Journal Pre-proof

Studies toward the comprehension of fungal-macroalgae interaction in cold marine regions from a biotechnological perspective

M.M. Martorell, M. Lannert, C.V. Matula, M.L. Quartino, L.I.C. de Figueroa, WP Mac Cormack, L.A.M. Ruberto

PII: S1878-6146(20)30173-2

DOI: https://doi.org/10.1016/j.funbio.2020.11.003

Reference: FUNBIO 1194

To appear in: *Fungal Biology*

Received Date: 22 August 2020

Revised Date: 2 November 2020

Accepted Date: 12 November 2020

Please cite this article as: Martorell, M., Lannert, M, Matula, C., Quartino, M., de Figueroa, L., Cormack, W.M., Ruberto, L., Studies toward the comprehension of fungal-macroalgae interaction in cold marine regions from a biotechnological perspective, *Fungal Biology*, https://doi.org/10.1016/j.funbio.2020.11.003.

Studies toward the comprehension of fungal-macroalgae interaction in cold marine regions from a biotechnological perspective

MM Martorell[1,2,3], M Lannert[1], CV Matula[1], ML Quartino[1], LIC de Figueroa [4,5], WP Mac Cormack[1,2,3], LAM Ruberto[1,2,3]

1, Instituto Antártico Argentino (IAA), Buenos Aires, Argentina

2, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

3, Instituto de Nanobiotecnología (NANOBIOTEC-UBA-CONICET), Buenos Aires, Argentina

4, Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina.

5, Universidad Nacional de Tucumán, Tucumán, Argentina

To whom correspondence should be addressed: María Martha Martorell**, marmartorellsaez@gmail.com** (ORCID ID 0000-0002-4266-8629)

ABSTRACT

In marine ecosystems, macroalgae are the habitat for several microorganisms, fungi being among them. In the Antarctic benthic coastal ecosystem, macroalgae play a key role in organic matter cycling. In this study, 13 different macroalgae from Potter Cove and surrounding areas were sampled and 48 fungal isolates were obtained from six species, four Rhodophyta *Ballia callitricha*, *Gigartina skottsbergii*, *Neuroglossum delesseriae* and *Palmaria decipiens*, and two Phaeophyceae*: Adenocystis utricularis* and *Ascoseira mirabilis*. Fungal isolates mostly belonged to the *Ascomycota* phylum (*Antarctomyces*, *Cadophora*, *Cladosporium*, *Penicillium*, *Phialocephala*, and *Pseudogymnoascus)* and only one to the phylum *Mucoromycota*. Two of the isolates could not be identified to genus level, implying that Antarctica is a source of probable novel fungal taxa with enormous bioprospecting and biotechnological potential. 73% of the fungal isolates were moderate eurypsychrophilic (they grew at 5-25°C), 12.5% were eurypsychrophilic and grew in the whole range, 12.5% of the isolates were narrow eurypsychrophilic, (growth at 15-25°C), and *Mucoromycota* AUe4 was classified as stenopsychrophilic as it grew at 5-15°C. Organic extracts of seven macroalgae from which no fungal growth was obtained (three red algae *Georgiella confluens*, *Gymnogongrus turquetii*, *Plocamium cartlagineum*, and four brown algae *Desmarestia anceps*, *D. Antarctica*, *D.*

menziesii, *Himantothallus grandifolius*) were tested against representative fungi of the genera isolated in this work. All extracts presented fungal inhibition, those from *P. cartilagineum* and *G. turquetii* showed the best results, and for most of these macroalgae, this represents the first report of antifungal activity and constitute a promising source of compounds for future evaluation.

Keywords: filamentous fungi, macroalgae, Antarctica, antifungal, psychrophile.

INTRODUCTION

The macroalgae community plays a key role all around the planet, this role being more important in temperate and cold seas (Dayton 1985) including the coastal Antarctic ecosystem (Wiencke and Amsler 2012) where, in contrast to the scarce diversity of terrestrial plants, coastal marine environments exhibit a large abundance of different species (Wiencke et al. 2007). As one of the most important primary producers, they supply food for the Antarctic benthic organism and contribute significantly to the amount of particulate and dissolved organic matter (Quartino and Boraso de Zaixso 2008; Braeckman et al. 2019). Also, macroalgae provide habitat and structural shelter for many microorganisms, mainly for symbiont, saprobe, and parasitic fungi (Ogaki et al. 2019). In fact, macroalgae are considered one of the main marine reservoirs of fungi (Rateb and Ebel 2011).

