three isolates which developed the highest ratio between total diameter (halo and colony) and the colony diameter were selected for further studies of enzymatic activity. Lipolytic and esterase activity were studied varying temperature and pH, two key variables that affect enzymes performances. The results obtained in this work, aim to contribute to the knowledge of Antarctic microbial populations and their potential biotechnological application.

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Biotechnological lactic acid production from renewable resources by Carnobacterium sp. isolated from Uruguay Lake, King George Island

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Antarctic psychrotolerant bacteria are promising sources of bioproducts with potential biotechnological applications, such as the production of value-added chemicals. In this work, an isolate (LUA) from a water sample from Uruguay Lake (Fildes Peninsula, King George Island), identified as Carnobacterium spp. was studied for lactic acid production. In the first instance, the optimum growth temperature was determined in synthetic MRS medium. Then, the consumption of different sugars (cellobiose, galactose, xylose, arabinose and fructose) was studied using a modified MRS medium, in which the glucose was replaced by the carbon source evaluated. As LUA consumed glucose and xylose successfully, a lignocellulosic residue, eucalyptus sawdust, was evaluated as a carbon source for the production of lactic acid. This material is an attractive substrate for bioprocesses due to its low cost and little competence with foods. Eucalyptus sawdust hydrolysate fermentations were carried out in a 5 L-bioreactor with and without pH control. Higher productivity was found when the pH was mainteined at 6.5 (0.32 g/Lh-1 and 0.15 g/Lh-1 with and without pH control, respectively). These preliminary results showed that the LUA isolate is a promising strain for lactic acid production. Other operational conditions and culture compositions, such as fed-batch mode and higher initial sugar concentrations, will be studied to improve lactic acid yields.

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Herbicidal activity of Penicillium spp. species obtained from ice and marine sediment of Antarctica

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In this study we evaluated the capacity of Penicillium spp. isolates obtained from ice and marine sediment of Antarctica. Fungi obtained from ice were processed in potato-dextrose-agar (PDA) while those from marine sediments in malt extract agar (MEA) at 15°C for 15 days. After incubation, the mycelia were macerated and transferred to Erlenmeyers, frozen at -20 °C for 72 h, followed by lyophilization for 96 h. The metabolites were extracted with dichlorometane for ice fungi and ethyl acetate for marine fungi. The extracts of all taxa showed strong to moderate herbicidal activity at a concentration of 1 mg mL-1 against the models Lactuca sativa (lettuce) and Allium schoenoprasum (chives). The active Penicillium were identified as P. crhysogenum, P. tardochrysogenum, P. kongii, P. solitum and P. palitans by sequencing the ITS, beta-tubulin and RNA polimerase 2 regions. The results showed that the extracts remained active. In addition, some of them will be selected for Ultra-high performance liquid chromatography (UHPLC) analysis, in order to characterize chemically the herbicidal substances.

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Screening of antioxidant, anti-melanin and cytotoxic activities of microalgal extracts with cosmeceutical potential

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Microalgae are known to be a potential candidate for cosmeceutical applications in human health due to the beneficial properties of bioactive compounds and cosmetic products from natural sources. Microalgae of different region produces secondary metabolites as an adaptive mechanism to maintain survivability against environmental stress. These bioactive compounds were reported to possess antioxidant and anti-melanin properties. Therefore, the aim of this study was to determine the antioxidant properties, anti-melanin properties and cytotoxicity effects of extracts from 11 microalgae strain from the polar, temperate, and tropical regions. The results showed that microalgae from different region exhibit varied growth rate, biochemical composition and antioxidant activities. Higher growth (μ = 0.383 - – 0.474 d^(-1)) was demonstrated by tropical microalgae compared to the polar microalgae (μ = 0.196 – 0.246 d^(-1)). Similar trend was observed for chlorophyll-a, carotenoid and lipid content. In addition, extracts from tropical microalgae (Chlorella UMACC 003) were shown to have lower cytotoxicity (75.87 ± 2.22) on HaCaT cells. Thus, tropical microalgae are potential to be use in cosmeceutical applications.

