DOI 10.12905/0380.sydowia70-2018-0185 Published online 30 August 2018

Biodiversity and antimicrobial activity of Antarctic fungi from the Fildes Peninsula, King George Island

Silvana Alborés[1,*], Paula Sanguiñedo[1], Benjamin H. Held[2], María Pía Cerdeiras[1] & Robert A. Blanchette[2]

[1] Área de Microbiología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Montevideo, Uruguay
[2] Department of Plant Pathology, University of Minnesota, St. Paul, MN, 55108, USA

* e-mail: salbores@fq.edu.uy

Alborés S., Sanguiñedo P., Held B.H., Cerdeiras M.P. & Blanchette R.A. (2018) Biodiversity and antimicrobial activity of Antarctic fungi from the Fildes Peninsula, King George Island. – Sydowia 70: 185–191.

Despite adverse conditions in Antarctica for life, organic materials can be successfully colonized by extremophilic microorganisms. This work describes the isolation of filamentous fungi from terrestrial and maritime habitats of King George Island, Antarctica, and the screening of their ability to produce antimicrobial compounds. A total of 44 samples were collected during the Antarctic expeditions from soil, sediments, terrestrial and marine waters and pieces of wood that had been brought into Antarctica. One hundred thirty-seven psychrotrophic fungi were isolated. Thirty-three strains selected from different samples and with different morphological characteristics were used in molecular identification and antimicrobial activity assays. The ability of all isolations to inhibit growth of different bacteria *in vitro* was tested using the agar plug diffusion method.

Most of the fungi identified were Ascomycota with only a few Zygomycota and no Basidiomycota isolated. Twenty-six cultures corresponded to known species. *Penicillium* was the most represented genus of identified fungal isolations (24 %) followed by species of *Cadophora, Mortierella, Pseudogymnoascus* and *Neurospora*. This is the first report of the presence of *Aspergillus pseudodeflectus* in this region. Of the isolates under study, eighteen were able to inhibit growth of at least one of the bacterial strains used.

Keywords: antarctic fungi, biodiversity, antimicrobial activity.

High radiation, low humidity, and extremely cold environments pose adverse conditions for microbial growth. In Antarctica, where some of the most extreme environmental conditions can be found, extremophilic microorganisms are able to successfully colonize organic materials. Two groups of microorganisms can be found in these environments. Psychrophiles, which are defined by an optimum growth temperature of 15 °C, a maximum growth temperature below 20 °C, and a minimum growth temperature equal to or below 0 °C, and psychrotrophic or psychrotolerant microorganisms, which are cold-adapted mesophiles, and compete well for resources (Mohamed Hatha et al. 2013). Psychrotrophic microorganisms have developed strategies to resist seasonal climate change as well as to protect themselves against nutritional and environmental stress of extremely cold regions, which sometimes can be an advantage over obligate psychrophiles. Interest in Antarctic microorganisms has increased due to their ability to grow in extreme environments and for their potential to be a source of novel bioactive compounds, such as antibiotics and enzymes (Nedialkova & Naidenova 2005). Although microorganisms representing all major taxonomic groups have been found in Antarctica, most studies on antimicrobial activity from the Antarctic region have focused on bacteria (Cheah et al. 2015, Leiva et al. 2015). Since psychrophilic microorganisms require specific conditions and may grow slowly, the costs for large-scale industrial production would likely be higher than those involved in the culture of psychrotrophes.

In spite of the great advances in chemotherapy, and the existence of a great variety of antibacterial and antifungal drugs in clinical use, infectious diseases remain one of the main causes of death in the world. One of the main reasons is the appearance of organisms that have developed resistance to various antibiotics. The global report by The World Health Organization indicates that antibacterial resistance threatens the prevention and treatment of various infections induced by microorganisms (OMS, 2014). Plant, fungal and bacterial natural products can be used as drugs against many diseases. Among the natural sources of antimicrobial compounds, fungi are very promising in terms of production of novel bioactive structures (Alborés et al. 2014, Barneche et al. 2016, Prior et al. 2017).

This work describes the isolation of filamentous fungi from terrestrial and maritime habitats of King George Island, Antarctica, the largest island within the South Shetland Island archipelago. As typical of this region of Antarctica, the island hosts a great diversity of ecosystems and habitats, including vegetation zones, pristine soils, as well as vertebrate-influenced habitats and sites under human impact (Krishnan et al. 2011). Fungal isolates obtained were identified by molecular characterization of their rDNA and their ability to produce antimicrobial compounds was evaluated.