Based on its geophysical and biological features, as well as the historic and temporal series of available abiotic and biotic data, Potter Cove (25 de Mayo/King George Island, Antarctica) is considered a model Antarctic coastal marine ecosystem l for studies related to global warming and its effects on the biota. Some such studies were focused on the description of macroalgal assemblages and their distribution in relation to abiotic factors (Quartino et al. 2005). The diversity of macroalgae in Potter Cove is represented by nearly fifty different species. In the last twenty years, the melting and the retreat of the bordering Fourcade Glacier have created newly ice-free areas available for benthic colonization (Quartino et al. 2013). In this scenario, macroalgae are winning new spaces, providing new shelters to fungi as well as more organic matter to the cove ecosystem.

Fungi ascribed to phyla, *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, *Chytridiomycota*, and *Glomeromycota*, are well represented in the Antarctic continent (Godinho et al. 2013) and have been isolated from several substrates such as soil, marine water, marine sediment, fresh water from lakes and snow. (Rosa 2019). It has been proposed that macroalgae and their associated microbiota interact in such a close way that they can be considered as a singular entity or holobiont (Egan et al. 2013). Several studies have focused on the bacterial partners of this holobiont (Spoerner et al. 2012; Wichard et al. 2015, 2018). However, few reports refer to fungi as members of these superorganisms (Vallet et al. 2018).

The search for and study of cold-adapted microorganisms have increased considerably during the last two decades because of the potential application of their metabolic products. In this sense, from a biotechnological point of view, both macroalgae and fungi separately can produce a myriad of compounds with diverse chemical structures and potential beneficial effects on human health. In the last few years, there have been several reports on the isolation

and description of secondary metabolites produced by these two kinds of eukaryotic organisms (Hasan et al. 2015; Stengel and Connan 2015). Interactions between macroalgae and fungi are a common event in the marine ecosystems and involve several biochemical mechanisms attractive for biotechnology (Vallet et al. 2018). It is interesting to note that despite lacking an immunological cell-mediated response, macroalgae can cope with microbes, mainly by using chemical compounds to stop or slow down microbial growth (Kubanek et al. 2003).

Nowadays, the search for novel antifungal compounds is a hot topic for biopharmaceutical as well as the food processing industry. As some fungi can damage microalgae tissues, these organisms are a potential source of natural, as well as novel, compounds showing antifungal activity. Due to the particular environmental conditions where these organisms live as well as the scarce knowledge of their physiology and biochemistry, macroalgae represent a promising source of novel antifungal compounds. Taking these ideas into consideration, the aim of the present study was the isolation, identification, and study of the growth of fungi associated with macroalgae obtained from Potter Cove, at 25 de Mayo/King George Island. Also, the antifungal activity of the macroalgae organic extracts was evaluated aiming to investigate their ecological role and also their potential biotechnological use.

MATERIALS AND METHODS

Macroalgae sampling and identification

Different macroalgae were collected from an intertidal rocky site at Peñón Uno in Potter Peninsula (62°14'49.9"S 58°40'54.5"W) and subtidal sites of Potter Cove (62° 14´ S, 58° 40´W), 25 de Mayo/King George Island, South Shetlands, Antarctica during the 2015–2019 austral summer expeditions at the Carlini Argentinean Scientific Research Station. Subtidal sampling was made by scuba diving at 5, 10, and 20 m depth whereas intertidal collection was performed during the low tide periods.

For fungal isolation purposes, after the divers collected the samples, the macroalgae were transported in seawater to the laboratory and identified. Three pieces of each sample (approximately 4x4 cm) were washed with filtered (0.44 µm) seawater to remove all particulate matter, such as epiphytes and sand particles, and maintained in sterile plastics containers until processed.