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Xerophilic activities of fungi associated with the Antarctic plants Deschampsia antarctica and Colobanthus quitensis

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We evaluate the diversity and xerophilic potential of the fungi present in leaves, roots and rhizosphere of Colobanthus quitensis and Deschampsia antarctica living in different sites of South Shetlands Islands, Antarctica. A total of 683 fungal isolates were obtained and identified as 60 taxa. Only 15 fungi were recovered in both plants. The assemblage of D. antarctica showed higher values of diversity, richness and dominance when compared with those of C. quitensis. Pseudogymnoascus destructans colonized systematically leaves, roots and rhizosphere of D. antarctica. Mortierella antarctica, Mortierella gamsii, Penicillium commune, Penicillium jamesonlandense, Penicillium raistrickii, Penicillium spathulatum, Penicillium swecickii and Pseudogymnoascus destructans grew at concentrations of glycerol ≥72% (approximately 0.13 water activity). Our results indicated that Antarctic angiosperms shelter cold-adapted cosmopolitan and endemic fungi. In addition, the xerophilic fungi may produce compounds or shelter genes to further use in agriculture biotechnological processes to help plants with economic potential to growth in drought and cold conditions.

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Identification and diversity of fungi associated with the Antarctic marine invertebrate Ophiuroidea sp.

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In the present study, we access the cultivable fungi associated with Ophiuroidea sampled in Antarctic sea. The Ophiuroidea were sampled at the islands King George and Deception islands using box core device and 29 specimens were analysed. The animals were washed in sterile seawater, the arms were surgically removed, homogenized in 1 mL of sterile seawater, and 100 µL were plated on Sabouread Agar and Marine Agar. Forty-eight fungal isolates were identified, 14 from Ophiuroidea of King George Island and 34 from Deception Island. Aspergillus versicolor, Penicillium tardochrysogenum and Fusarium beomiforme were isolated from both areas. Penicillium rubens was only isolated from King George samples. Therefore, the conclusion is although the fungal diversity has been low, the taxa which were found were already described as source of bioactive compounds, can be used in future biological essays.

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Fungal associated with the Antarctic Diptera Parochlus steinenii (Gerke): taxonomy, ecology and search of bioactive compounds

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We accessed the fungal associated with the Antarctic chironomid Parochlus steinenii, which were captured on King George Island, Antarctica. Fifty individuals were surface disinfested and other 50 were not disinfested. All chironomids were plated on the DRBC, Sabouraud, and Minimum Medium media at 15 °C for up to 60 days. Forty-two filamentous fungi were obtained and identified as Penicillium and Aspergillus taxa. All fungi were growth using solid-state fermentation at 15 °C for 7 days. The media and fungi were lyophilised and submitted to extraction using the solvent dichloromethane. All fungal extracts obtained were submitted to detection of herbicidal activity against Lactuca sativa and Allium schoenoprasum. The extracts of P. chrysogenum UFMGCB 16811 (4.5±0), P. chrysogenum UFMGCB 16803 (3.5±0) and P. commune UFMGCB 16834 (4.0±0.7) had the best results against L. sativa. There wasn't inhibition against A. schoenoprasum. As conclusion, this work shows that the Antarctic chironomid Parochlus steinenii works as a good source for fungal species with high herbicidal activity.

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Temporal monitoring of fungal richness in moss ‘fairy rings’ on the Antarctic Peninsula

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In order to identify the fungi that may be causing the fairy ring disease, we monitored rings and the fungi present in two locations in the South Shetland Islands, Antarctica. Over two years, we collected, photographed, and counted rings on a carpet of Sanionia uncinata showing signs of the disease on King George Island. We also monitored a turf of the moss Polytrichastrum alpinum on Deception Island, visually apparently free of the disease. Over the period the incidence of rings increased on the S. uncinata carpet, while the P. alpinum turf remained visually unaffected. Using molecular approaches, we identified a rich fungal assemblage associated with the rings on S. uncinata, composed of 35 taxa and dominated by Vishniacozyma victoria, Pseudogymnoascus destructans, Cystobasidium larynges, and Mortierella sp. 1. In contrast, that associated with P. alpinum comprised only nine taxa, dominated by Penicillium sp. 1, Phenoliferia glacialis, Antarctomyces psychrotrophicus, and Mrakia frigida. Only P. glacialis, A. psychrotrophicus, P. destructans, Clathrosphaerina sp. 1, and Chalara pseudoaffinis were detected in association with both mosses, but none of these were dominant taxathere. However, fungal species previously reported as causal agents of the fairy rings were not detected. Our data confirm that the fungal assemblage of diseased carpets of S. uncinata is very different of that of non-infected P. alpinum. The high fungal richness associated with S. uncinata suggests that fairy rings may act as an initial gateway for fungal colonization that can then accelerate the spread of disease in mosses in Antarctica.