Materials and methods

Sample collection

Filamentous fungi were isolated from soil and terrestrial and marine waters from different sites on the Fildes Peninsula, King George Island (Lat 62° 11′ 04″ S; Long 58° 54′ 25″ W), situated 120 km off the coast of the Antarctic Peninsula in the Southern Ocean. Samples were collected during expeditions organized by the Instituto Antartico Uruguayo in December 2014 and January 2016. Different samples were taken from soil, sediments, water, moss, lichens, feathers, penguin feces, pieces of wood, in the various locations of Norme Cove, Ardley Island, Uruguay Lake, Collins Glacier, Collins Bay, Drake Sea (Fig. 1). They were collected in sterile tubes and kept at 0 °C until processed.

Fungal isolation

In order to obtain pure cultures, 10 g of the different terrestrial samples were suspended in 90 ml of sterile water and 100 µl of the obtained suspension and 1/10 and 1/100 dilutions were spread onto the surface of Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) (Merk, Darmstadt, Germany) plates. For water samples, 100 ml were filtered and the membrane filters (0.45 µm pore, Millipore)

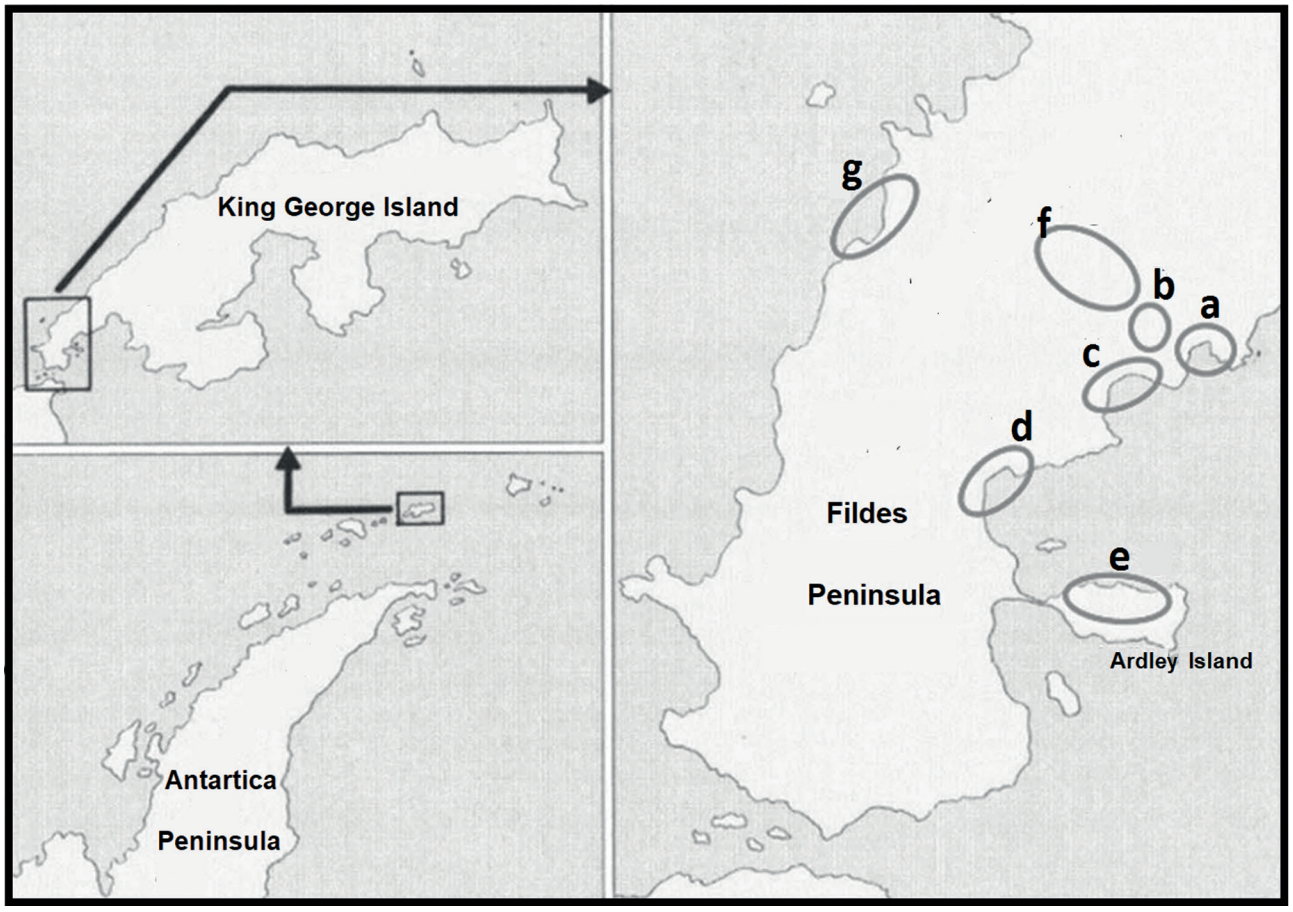

Fig. 1. Map of King George Island showing the sites where samples were collected. **a** Collins Bay, **b** Uruguay Lake, **c** Norme Cove, **d** Ardley Cove, **e** Ardley Island, **f** Collins Glacier, **g** Drake Bay.

were placed in petri dishes with DRBCA. Plates were incubated at 20 °C for one week after which colony forming units (= CFUs) were counted and subcultures made of all morphologically distinct colonies from each sample in Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK). Plates that had no visible CFUs after one week were kept for at least two months to ensure slow growing isolates were not excluded. For preservation, all isolates in PDA were transferred to tubes with PDA and the grown cultures were covered with sterile mineral oil.

Thirty-three isolates from a total of 100 isolates were selected, based on their different morphological characteristics and different sample sites, for the antimicrobial activity assays.

Fungal identification

For molecular identification, DNA isolation by a cetyltrimethylammonium bromide (CTAB) extraction procedure was done from pure cultures. Fungal hyphae from approx.. ¼ of a Petri dish were scraped from the surface of an actively growing culture (on 15 % malt extract agar) and suspended in 500 µl of CTAB lysis buffer with glass beads and vortexed for 1 min. After brief centrifugation to aggregate hyphal material, supernatant was transferred to a new microcentrifuge tube and placed in a hotwater bath (65 °C) for 