Fungal isolation

Isolation was carried out using two different methods: culture on solid media and moist chamber (Krug 2004). For the solid media isolation scheme, Diluted Marine Yeast Morphology Medium (DMYM, composition in g L^{-1}: yeast extract 0.03, malt extract 0.03, peptone 0.05, dextrose 0.1, agar 15) was prepared using filtered (0.44 um) seawater; pH was adjusted to 4.5 by the addition of HCl 1N (to prioritize the growth of fungi instead of bacteria). In order to minimize the presence of opportunistic propagules merely attached to the algae surface, A portion of each macroalgae sample was washed vigorously five times with sterile seawater (one liter in each wash) and then fractionated in small pieces under aseptic conditions and placed in both, DMYM agar plates and a moist chamber. The plates were incubated at 10°C for 7-21 days under natural lighting conditions. Actively growing fungi colonies were taken from the plates or moist chamber and subcultured onto fresh PDA (Potato Dextrose Agar) plates as individual isolates. All pure isolates were cryopreserved in glycerol 20% and sent to the Argentinean Antarctic Institute (Buenos Aires, Argentina) at -20°C and then, they were maintained on PDA medium at 4°C.

Fungal Growth Temperature range

The effect of temperature on the growth of the isolates was observed on PDA agar plates (90 mm). Isolated fungal strains (pre-grown on PDA agar plates) were inoculated (three replicates) and incubated at temperatures 5, 15, 25, and 35°C. Growth was monitored periodically up to a maximum incubation time of 25 days, to avoid missing out on any slow-growing fungi at a specific temperature. Growth was expressed as the fungal colony diameter in mm, as reported by Brancato and Golding (1953). For the growth temperature analysis, a modification of the classification proposed by Feller and Gerday (2003) was used. They classify cold-loving organisms into two groups: stenothermal psychrophiles (true or obligated psychrophiles) and eurythermal psychrophiles (facultative psychrophiles of psychrothrops). To ensure a more thoroughly descriptive analysis, in this work four categories were used: 1) stenopsycrophilic (minimal growth temperature of 5°C or lower, optimal near 15°C and maximal at approximately 25°C), 2) moderate eurypsychrophilic (a minimal growth temperature of 5°C or lower, maximal below 35°C), 3) narrow eurypsychrophilic (minimal growth temperature above 5°C, maximal below 35°C) and 4) eurypsychrophilic (minimal growth temperature of 5°C or lower, maximal above 35°C, with better growth in the 15-25°C range) (Deming 2019). This classification aimed to provide a tool for a deeper understanding of the different growth temperature behavior shown by several of the tested microorganisms that were initially classified as eurypsychrophile.

The growth rate was estimated using the method proposed by Laszlo and Silman (1993) with slight modifications. The radial growth rate is a simple method to evaluate fungus development on solid media. Although the method has the limitation that it only considers the diameter increase and not the vertical growth, it provides a good estimate of growth capacities. Colony diameter was measured for each plate (3 plates per fungus) at 4, 7, 11, 14, 18, 21, and 25 days. For each plate, the average of three measurements was used to consider the irregular colony shape. Linear regression was built for each sample using the equation $d(t)= a+ rgr.t$, where d is the diameter of the colony in millimeters, a is the linear regression constant, rgr is the radial growth rate ($mm.d^{-1}$) and t represents the time in days. The maximum radial growth rate (MRGR) was obtained from the regression considering only the period where the highest change was recorded. Results represent the average of slopes obtained from regressions of three replicates per fungal strain.

Molecular identification of fungi

For obtaining fresh biomass for molecular identification, each fungal isolate was grown on PDB (Potato Dextrose Broth) at 15°C and 200 rpm for 7 days. Biomass was collected by centrifugation (10,000 rpm for 10 minutes) and washed twice with sterile distilled water. The genomic DNA extraction was performed using a commercial kit (FastDNA™ Spin Kit, MP Biomedicals). The ITS region and the divergent domain at the 5′ end of the LSU rDNA gene (including the D1-D2 region) was symmetrically amplified with primers ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3´) according to standard methods (Schoch et al. 2012). The PCR products were purified and sequenced by MACROGEN (Korea). Sequences were analyzed and edited, when necessary, using DNA Dragon software (Hepperle 2011). DNA sequences were submitted to GenBank under Accession Numbers listed in Table 1. Strains identification was performed by comparison with the NCBI and UNITE databases. A ≥99% identity criterion was employed to identify strains at the species level. Sequences showing 97–98% identity were tentatively identified to the genus level. Sequences showing less than 97% identity were considered unidentified (Schoch et al. 2012).