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Diversity, distribution, and bioprospecting of bioactive compounds in fungi of glacial ice Antarctica

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In this study, we recovered and identified the cultivable fungi present in glacial ice fragments collected at nine sites across Antarctic Peninsula. We also evaluated their potential as producers of bioactive secondary metabolites, useful against neglected tropical diseases, and as the use of herbicides in agriculture. Approximately 20 kg of each bergy bit was collected and ice samples were broken into smaller pieces for surface decontamination with sodium hypochlorite at 5%, sterilized distilled water, and exposed to ultraviolet radiation. We filter 3 L of melted ice through of 0.45 µm membrane in duplicate. The membranes were placed in media different and incubated at 10 °C for 60 d. In total 66 isolates were collected, classified into 27 taxas of 14 genera. Penicillium palitans, Penicillium sp. 1, Thelebolus balaustiformis, Glaciozyma antarctica, Penicillium sp. 7, Rhodotorula mucilaginosa, and Rhodotorula dairenensis had the highest densities. The diversity and richness of the fungal community were high with moderate dominance. Penicillium species were present in all samples, with Penicillium chrysogenum showing the broadest distribution. P. chrysogenum, P. palitans, and Penicillium spp. had trypanocidal, leishmanicidal, and herbicidal activities, with P. chrysogenum having the broadest and highest capability. The ^{1}H NMR signals showed the presence of highly functionalized secondary metabolites. Despite extreme and ultraoligotrophic environmental conditions, glacial ice harbours a diverse fungal community, including species never before recorded in the Arctic and Antarctica. The genus Penicillium may represent wild fungal strains with genetic and biochemical pathways that can produce new secondary bioactive metabolites or not described.

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Isolation of Antarctic and sub-Antarctic microorganisms, for the treatment of soils contaminated by hydrocarbons and their application in soils of Patagonia

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In sub-Antarctic and Antarctic regions it is possible to find historically contaminated soils with hydrocarbons. These soils are interesting for the isolation of bacteria.
Agar cultures of 13 samples of Antarctic soils chronically contaminated with hydrocarbons and 2 samples of subantarctic soils from collection pits of soils contaminated with crude oil were made. Basal saline medium (BSM) with the addition of petroleum crude and Diesel was used to select hydrocarbon degrading bacteria. Antarctic soil plates with the highest number of colonies and subantarctic soil plates were selected for cultivation in liquid medium at 10 ° C. In the liquid media with crude oil, the bacteria of antarctic origin showed the fastest growth.
Liquid cultures were subsequently inoculated into microcosms of subantarctic soils chronically contaminated with crude oil (E1 and E2) and commercial diesel (D). Preliminary, tests showed that the degradation reached 46 and 16% for E1 and E2 respectively, while the degradation for D exceeded 90% in 50 days.
Despite reaching the same degradation, the microcosms inoculated with bacteria showed a degradation kinetics superior. For the E1 soil, the bacteria isolated from the same soil showed the best performance. For the E2 soil, the set of Antarctic bacteria was the one that reached the maximum degradation in the shortest time (20 days).
These preliminary results show that the addition of Antarctic bacteria or isolated from the same soil can accelerate the biodegradation process, which presents an interesting potential to improve the recovery processes of contaminated soils in cold areas.

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Collecting Samples from Northern Islands in Antarctica for Bioprospecting Action

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Bioprospecting is a term that could be explained as “A research interest on plants and microorganism for producing large scaled pharmaceutics” first for scientists then industry. As a result of lack of resources from over-populated human wealth and global warming, it is in need to find new bio-catalyzers and other metabolites for large-scale industrial production. As a results, identification and revelation of potential production capacity of the extramophile organisms. For this kind of specific approach, phylogenic information and preliminary characterization is needed.

Two sampling methods were processed on site from Northern Islands in Antarctica, one sampling method is based on taking samples from the organisms avoiding serious damage to them; second sampling technique is to make 0.20/0.22 µm filtration on both fresh and marine water as 3 liters of sample to obtain eDNA. All sampled material were being stored in RNA/DNA Shield™ until DNA isolation date. Sampling route is started from King George Island (-62,18; -58,89) to Doumer Island (-64,87; -63,58) for all kind of material including water, soil and organisms.

DNA isolation is planned from all samples and DNA-sequencing will be performed in Oxford Nanopore™ Sequencer. For achieving the goal of gene hunting, obtained (consensus) sequence data will be processed on high-end cluster system. Resulting data is planned to be operated for identify organisms that could potencial to produce pharmaceutics. At collection points, GPS and meteorological data were also recorded for further analysis in order to create DNA based phylogenic network.