20 min. Then 500 µl of chloroform/phenol/isoamyl alcohol (25:24:1) was added to each tube and mixed vigorously and centrifuged for 5 min at 15871 rcf. The supernatant was then removed and transferred to a clean microcentrifuge tube and isopropanol (stored at –20 °C) was added, gently mixed and incubated, for 5 min, at room temperature. Tubes were centrifuged for 7 min at 21130 rcf and isopropanol was then carefully removed. The DNA pellet remaining was washed with 500 µl of 70 % ETOH (stored at –20 °C) and centrifuged for 3 min at 21130 rcf after which the ETOH was removed and tubes were left in a sterilized bio-safety cabinet to air dry. Finally, DNA was rehydrated with 100 µl of sterile water. The internal transcribed spacer region (ITS) of rDNA was amplified using the primer combination ITS1F/4 via PCR (Gardes & Bruns, 1993). One ml of DNA template was used in each PCR reaction containing1 µl of each primer (5 µM), 0.5 µl BSA, 12.5 µl of GoTaq® Green Master Mix and 9.5 µl of sterile water in a thermocycler with the following program: 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 5 min. Amplicons were verified by electrophoresis on a 1 % agarose gel with a SYBR green 1 prestain and transilluminated with a Dark Reader DR45 (Clare Chemical Research, Denver, Colorado). Sequencing was carried out using both forward and reverse primers using an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA). A consensus sequence was assembled using Geneious 7.0 (Kearse et al. 2012), sequences were aligned with MEGA version 7, visually corrected and compared to NCBI databases using BLAST. DNA sequences were aligned with sequences of homologous regions of closely related strains retrieved from GenBank using, if possible, accessions from taxonomic publications. Moreover, morphological (macro- and microscopic) observations were done.

Antimicrobial activity

The ability of all isolations to inhibit growth of different bacteria *in vitro* was tested using the agar plug diffusion method as described by Balouiri et al. (2016). Briefly, a culture of each Antarctic fungus was grown on PDA for 10 days. After incubation, an agar-plug was cut aseptically with a sterile cork borer and placed on the agar surface of another plate with nutrient agar medium (Oxoid) previously inoculated with test bacteria. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC *15442, Staphylococcus aureus* ATCC 6538p and *Bacillus subtilis* ATCC 6633 were used for the assay bacteria tested. The antimicrobial activity of the fungal secreted molecules was detected by the appearance of an inhibition zone around the agar plug and the zone of inhibition was measured.