Preparation of organic extracts from selected macroalgae

Macroalgae samples were washed first with filtered seawater, then with sterile distilled water, and finally freeze-dried and stored at -20°C until extracts preparation. Three different solvents, hexane (HX), ethyl acetate (EA), and

methanol (ME) were used to extract macroalgae metabolites considering a wide range of polarity. Three grams of finely powdered lyophilized macroalgae were mixed with 15 ml of each solvent separately and kept at 15°C under shaking conditions overnight (12 h). This procedure was repeated 3 times, and fractions were pooled, getting 45 ml of extract from each macroalga for each solvent used. Extracts were dried under reduced pressure, at 30°C, using a rotary evaporator and N_2 stream. Extracts were weighed, resuspended in a small volume (between 1 and 2 ml) of the same solvent used for extraction, and stored at −20°C until analysis (Shobier et al. 2016; Shedek et al. 2019).

Antifungal assay

The antifungal analysis of the macroalgal extracts was carried out using the well-cut technique (Bodet et al. 1985). Nine fungal isolates belonging to genera *Penicillium*, *Cladosporium*, *Cadophora*, *Antarctomyces*, *Pseudogymnoascus*, and *Phialocephala* were selected from those obtained in the 2015/2016 austral summer expedition. The selected isolates were cultured on PDA at 15°C for 7 days. The fungal colonies were suspended in sterile saline solution up to 0.5 MacFarland scale turbidity standard (10^7 spores ml^{-1} suspension). Each fungal suspension (100 µl) was separately inoculated on PDA plates using a Drigalski spatula.5 mm-diameter wells were punched in each plate and 100 µl of each extract was tested by duplicate in a concentration of 10 mg/ml (10 mg of the dried extract resuspended in 1 ml of the used solvent). The solvents (HX, EA, ME) and a 10 $mg.ml^{-1}$ ethanolic solution of cycloheximide were used as control. Plates were incubated at 15°C for 7 days and the results were expressed as absence or presence of growth and by the ratio of the inhibition zone.

RESULTS

Identification of macroalgal material

Thirteen macroalgae from different areas and depths of Potter Cove were identified to species level using the criteria previously described by Wiencke and Clayton (2002) and Hommersand et al. (2009). Seven species were classified as red algae (Rhodophyta): *Ballia callitricha* (C.Agardh) Kützing, *Gigartina skottsbergii* Setchell & N.L.Gardner, *Georgiella confluens* (Reinsch) Kylin, *Gymnogongrus turquetii* Hariot, *Neuroglossum delesseriae* (Reinsch) M.J.Wynne, *Palmaria decipiens* (Reinsch) R.W.Ricker and *Plocanium cartlagineum* (Linnaeus) P.S.Dixon, and six were brown algae (Phaeophyceae): *Adenocystis utricularis* (Bory) Skottsberg, *Ascoseira mirabilis* Skottsberg,