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Antibacterial activity of Antarctic's Lichen and Seaweeds against human and rainbow trout bacterial pathogens

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Lichens and seaweeds synthesize a great variety of chemically complex. Lichen (Usnea aurantiaco-atra) and seaweeds (Himantothallus grandifolius and Desmarestia confervoides) were collected in 23rd and 25th Peruvian Scientific Expedition from Antarctica, respectively, were transferred to Instituto Tecnologico de la Produccion (Callao-Peru) for processing. Samples were dried, grounded, and ultrasound-assisted extraction with acetone-methanol 1:10 (w/v) (lichen) and methanol 1:3 (w/v) (seaweeds) was performed. Methanol-acetone Extract (MAE) and Methanol Extract (ME) were obtained by vacuumed dried (30 °C).
The antibacterial activity of MAE against Staphylococcus aureus ATCC 14775, Pseudomonas aeruginosa ATCC 27853, Vibrio alginolyticus ATCC 17749, methicillin-resistant S. aureus (MRSA) and multidrug-resistant S. aureus; and MEs against Yersinia ruckeri biotype 1 and 2 from rainbow trout were evaluated by microdilution broth in 96-well plate. The minimum inhibitory concentration (MIC) and the inhibition of bacterial growth (IBG) were determined. Antibacterial activity of MAE was demonstrated against S. aureus (98.43% of IBG at MIC of 31.25 µg/mL), MRSA (98.76% of IBG at MIC of 250 µg/mL) and multidrug-resistant S. aureus (93.90% of IBG at MIC of 62.50 µg/mL). ME of H. grandifolius at MIC value of 96.00 mg/mL showed 98.50 and 98.80% of IBG against Y. ruckeri serotype 1 and 2, respectively and ME of D. confervoides at MIC value of 96.00 mg/mL showed 92.00 and 95.00% of IBG against Y. ruckeri 1 and 2, respectively.
It concluded that Antarctic's lichen and seaweeds present antibacterial property. Besides, U. aurantiaco-atra is a natural and potential source of antibacterial compounds against MRSA even multidrug-resistant S. aureus.

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Fungi-macroalgae associations in Potter Cove

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Aiming to study fungi-macroalgae associations in Potter Cove (25 de Mayo/King George Island, Southern Shetlands) 18 different species of macroalgae were sampled and screened for fungal growth. Fungal isolates (48) were recovered only from six macroalgae species (Gigartina skottsbergii, Palmaria decipiens, Neuroglossum delesseriae, Adenocystis utricularis, Ballia callitricha, Ascoseira mirabilis). These isolates were characterized considering their morphology and growth performance (colony diameter) on potato dextrose agar (PDA) at different temperatures (5 to 35°C). Only 12.5% of the isolates were able to grow at 35°C and, surprisingly, 18.75% were not able to grow at 5°C. All the isolates were able to grow at 15 and 25°C, while 75 % presented the largest colony diameter after 25 days at 25°C. Nevertheless, some isolates grew faster during the first incubation period at a temperature different from that where they showed the largest diameter at 25 days. This could be related to different metabolic adaptation to temperature that each isolate can put forth. After redundancy checking, 38 isolates were identified by molecular biology. Most of them belonged to Penicillium and Cladosporium genera, while others showed to be Cadophora, Antarctomyces, Mycochaetophora, and Pseudogymnoascus. No fungal growth was obtained from the other 12 macroalgae sampled, suggesting a possible antagonistic relation between some of them and the marine fungi in Potter Cove.

1707

The biological potential of Antarctic subsurface brines

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Groundwater systems, subglacial wetlands and hundreds of subglacial lakes exist below the ice sheets of Antarctica. These subice environments are proving to be diverse microbial habitats driven by unique geochemical and hydrological settings. In the McMurdo Dry Valleys of Antarctica, subsurface liquid exists as chemically diverse brines. Two locales where this brine leaks to the surface, enabling direct sampling, are features known as Blood Falls and Don Juan Pond. Extreme environments, such as these unique subglacial and sub-permafrost brines, are a potentially untapped resource for natural products such as enzymes, bioactive compounds or nano-structures with possible biotechnical applications. Here I will highlight recent molecular gene sequencing and cultivation data from these two unique brines, with a focus on intriguing physiological and genomic features of microbial isolates such as diverse pigmentation and gas vesicle production. In a sense, bioprospecting overlaps with research on elucidating the ecological function of natural communities. For example, persisters are a subpopulation of microbial cells that, in clinical settings, are transiently antibiotic tolerant or able to resume growth after a lethal stress exposure. In Antarctica, evidence of 'persistence' has been observed when dormant microbes are revived from ice cores 100s of thousands of years old or awaken from desiccated sediments. Understanding the ecological process of persistence borrows tools and insight from the clinical studies and vice versa. Thus, can exploring clinical applications and ecological understanding go hand-in-hand when studying Antarctic microbial isolates?