Results

Fungal isolations

A total of forty-four samples were collected during the Antarctic expeditions from soil, sediments, terrestrial and marine waters and pieces of wood. Fungal counts in all samples were between 3×10^2 and 6×10^4 cfu/g, showing filamentous colonies in most cases, except for samples of water from Uruguay Lake, where no filamentous colonies were recovered. One hundred thirty-seven psychrotrophic fungi were isolated in PDA, subcultured and preserved for future applications. Thirty-three strains selected from different samples and with different morphological characteristics were used in molecular identification and antimicrobial activity assays.

Fungal identification

Thirty-three isolates were identified by sequencing of the ITS region of rDNA. All sequences showed 98–100 % homology to sequences in GenBank when

Tab 1. Strains isolated in this study with GenBank accession no. and the best BLAST match with percent identity of the ITS gene region and source. *

Strain	GenBank No.	Source	Best BLAST match	GenBank accession	% query coverage	% identity
CCMGE406	MH791332	Ardley Island (piece of wood)	*Alternaria alternata*	KY609180	99	100
CCMG111	MH790295	Ardley Island (soil, moss, bird feces)	*Aspergillus pseudodeflectus*	NR_135372	90	100
CCMGE413	MH791350	Norme Cove (piece of wood)	*Cadophora fastigiata* E18	FJ903336	90	100
CCMGE402	MH790979	Ardley Island (piece of wood)	*C. melinii*	DQ404351	97	98
CCMGE404	MH791324	Ardley Island (piece of wood)	*Cadophora* sp. ICMP 18087	HM116752	99	98
CCMGE408	MH791342	Ardley Island (piece of wood)	*Cadophora* sp. ICMP 18087	HM116752	99	98
CCMGE45	MH790419	Ardley Island (moss)	*Cladosporium cladosporioides*	KU182497	99	99
CCMG4410	MH790406	Norme Cove (soil, moss, bird feces, feathers)	*Cordyceps confragosa*	AB111495	99	99
CCMG4412	MH790411	Norme Cove (soil, moss, bird feces, feathers)	*Cosmospora viridescens*	KC291731	94	99
CCMGE112	MH790549	Collins Bay (moss and lichen)	*Neurospora crassa*	FJ360521	99	99
CCMGE12	MH790416	Ardley Island (lichens)	*N. crassa*	FJ360521	100	99
CCMGE72	MH790467	Ardley Island (soil and defrost water)	*N. crassa*	JN188473	89	99
CCMGE181	MH790978	Norme Cove (moss)	*N. crassa*	FJ360521	99	99
CCMG203	MH791153	Ardley Island (soil)	*Paecilomyces variotii*	JN227050	61	99
CCMG323	MH790323	Collins Bay (moss)	*Penicillium carneum*	DQ339566	100	99
CCMG95	MH790294	Ardley Island (soil, moss)	*P. chrysogenum*	HQ026745	99	99
CCMG362	MH790330	Collins Bay (pieces of wood)	*P. chrysogenum*	HQ026745	99	99
CCMG395	MH790329	Uruguay Lake (soil)	*P. chrysogenum*	HQ026745	99	99
CCMG202	MH790322	Ardley Island (soil)	*P. chrysogenum*	KR233462	97	99
CCMG91	MH790116	Ardley Island (soil, moss)	*P. polonicum*	GU566208	99	99
CCMG341	MH790328	Norme Cove (soil)	*P. polonicum*	GU566208	99	99
CCMG131	MH790319	Collins Glaciar (soil)	*P. polonicum*	GU566208	100	100
CCMG454	MH790405	Uruguay Lake (soil)	*Pseudogymnoascus* sp. TW 236	KP902683	99	98
CCMGE55	MH790447	Drake Bay (soil and defrost water)	*Pseudogymnoascus* sp. TW 236	KP902683	100	99
CCMGE59	MH790448	Drake Bay (soil and defrost water)	*Pseudogymnoascus* sp. TW 236	KP902683	99	99
CCMG441	MH790391	Norme Cove (soil, moss, bird feces, feathers)	*Sarocladium kiliense*	AJ621775	89	100
CCMG204	MH790324	Ardley Island (soil)	*Talaromyces radicus*	HM469413	94	99
CCMG361	MH790327	Collins Bay (pieces of wood)	*T. stollii*	JX965246	94	99
CCMGE412	MH791373	Norme Cove (piece of wood)	*Truncatella angustata*	KT963797	99	99
CCMGE41	MH790415	Ardley Island (moss)	*Mortierella alpina*	KJ469836	97	99
CCMGE171	MH790889	Norma Cove (soil)	*M. antarctica*	KP714645	93	100
CCMGE172	MH790905	Norma Cove (soil)	*Mortierella* sp. 03VT03	JX270364	98	99
CCMGE103	MH791155	Ardley Island (soil)	*M. turficola*	JX975896	90	99