176 *Desmarestia anceps* Montagne, J.Agardh, *D. antarctica* R.L.Moe & P.C.Silva and *Himantothallus grandifolius*
177 (A.Gepp & E.S.Gepp) Zinova.
178
179 **Fungal isolates identification**
180 After 7-21 days of incubation, 48 fungal isolates were recovered as pure cultures from macroalgae samples (Table 1).
181 In the case of *A. utricularis*, the liquid inside the globose thalli was extracted with a sterile syringe and inoculated on
182 the isolation media plates using a Drigalski spatula. The isolates recovered from this liquid were coded as AUi and
183 those from the direct spread of the macroalgae sample on isolation media or moist chamber as AUe. When the origin
184 of the samples was analyzed, it was noticed that most isolates were obtained from *A. utricularis* (n=25), followed by
185 *G. skottsbergii* (n=10), *N. delesseriae* (n=6), *A. mirabilis* (n=3), *P. decipiens* (n=2) *and B. callitricha* (n=2).
186 Surprisingly, the other species presented no fungal growth at the end of the isolation scheme. To confirm this
187 observation and discard any non-controlled artifact, new samples of these macroalgae (*P. cartlagineum*, *G. turquetii*,
188 *G. confluens*, *H. grandifolius*, *D. anceps*, *D. menziesii* and *D. antarctica*) were tested again in the subsequent austral
189 summer expeditions of 2016/2017 and 2017/2018. In accordance with the results from the 2015/2016 campaign, no
190 fungal isolates were recovered from these macroalgae species, suggesting the presence of an antifungal activity on
191 them.
192 Most of the fungal isolates (47 out of 48) proved to belong to the phylum *Ascomycota* and the remaining one to the
193 phylum *Mucoromycota*. The former was distributed in only 6 different genera: *Antarctomyces*, *Cadophora*,
194 *Cladosporium*, *Penicillium*, *Phialocephala*, and *Pseudogymnoascus*, (Table 1). The isolates named AUe2, AUe3 and
195 PD2 were identified as *Cladosporium* based on morphology. Nevertheless, their molecular identity was less than
196 97%. The same situation resulted for isolate GS 2, which was identified as *Penicillium* sp. using the same
197 morphology-based criteria. Two isolates (AUe4 and ND5) could not be identified to the genus level. The closest
198 relative of isolate AUe4 was *Mortierella stylospora*, which indicates that AUe4 belongs to the phylum
199 *Mucoromycota* and was identified as *Mucoromycota* AUe4. In the case of isolate ND5, its closest relative for both
200 NCBI and UNITE databases was an uncultured fungus clone, that belongs to the *Ascomycota* subphylum
201 *Pezizomycotina*. Based on this, ND5 was identified as *Pezizomycotina* ND5. Further molecular characterization and
202 physiological tests are currently in progress for these two isolates to investigate the potential presence of a new
203 fungal species.

Most of the identifications were based on the UNITE database, as it provides more diversity of fungal strains, particularly in the case of *Cladosporium*. Of the 24 isolates belonging to the *Cladosporium* genus, nine were identified to species level; two *C. halotolerans*, four *C. cladosporoides* and three *C. sphaerospermum*. This genus was the most abundant in this study and was isolated in four out of six macroalgae that presented fungal growth. The ITS region proved to be insufficient for the identification of some genera such as *Aspergillus, Fusarium, Penicillium,* and *Trichoderma*, which have narrow or no barcode gaps in their ITS regions (Raja et al. 2017). For this reason, in this work, several isolates showing a 99% identity with a species of *Penicillium* were presumptively classified as *Penicillium* sp. This genus was the second most abundant in this study with 16 isolates. When the similarity in percentage with the UNITE sequences was considered, six different types of *Penicillium* were found, with several isolates showing to be the same (Table 1).

The two *Phialocephala sp*. (AM1 and AM2) were isolated from the same macroalgae and proved to be identical. In the case of isolates GS2.2 and AM4, they were identical too but were isolated from different macroalgae. As in the cases described above, they did not exhibit a 99% identity with *Cadophora malorum* and then were classified as *Cadophora* sp. GS2.2 and *Cadophora* sp. AM4. In the case of *Antarctomyces psychrotrophicus* BC1 and *Pseudogymnoascus pannorum* GS4, they were only isolated once in this work.

Growth temperature characterization

Based on the classification explained above, 73% (n=35) of the isolated fungi proved to be moderate eurypsychrophilic and only grew between 5 and 25°C, 12.5% (n=6) were eurypsychrophilic and grew in the whole range of tested temperatures (5 to 35°C), 12,5% of the isolates (n=6) was classified as narrow eurypsychrophilic, meaning that they only presented growth between 15 and 25°C. Only one isolate, *Mucoromycota* AUe4, was classified as stenopsychrophilic as it grew only at 5 and 15°C (Table 2).