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Screening of soil yeasts with fermentative capacity from the Antarctic Continent for their application in the Chilean wine industry.

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The Antarctic Continent has become a crucial resource for the isolation of new microorganisms and secondary metabolites, given the competitive conditions of this environment and the high ultraviolet irradiation.
Considering this, we propose the search of new microorganisms for their use in the wine and beer industry. Considering the selective pressure of the Antarctic continent, we expect to discover new and diverse yeast species with the production of different secondary metabolites as a result of low fermentation temperature that will provide a distinctive set of aromas to the wine. We expect to generate conclusive results, which may contribute to an improvement of Chilean quality, by refining the production and variety of wines which will generate a high impact in this industry.
To achieve this objective, 6 soil samples collected in Fildes Bay, west of King George Island and 3 soil samples from the rhizosphere of Deschampsia antarctica Desv in King George Island, were processed for yeast isolation.
We obtained 125 yeasts from the soil samples, with a growth temperature of 10ºC. Overall, 25 yeasts (31%) have fermentative activity and are able to tolerate a culture medium with at least 20% glucose and up to 6% of ethanol.
The isolates were also characterized by optical microscopy assays and fingerprinting using PCR with arbitrary primers to discard identical strains.
The fermentative yeasts with high alcohol tolerance and fermentation at high concentrations of sugar will be used for micro-fermentation of synthetic must to determine their potential use in the production of Chilean wine.

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Whole genome hybrid assembly for comparative genomics to understand the adaptability traits of Antarctic bacteria

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Antarctic microorganisms might harbor unique features to survive in the extreme environment, which can be exploited for multiple biotechnological applications. However, bacterial adaptative mechanisms to the Antarctic polar region is not completely understood. In fact, reference genomes from Antarctic isolates are scarce on the databases, limiting the possibilities for comparative genomics studies. In this work, we applied a novel approach on whole genome sequencing to obtain reference genomes from bacterial Antarctic strains for comparative genomics analysis. Antarctic bacterial strains (x10) were sequence both by Illumina and Oxford Nanopore technologies. Low quality reads were filtered, and adapters were eliminated using Trimmomatic and NanoFilt/Porechop, respectively. Hybrid assembly was conducted with Unicycler and/or SPAdes and quality was assessed by Quast and CheckM. Pangenome analysis was performed with Roary or Pirate. The reference genomes were successfully assembled in one unique contig for all the strains. Preliminary comparative genomics analysis showed that Antarctic Streptomyces fildesensis devoted a higher percentage of its total genome for the biosynthesis of secondary metabolites. Genes associated with cold-adaptation were also identified in all the strains, including the exoribonuclease R, which is essential at low temperatures as part of the degradosome of some bacterial species. Other bacteria such as Sphingomonas alpina —non-previously reported in the Antarctic— showed unique metal and antibiotic resistance genes when compared to the other members of this genus. Our work highlights that ONT+Illumina hybrid assembly generated high-quality complete genomes, suitable for characterization of unique adaptative mechanisms and subsequent evolution studies on Antarctic bacteria.

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Multi-omics approach revealed culture and elicitation conditions of Antarctic bacteria for the production of antimicrobial compounds

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Concern about finding new antibiotics against drug-resistant pathogens is increasing every year. Antarctic bacteria have been proposed as an unexplored source of bioactive metabolites, however, most biosynthetic gene clusters (BGCs) producing secondary metabolites remain silent under common culture conditions. Our work aimed to characterize elicitation conditions for the production of antibacterial secondary metabolites from 34 Antarctic bacterial strains based on MS/MS metabolomics and genome mining approaches. Each bacterial strain was cultivated under nutrient and elicitation treatments, in a screening of 36 culture conditions, including the addition of lipopolysaccharide (LPS), sodium nitroprusside (SNP) and coculture. Metabolome were obtained by HPLC-QTOF-MS/MS and analyzed through molecular networking. Antibacterial activity was determined for each extract, and seven strains were selected for genome sequencing and analysis. Biosynthesis pathways were activated by all the elicitation treatments, which varies among strains and dependents of culture media. Increased antibacterial activity was observed for few strains and addition of LPS was related with inhibition of Gram-negative pathogens. Selected promising bacterial strains for drug discovery belongs to actinobacteria (Streptomyces fildesensis and Microbacterium sp.), proteobacteria (Sphingomonas alpina, Stenotrophomonas maltophilia and Massilia sp.), and firmicutes phyla (Bacillus subtilis). Antibiotic BGCs were found for all selected strains and the expression of Actinomycin, Carotenoids and Bacillibactin was characterized by comparison of genomic and metabolomic data. This work stablished the use of potential new elicitors for bioprospection of Antarctic bacteria and highlights the importance of new -omics comparatives approaches for drug discovery applied to this extreme and untapped environment.