* Duplicate copies of strains were deposited in the fungal collection (CCMG) in the Department of Bioscience, Universidad de la Republica (Uruguay) and in the Department of Plant Pathology in the University of Minnesota (UMSP) (USA).

using Blast searches (Tab. 1). Most of the fungi identified were Ascomycota with only a few Zygomycota. No Basidiomycota were isolated. The Zygomycota were all identified as different species of *Mortierella*. Among the Ascomycota, fungal isolates were affiliated with 13 different genera. Twenty-six cultures corresponded to known species. Sequences of isolates 91, 95, 131, 202, 341, 362 and 395 showed 99 % homology with several species of *Penicillium* which were identified as three different species. Isolates identified as *Penicillium* were found in most samples collected from different sites on the Fildes Peninsula (Tab. 1). Furthermore, *Penicillium* was the most represented genus of the identified fungal isolations (24 %).

Antimicrobial activity

Antimicrobial activity of all identified strains was evaluated against four different bacteria: *S. aureus*, *P. aeruginosa*, *E. coli* and *B. subtilis*. From 33 fungal strains evaluated, 18 showed an inhibition halo in the dual cultures against at least one of the bacteria (Tab. 2). Most of these fungi were active against the Gram-positive bacteria *B. subtilis* and *S. aureus* (14 and 12, respectively). The Gram-negative bacteria *P. aeruginosa* and *E. coli* growth were inhibited by six and two fungal strains, respectively. These fungi were isolated from different substrates and identified as *Pseudogymnoascus* sp., *Cadophora melinii*, *Alternaria alternata*, *Penicillium polonicum* and *Sarocladium kiliense*.

Discussion

In this work, filamentous fungi were isolated from different sites at King George Island. A culture base method for fungal detection was used so that cultures would be available for antimicrobial testing. Although many species of non-culturable fungi have been detected in previous studies using molecular techniques, previous research on soils from Antarctica using similar media types as well as the molecular detection technique of denaturing gradient gel electrophoresis found that approximately 2/3 of total taxa could be detected using culturing-based investigations (Arenz et al. 2006). Although our methodology may not have identified all the fungi that might have been present in the various samples, this study provided a large number of diverse isolates for antibiotic evaluations.

As previously reported in studies conducted in different regions of Antarctica, also in our investigation the most frequently encountered fungi are asexual morphs of Ascomycota. These fungi have short life cycles, often produce asexual spores, and limit their metabolic investment in sexual reproduction. They can colonize substrates in extreme environments and tolerate various toxic substrata, like salts, unlike other groups of fungi (Krishnan et al. 2016).

Pseudogymnoascus species are common in cold environments, have been frequently recorded in Antarctica with a ubiquitous distribution, and can be isolated from many different substrates (Minnis & Lindner 2013, Godinho et al. 2015). In our work, *Pseudogymnoascus* strains were isolated from two different samples (from Uruguay Lake and Drake Bay) collected at different times. At present, it is difficult to determine species of this genus using just ITS sequences. To accurately identify *Pseudogymnoascus* isolates, sequencing of additional genes is necessary as the ITS region is insufficient to resolve to the species level (Muller et al. 2013).

Penicillium has been described as a very common fungal genus in Antarctic ecosystems, as it has been found in samples from soils (Marfenina et al. 2016), wood (Arenz et al. 2006), marine sediments (Gonçalves et al. 2013), sponges (Henríquez et al. 2014), and macroalgae (Godinho et al. 2013). Similarly, in the work presented here, *Penicillium* strains were isolated from different sources (moss, wood and soil) at several different sites on King George Island.