Another analysis of this assay referred to the evaluation of the temperature (among the tested range) in which the fungal isolates presented their largest growth. While these fungal isolates are considered cold-loving or psychrophilic (either eury- or steno-), 69% (n=33) showed the largest growth at 25°C and 23% (n=11) at 15°C. In some cases, the largest growth was recorded indistinctly at two temperatures (15 and 25°C), which would mean that their optimal temperature is probably within that range. No isolate showed its largest growth at 35° or 5°C (Table 2). At 35°C all eurypsychrophiles showed similar or smaller colony diameters than those observed at 5°C.

We also evaluated the intrinsic growth ability of the fungal isolates at each temperature. For this purpose, we considered the time needed for the displayed growth, and expressed the results as the maximum radial growth rate (MRGR). Results reinforced the grouping criteria described above. The stenopsychrophile AUe4 showed the highest MRGR (12.25±0.14 $mm.d^{-1}$) at 5°C while at 15°C it reached a value of 6.64±0.08 $mm.d^{-1}$. Narrow eurypsychrophiles showed similar MRGR at 15 and 25°C, which were 3 to 4 times lower than those observed for AUe4 at 5°C. Moderate eurypsychrophiles showed different growth patterns. All of them grew at 5, 15 and 25°C but not at 35°C. Among them, ND5 exhibited an MRGR of 11.13±0.19 $mm.d^{-1}$ at 15°C which fell to 7.43±0.13 $mm.d^{-1}$ at 25 °C and 6.81±0.88 $mm.d^{-1}$ at 5°C. ND2 showed an MRGR pattern with the highest values at 15 or 25°C indistinctively (3.73±0.13 and 3.85±0.17 $mm.d^{-1}$). Meanwhile, BC1 (*A. psychrotrophicus*) presented a similar MRGR at 5, 15 and 25°C (1.94±0.11, 1.87±0.21 and 2.21±0.21 $mm.d^{-1}$), being the only isolate displaying such a constant behavior. Among eurypsychrophiles, ND3 showed the highest MRGR (7.93±0.04 $mm.d^{-1}$) at 25°C. All of them displayed bell-shaped MRGR vs temperature curves, the sharpest being that observed for ND3 and the flattest those from GS3 and GS23, suggesting that MRGR at 15 and 25°C was similar for these isolates. Fig.1 shows the pattern of MRGR versus temperature for some of the representative isolates of each group.

Antifungal activity of macroalgae extracts

Table 3 shows the results for the antifungal screening of the selected macroalgae organic extracts and the positive (cycloheximide 10 $mg.ml^{-1}$) and negative (hexane, ethyl acetate, and methanol) controls against a panel of selected fungi isolated in this work. These fungal isolates comprised all the genera obtained after the isolation scheme, and in one case (*Penicillium*), three different isolates (according to BLAST results).

The extracts were obtained from those macroalgae for which no fungal growth was observed. The macroalgae included in this group were: three red algae (*G. confluens*, *G. turquetii*, and *P. cartlagineum*) and four brown algae (*D. anceps*, *D. Antarctica*, *D. menziesii* and *H. grandifolius*). Besides, the *A. mirabilis* extract was used as a control, considering that fungal isolates included in the testing group were obtained from this macroalgae.

The three extracts (HX, EA and ME) from *P. cartlagineum* showed the largest inhibition haloes with all the tested fungal isolates. The HX extract was the one presenting the best performance in this assay, suggesting that the active compounds in *P. cartilagiueum* are probably strongly non-polar and thus they were more efficiently extracted with

HX. Also, the HX extract from *G. turquetii* presented large inhibition haloes: 17 ± 0.71 mm in *C. cladosporoides* AUi7 and 10,5 ± 2.47 mm in *Penicillium* sp. AUe8.