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Diversity and bioprospecting of fungi present in impacted lakes of Fildes Peninsula, King George Island, Antarctica

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Antarctica has been subjected to global climatic changes over the last 50 years. Many of Antarctic environments are impacted by human activities by pollution problems, due to fuel, oil, waste contamination, and other activities associated with research stations and tourism. In this study, we characterized the diversity of fungi present in sediments samples of three lakes in Fildes Peninsula, King George Island, Antarctica under effect of anthropogenic activities. in addition, we evaluated the production of bioactive compounds by the resident fungi. We identified a fungal community composed by 63 taxa, which Cladosporium sp., Pseudeurotium hygrophilum, and Pseudogymnoascus verrucosus were the most abundant. A statistical comparison of hydrocarbon and heavy metals in sediment analysis demonstrated that the high concentrations of metals coincided with the lowest fungal diversity indices in Central Lake, and it may be influenced by human activities next to research stations, differently to those of the other two lakes, which were far from the stations. These results suggest that increasing anthropogenic activities in the Fildes Peninsula might be affecting microbial diversity and the fungal diversity may be a model to study the impact of human activities in Antarctica. In addition, the fungal isolates were evaluated about their biological activity against different clinical and agricultural pathogens. Among them, 40 fungal isolates demonstrated strong trypanocidal, herbicidal, and antifungal activities, and may represent cell factories for bioactive compounds to develop new drugs and less toxic herbicides.

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Isolation and identification of some microorganism species collected from Horseshoe Island, Skua Lake, Antarctica and microalgae cultivation

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The territory of Antarctica is the ideal place to study specific features of microorganisms that have not discovered yet. Despite significant progress in climatology, fisheries and biodiversity researches in the poles, a little is known about discovering the biological sources and ecological structure of the ocean surrounding Antarctica. Unique ecogeographic and climatological structure of the polar region have led to the microorganisms' adaptation to the region. In this study, some microorganisms were isolated from Horseshoe Island, Skua Lake-Antarctica, collected within the scope of 3rd Turkish Antarctic Expedition between January 25 and March 9, 2019, and identified as Chlorella variabilis (GenBank accession No:MN372092), Blastomonas sp. (GenBank accession No:MN384971) and Achromobacter sp. (GenBank accession No:MN396385). Isolated microalgae (Chlorella variabilis) were cultivated and characterized in the Algal Biotechnology and Bioprocess laboratory, Bioengineering Department of Yildiz Technical University. In this context, microalgae were cultivated in a lab-scale photobioreactor under moderate environment conditions. During cultivation, microalgae growth graph was obtained by spectrophotometric method and growth kinetics were calculated using these data to clear the effect of difference in cultivation conditions of algae. And also, biochemical characterization of microalgae was carried out after harvesting process by centrifugation. When the results of this study were examined, it was seen that isolated photosynthetic microorganisms can live in extreme pole conditions according to their strong adaptability. Besides, it was also determined that polar microalgae are highly rich in bioactive substance content.

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Investigation of antibacterial and antifungal effects of C. variabilis isolated from Horseshoe Island, Skua Lake-Antarctica

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Today, development of algal biotechnology and increasing its importance continues rapidly due to the recent trends and needs. Algae have been used in many different fields for many years due to their rich content of protein, fatty acids, vitamins, minerals etc. Many of these compounds have antimicrobial, antioxidant, antifungal, antiviral and anti-inflammatory properties and play an important role in preventing and treatment of diseases. When some types of microalgae are grown under various stress conditions such as low temperature and low light intensity, they can produce and accumulate substances with antimicrobial and antioxidant properties in the cell. The aim of this study is to investigate the antibacterial and antifungal effects of microalgae isolated from Horseshoe Island, Skua Lake, Antarctica. In this context, Chlorella variabilis (GenBank Accession number: MN372092), isolated from samples collected within the scope of the Turkish Antarctic Expedition-III, was cultivated in a photobioreactor, harvested and extracted. Antibacterial and antifungal effects of obtained extracts from polar microalga were investigated with antimicrobial activity tests by agar disc diffusion assay against bacteria (Escherichia coli ATCC 43888, Bacillus cereus ATCC 11778, Listeria monocytogenes ATCC 13932 and Salmonella typhimurium ATCC 14028) and fungi (Aspergillus brasiliensis ATCC 16404, Candida albicans ATCC 10231). The results of this study showed that the polar microalgal extracts have higher antibacterial and antifungal activities against some bacterial and fungal pathogens than microalgae isolated in moderate environment conditions.