Although many *Aspergillus* species have been frequently found in investigations from different places in Antarctica (Godinho et al. 2015, Henríquez et al. 2014, Blanchette et al. 2016), this is the first report of *Aspergillus pseudodeflectus* in this region. This fungus was isolated from a sample collected in Ardley Island, which is one of the Antarctic Specially Protected Areas. *Cladosporium* is one of the largest genera of dematiaceous hyphomycetes, which includes saprobic and parasitic species, with a worldwide distribution. In Antarctica, it has been found associated with plants and is one of the dominant genera in the soil as well as historic woods (Arenz et al. 2006, Blanchette et al. 2010, Godinho et al. 2015). We isolated a strain of *Cladosporium cladosporioides* from a sample of moss from Ardley Island. Godinho et al. (2015) recovered this species from soil samples in Continental Antarctica.

The fungi able to survive the harsh extreme environment of Antarctica may have different biochemical pathways used to generate new compounds that could be used for new pharmaceuticals (Santiago et al. 2012). In this study, we screened the

antibacterial activity of the fungal isolates we obtained, of which 18 showed active inhibitions. The agar plug diffusion method is a relatively simple and efficient way of screening for antibacterial activity. No visible inhibition zones may indicate absence of antimicrobial agent, complete resistance, or be attributed to the fact that the bioactive metabolite might not diffuse far enough into the agar to form a visible inhibition zone. Normally, a large zone indicates more effective antimicrobial activity or greater diffusion of the inhibitory compounds that were produced, or both (Cheah et al. 2015).

Intrinsic resistance is the innate ability of bacteria to resist activity of a particular antimicrobial agent through its inherent structural characteristics, such as the outer membrane of Gram-negative bacteria. In general, in our work, Gram-negative bacteria (*P. aeruginosa* and *E. coli*) were inhibited by a smaller number of fungal strains than the Gram-positive bacteria (*B. subtilis* and *S. aureus*). These results could be attributed to intrinsic resistance to antimicrobial compounds of Gram-negative bacteria (Perricone et al. 2015).

Aspergillus and *Penicillium* species are well-known producers of many bioactive compounds, but few species found in Antarctica have been chemically investigated. Extracts from *Penicillium* and *Aspergillus* species present in the cold-arid oligotrophic soil of Antarctica were able to produce antibacterial compounds (Godinho et al. 2015). According to our results, all strains of these genera, except for one, inhibited the growth of at least one bacterium.

Results showed discrepancies among the antibacterial activity of different isolates of the same fungal species (*Cadophora melinii, Penicillium chrysogenum* and *Penicillium polonicum*). This result is not uncommon, as distinct secondary metabolites can be produced by conspecific isolates in other fungi, as previously reported with fungal strains isolated from soil of continental Antarctica (Godinho et al. 2015). Thus, if secondary metabolic diversity is of interest, for potential future applications it is important to keep different isolates of the same species in the culture collections.

In the study reported here, we demonstrate that *Cordyceps confragosa* was also able to inhibit *B. subtilis* growth. A recent study reported a strain of this genus collected from moss in Antarctica as a powerful producer of cold tolerant enzymes (Fenice, 2016).

Several fungal isolates demonstrated to be a source of antibacterial compounds that may confer advantages during competition with dominant species and help the species survive in the extreme environmental conditions. These bioactive fungi also represent a potential source of molecules to use in drug and agrochemical studies. Additional characterization of these isolates to produce potentially useful antibiotic compound(s) is warranted to better elucidate their identity, structure and function.

Acknowledgements

This work was supported by the Comisión Sectorial de Investigación Científica (CSIC, Universidad de la República), Program for the Development of Basic Sciences (PEDECIBA-Química) and Instituto Antártico Uruguayo (IAU), Uruguay.

References

Alborés S., Mora P., Bustamante M.J., Cerdeiras M.P., Franco Fraguas L. (2014) Purification and applications of a lectin from the mushroom *Gymnopilus spectabilis*. *Applied Biochemistry and Biotechnology* **172**: 2081–2090.

Arenz B.E., Held B.W., Jurgens J.A., Farrell R.L., Blanchette R.A. (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. *Soil Biology Biochemistry* **38**: 3057–3064.

Balouiri M., Sadiki M., Ibnsouda S.K. (2016) Methods for in vitro evaluating antimicrobial activity: a review. *Journal of Pharmaceutical Analysis* **6**: 71–79.