By the observed absence of culturable fungi on their surface, extracts from all the studied algae showed antifungal activity against some of the selected isolates. The other red alga evaluated was *G. confluens*, and its extracts presented inhibition growth against *Phialocephala* sp AM1 (all extracts) and *A. psychrotrophicus* BC1 (EA and ME extracts). For both fungi, the EA extract showed the highest inhibitory activity. Among the Phaeophyceae macroalgae, EA and ME extracts from *D. antarctica* inhibited the growth of *Penicillium* sp. GS2, *Penicillium* sp. AUe8, *Phialocephala* sp. AM1 and *A. psychrotrophicus* BC1, the ME extracts also showed inhibition haloes in *C. cladosporoides* AUi7 and *P. pannorum* GS4. The EA and ME of *D. anceps* presented growth inhibition for *C. cladosporoides* AUi7 and *Phialocephala* sp. AM1, and its ME extract for *Penicillium* sp. PD1, *Penicillium* sp. GS2 and *Penicillium* sp. AUe8. *D. menziesii* EA and ME extracts inhibited *Penicillium* sp. GS2, *C. cladosporoides* AUi7 and *A.psychrotrophicus* BC1. The ME extract also showed inhibition for *Penicillium* sp. PD1, *Penicillium* sp. AUe8 and *Phialocephala* sp. AM1. Finally, *P. pannorum* GS4 was inhibited by the EA extract. Even the extracts from *A. mirabilis*, that was used as control of a macroalga which allows fungal growth, presented some fungal growth inhibition. Its HX extract was active against *Phialocephala* sp. AM1, the EA extract against *C. cladosporoides* AUi7, *A.psychrotrophicus* BC1 and *Pezizomycotina* ND5, and the ME attract against *Penicillium* sp. GS2, *Penicillium* sp. AUe8, *Phialocephala* sp. AM1 and *Pezizomycotina* ND5.

As explained, the extracts of *A. mirabilis*, *D. antarctica*, *D. anceps*, *D. menziesii*, *H. grandifolius* and *G. confluens* presented some inhibition, but with a smaller halo, in comparison with the extract of *P. cartilagenum* and *G. turquetti*. This could be related to the concentration used in this experiment (10 $mg.ml^{-1}$). However, in all the extracts (except those from *P. cartlagineum*), relationships among the size of the haloes and the polarity of the solvent were not observed. This observation could indicate the presence of diverse antifungal compounds in these macroalgae species that are extracted efficiently with solvents of different polarity.

DISCUSSION

As the first step for a deeper understanding of the processes involved in the fungi-algae interactions existing in cold marine waters, we studied the taxonomic assignment and some growth properties of the fungi isolated from macroalgae living in Potter Cove and surrounding areas, Antarctica. In the last decades, several researchers

contributed to the knowledge of Antarctic fungal diversity and most of the fungal genera described in this work were previously reported as inhabitants of some Antarctic macroalgae in other areas of the continent (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2018; Ogaki et al. 2019).

Cladosporium is a genus previously found only in few Antarctic macroalgae hosts. Based on the extensive review reported by Ogaki et al. (2019) *Cladosporium* strains were isolated from *A. mirabilis*, *G. confluens* (Furbino et al. 2018), *Pyropia endiviifolia* (A.Gepp & E.Gepp) Y.M.Chamberlain, *Monostroma hariotii* Gain 1911 (Furbino et al. 2014) and *Acrosiphonia arcta* (Dillwyn) Gain 1912 (Godinho et al. 2013). In the mentioned review, the authors described *Cladosporium* as the most abundant and ubiquitous genera. Besides this, our work represents the first report of *Cladosporium* as a member of the fungal community associated with *G. skottsbergii*, *A. utricularis*, *P. decipiens*, and *N. delesseriae*. None of the isolates that were identified as *Cladosporium* (n = 26) in this work grew at 35°C, and most of them (n=21) were able to grow at 5°C. Due to this behavior, these 21 isolates were classified as moderate eurypsychrophilic, the 5 remaining being classified as narrow eurypsychrophilic. *Cladosporium* has a worldwide distribution and eurypsychrophilic representatives of this genus have been isolated from both, terrestrial (oligotrophic soil) and marine (benthic mats, marine sponges and seawater) cold environments of Antarctica, the Tibetan plateau, the deep Pacific Ocean, and the Arctic. Currently, 205 species are accepted as belonging to *Cladosporium* (Ma et al. 2018).