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Low temperatures biosynthesis of photostable CdS quantum dots by UV-resistant psychrophilic bacteria isolated from Union Glacier, Antarctica.

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Fluorescent semiconductor nanocrystals (Quantum Dots, QDs) synthesized by living organisms have gained considerable interest during the last decade because of their unique properties. Extremophile microorganisms can biosynthesize QDs with improved properties such as salt and pH stability in the case of halophilic and acidophilic bacteria, respectively. A common problem of QDs is that constant UV-exposure induces photochemical reactions that affect their structure and stability. Thus, we hypothesized that UV-resistant psychrophilic bacteria from the Union Glacier, Antarctica, a glacier located about 1000 km from the South Pole, could biosynthesize QDs at low temperatures with increased tolerance to UV radiation. The Union Glacier presents high levels of UV radiation during the summer and temperatures below freezing throughout the year. These hyper-extreme conditions favor the development of unique psychrotolerant microorganisms with increased tolerance to UV-radiation. The aim of this study was to evaluate the photostability of CdS QDs produced at low temperatures by UV-resistant bacteria inhabiting the Union Glacier. UV-resistant psychrophilic bacteria were isolated from soil samples of two different sites at the Union Glacier. Three isolates capable of tolerating UV-C doses up to 100 J/m2 were obtained and identified as Paracoccus and Arthrobacter by 16s sequencing. Isolates were capable of biosynthesizing QDs at 4 and 20 °C with excellent optical properties (quantum yield, FWHM, band gap, size) and high photostability when compared with those produced by mesophilic bacteria (Escherichia coli). Finally, the QDs biosynthesized by UV-resistant bacteria showed decreased phototoxicity to E. coli cells when compared with those produced by mesophilic bacteria.

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Advanced biochemical and microbial diversity monitoring system based on micro/nano sensors and micro structure aerators , IoT compatible.

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There is a growing interest in the development of accurate, rapid and mobile methods of bioprospecting, but also for detecting pollutants responsible for contamination of waters with toxic substances. Biosensors are devices subject to continuous development as the detection of biomolecules in a real-time, label-free, highly sensitive and economical manner provides the basis of an attractive technology. We hereby propose acoustic IoT compatible sensors as means of detection and measurement of bioactive molecules.

Typically, a shear horizontal surface acoustic wave device (SH-SAW) consists of a piezoelectric substrate (ST-Z Quartz, Lithium tantalate LiTaO3 (360YX), lithium niobate LiNbO3 and Langasit) with a planar structure of interdigital electrodes transducers (IDT). The acoustic wave sensor uses the piezoelectric effect to electrically excite the acoustic wave at the input transducer (input IDT), which is then received at the output transducer (output IDT). Any biological material containing the analyte is put into contact with the piezoelectric material, that has on its surface a selective bioreceptor layer which can be added as required.

The mechanical and/or electrical interaction between the measurement analyte and the bioreceptor produces a change in the attenuation and/or in the propagation speed of the acoustic wave, which is then registered as the response of the sensor, IoT compatible and capable of transferring real-time measurements data to an online database.

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Characterization of fungi present in sulfide soils in Antarctica and their evaluation for studies in metal bioleaching processes

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We characterized the diversity of cultivable fungi present in sulfide soils located on the Keller Peninsula, King George Island, Antarctica and evaluate them for future studies of metal bioleaching. A total of 85 fungi were isolated and Mortierella amoeboidea, Mortierella globalpina, Mortierella turficola, Penicillium chrysogenum, Penicillium rubens, and Leucosporidium creatinivorum were the most abundant taxa. The fungi Hyaloscypha hepaticicola, Leptobacillium leptobactrum, M. turficola, P. chrysogenum, Penicillium rubens, Periconia prolifica, Pseudogymanoascus destructans, L. creatinivorum, Leucosporidium sp. and R. mucilaginosa were able to grow at pH 3 and also showed marked growth profiles for high temperatures up to 35 ºC and considered promising for future studies on the bioleaching of metals of interest. In the bioleaching test using a mining tailing complex, P. chrysogenum UFMGCB 17938 appears to have absorbed some metals of interest (Sr and Zn), but the bioleaching of Fe and Mn (metals most abundant in the tailings) was inconclusive. Finally, from the data sets obtained, acidophilic and mesophilic fungi present in the sulfide soils of Antarctica can be explored in the future with organisms capable of acting in processes of bioleaching of metals of interest in the mining industry.