Barneche S., Jorcin G., Cecchetto G., Cerdeiras M.P., Vázquez A., Alborés S. (2016) Screening for antimicrobial activity of wood rotting higher basidiomycetes mushrooms from uruguay against phytopathogens. *International Journal of Medicinal Mushrooms* **18**: 261–267.

Blanchette R.A., Held B.W., Arenz B.E., Jurgens J.A., Baltes N.J., Duncan S.M., Farrell R.L. (2010) An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. *Microbial Ecology* **60**: 29–38.

Blanchette R.A., Held B.W., Hellmann L., Millman L., Büntgen U. (2016) Arctic driftwood reveals unexpectedly rich fungal diversity. *Fungal Ecology* **23**: 58–65.

Cheah Y.K., Lee L.H., Chieng C.Y.C., Wong V.L.C.M. (2015) Isolation, identification and screening of actinobacteria in volcanic soil of deception island (the Antarctic) for antimicrobial metabolites. *Polish Polar Research* **36**: 67–78.

Fenice M. (2016) The psychrotolerant antarctic fungus *Lecanicillium muscarium* CCFEE 5003: A powerful producer of cold-tolerant chitinolytic enzymes. *Molecules* **21**: 447.

Gardes M., Bruns T.D. (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Godinho V.M., Furbino L.E., Santiago I.F., et al. (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. *International Society for Microbial Ecology Journal* **7**: 1434–1451.

Godinho V.M., Gonçalves V.N., Santiago I.F., et al. (2015) Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. *Extremophiles* **19**: 585–596.

Gonçalves V.N., Campos L.S., Melo I.S., Pellizari V.H., Rosa C.A., Rosa L.H. (2013) *Penicillium solitum*: A mesophilic, psychrotolerant fungus present in marine sediments from Antarctica. *Polar Biology* **36**: 1823–1831.

Henríquez M., Vergara K., Norambuena J. et al. (2014) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. *World Journal of Microbiology and Biotechnology* **30**: 65–76.

Kearse M., Moir R., Wilson A. et al. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Krishnan A., Convey P., Gonzalez-Rocha G., Alias S.A. (2016) Production of extracellular hydrolase enzymes by fungi from King George Island. *Polar Biology* **39**: 65–76.

Krishnan A., Alias S.A., Wong C.M.V.L., Pang K.L., Convey P. (2011) Extracellular hydrolase enzyme production by soil fungi from King George Island, Antarctica. *Polar Biology* **34**: 1535–1542.

Leiva S., Alvarado P., Huang Y., Wang J., Garrido I. (2015) Diversity of pigmented Gram-positive bacteria associated with marine macroalgae from Antarctica. *FEMS Microbiology Letters* **362**: 1–6.

Marfenina O.E., Nikitin D.A., Ivanova A.E. (2016) The structure of fungal biomass and diversity of cultivated micromycetes in Antarctic soils (progress and Russkaya Stations). *Eurasian Soil Science* **49**: 934–941.

Minnis A.M., Lindner D.L. (2013) Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology* **117**: 638–649.

Mohamed Hatha A.A., Rahiman Mujeeb R.K., Krishnan K.P., Saramma A.V., Saritha G., Lal D. (2013) Characterization and bioprospecting of cold adapted yeast from water samples of Kongsfjord, Norwegian Arctic. *Indian Journal of Marine Science* **42**: 458–465.

Muller L.K., Lorch J.M., Lindner D.L., O'Connor M., Gargas A., Blehert D.S. (2013) Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic region of *Geomyces destructans*. *Mycologia* **105**: 253–259.

Nedialkova D., Naidenova M. (2005) Screening the antimicrobial activity of Actinomycetes strains isolated from Antarctica. *Journal of Culture Collections* **4**: 29–35.

OMS (2014) Estadísticas Sanitarias Mundiales 2014: Una mina de información sobre salud pública mundial. *http://www.who.int/gho/publications/world_health_statistics/2014/es/*.

Perricone M., Arace E., Corbo M.R., Sinigaglia M., Bevilacqua A. (2015) Bioactivity of essential oils: a review on their interaction with food components. *Frontiers in Microbiology* **6**: 1–7.

Prior R., Feige, A., Begerow, D. (2017) Antagonistic activity of the phyllosphere fungal community. *Sydowia* **69**: 183–198.

Santiago I.F., Alves T.M.A., Rabello A. et al. (2012) Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. *Extremophiles* **16**: 95–103.

(Manuscript accepted 22 August 2018; Corresponding Editor: I. Krisai-Greilhuber)