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Conservation and characterisation of Polar Cyanobacteria by the BCCM/ULC collection

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The BCCM/ULC public collection funded by the Belgian Science Policy Office since 2011 aims to gather a representative portion of terrestrial, freshwater and marine cyanobacterial strains with a focus on the polar diversity with different ecological origins (limnetic mats, soil crusts, cryoconites, endoliths…). The collection's aim is to preserve the deposited biological material, to valorize it by performing research on it, to provide it to interested parties for fundamental and applied research, and to provide services linked to the identification of the Cyanobacteria for the scientific community. An ISO 9001 certificate was obtained for the public deposition and distribution of strains, as part of the multi-site certification for the BCCM consortium.
Currently, the ULC collection contains 253 cyanobacterial strains, with 134 being of Polar origin. Cyanobacteria in the Polar Regions represent key primary producers and are the main drivers of the food webs in a wide range of aquatic to terrestrial habitats. Due to their harsh environments, all these Polar Cyanobacteria may present interesting features to survive, for example, freeze/thaw cycles, fluctuating salt concentrations, high UV radiations, desiccation and other stresses. Morphological identification shows that the strains of BCCM/ULC belong to the orders Synechococcales, Oscillatoriales, Pleurocapsales, Chroococcidiopsidales and Nostocales. The 189 BCCM/ULC strains, for which 16S rRNA sequences were analyzed correspond to 69 OTUs (sequences with > 99 % 16S rRNA similarity), representing a large diversity

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The first report of microcystin producing Wilmottia murrayi from cryopreserved Antarctic cyanobacterial mats

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A new microcystin producing benthic cyanobacterium Wilmottia murrayi was isolated from a deep-frozen (-15^{o}C) sample collected from a cyanobacterial mat in a pond at Northern Victoria Land, Antarctica in 1985. Two strains successfully isolated from two different sites were characterised using both morphological and molecular approaches. Morphologically, the strains resemble Phormidium Kützing ex Gomont (1892). However, phylogenetic analyses using partial 16s rRNA sequences of the two strains showed that they formed a well-supported monophyletic clade with other Wilmottia murrayi and were well separated from Phormidium. Amplification of a fragment of the mcyE gene involved in microcystin biosynthesis from both Wilmottia murrayi strains confirmed the presence of this genetic determinant.This is a new report of microcystin synthesis from the cyanobacteria species Wilmottia murrayi both in Antarctica and worldwide.

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Heterocyclic compounds degradation using bacteria isolated from Antarctic soil

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Oil contamination in soil and seawater is undeniably a serious environmental concern with prolonged consequences towards the ecosystem. Oil contains heterocyclic compounds such as carbazole (CAR), dibenzothiophene (DBT) and dibenzofuran (DBF), are highly stable due to their planar ring structures, will reside in the environment for long periods unless treated effectively. The objective of this study is to isolate heterocyclic compounds degrading bacteria from the Antarctic environment for future development of bioremediation solution for cold environments. Enriched culture approach was chosen to isolate bacterial strains with ability to utilize CAR as sole carbon sources in minimal salt medium. The culture enrichment cycle were repeated at least 3 times at 15 ℃ using 100 rpm rotary shaker. As a result, a total of six strains were isolated after 2 months of shaking. The isolates showed CAR utilization at temperatures ranging from 5 ℃ to 35 ℃ with optimum growth between 15 to 20 ℃. Analyses of residual CAR using GC showed complete substrate utilization after 9 days. Utilization of DBT, DBF, biphenyl, fluorene and phenanthrene were also confirmed by agar media growth. These results indicated that these isolates posses wide range substrate utilization ability. Further studies on these strains will lead to more understanding for application of bioremediation in the future.

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Seiryu Take[1], Tatsuya Niwano[1], Siti Aqlima Ahmad[2], Peter Convey[3], Claudio Gomez-Fuentes[4], Azham Zulkharnain[1]

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