The second most abundant isolated genus in our work was *Penicillium*, with 16 representatives, from four different macroalgae: *A. utricularis*, *B. callitricha*, *G. skottsbergi* and *N. delesseriae*. Interestingly, some of the *Penicillium* isolates were the only tested fungi able to grow at 35°C. Isolation of *Penicillium* from different Antarctic environments has been frequently reported, such as soils (Martorell et al. 2019), wood remains (Arenz et al. 2006), marine sediments (Ogaki et al. 2020), and even permafrost (Zucconi et al. 2012). Because of its distribution, this genus is rightfully considered a cosmopolitan one, and it is also one of the most frequently isolated from macroalgae. This wide distribution brought into the discussion whether *Penicillium* establishes a permanent association with the host or if its presence on macroalgae is just attributable to eventual spore contamination. Fungi belonging to *Penicillum* are considered versatile microorganisms with a protagonist role in intertidal zones (Park et al. 2019). Considering this and the thoughtful surface-sterilization protocols usually applied in this study, which included the use of ethanol, chlorine, or several washes with sterile sea or distilled water, the permanent association *Penicillum*-

macroalgae seems to be the most probable one (Ogaki et al. 2019). To our knowledge, this is the first report of *Penicillium* isolates from *B. callitriche*, *G. skottsbergii*, and *N. delesseriae.*

The genus *Pseudogymnoascus* is worldwide considered polar, being found in Antarctica (Ding et al. 2016; Kochkina et al. 2019; Martorell et al. 2019), the Arctic and even in the Alps (Hayes et al. 2012). Following these reports, the isolate identified as *P. pannorum* in this work was classified as a moderate eurypsychrophilic, with an optimal growth temperature of 15°C. It was isolated from *G. skottsbergii*, representing the first report of *P. pannorum* in association with this macroalgae.

The genus *Phialocephala* belongs to the class Leutuomycetes. This genus was commonly reported as a plant roots endophyte with widespread distribution in sub-Antarctic ecosystems and in soils from continental Antarctica (Newsham et al. 2009; Martorell et al. 2019). The two isolates ascribed to *Phialocephala* sp. (AM1 and AM2) were obtained from *A. mirabilis* and both grew better at 25°C. For this reason, we classified these isolates as moderate eurypsychrophilic. This is the first report for this genus in association with Antarctic macroalgae.

The presence of *Cadophora* has been reported in several substrates from Antarctica (Onofri et al. 2004, 2007; Arenz et al. 2006; Stchigel et al. 2017; Martorell et al. 2019). Its presence on Antarctic macroalgae was previously reported only on *P. endiviifolia* (Furnino et al. 2014). The isolates in this work were found on *A. mirabilis* and *G. skottsbergii* and can be considered as new fungal-macroalgae associations for Antarctica.

In relation to *A. psychrotrophicus*, the BC1 isolate is the first report of this fungi in *B. callitricha*. This fungal genus, *Antarctomyces* Stchigel & Guarro (Stchigel et al. 2001) is considered endemic to Antarctica, and has been isolated from different substrates such as soil, Antarctic grass (*Deschampsia antarctica*), freshwater lakes, lichens and other macroalgae, as *A. mirabilis, Ulva intestinalis* Linnaeus and *P. endiviifolia* (Stchigel et al. 2001; Rosa et al. 2009; Gonçalves et al. 2012; Godinho et al. 2013; Furbino et al. 2014, 2018; Santiago et al. 2015).

Potter Cove seawater temperature during summer ranges between 0 and 2.5°C, reaching -2°C in winter (Krock et al. 2020). It seems interesting to consider that 6 of the isolates were not able to grow at 5°C (narrow eurypsychrophiles) and most of those able to grow at that temperature, showed their optimum value at higher temperatures (15 and 25°C), which will probably never happen during their whole life cycle in the Antarctic marine environment. The maximal radial growth rate (MRGR) provides a tool for screening fungal growth fitness on solid media. Despite its limitations (Hendricks et al. 2017), it is a useful method to obtain information about growth rates in fungi, organisms in which biomass development is difficult to quantify, especially when different genera are involved. The MRGR

analysis revealed that the stenopsychrophile isolated in this work (*Mucoromycota* AUe4) can grow on solid PD agar at a rate of 12.25±0.14 mm.d-^{1} when incubated at 5°C. This growth rate seemed to be more than 10 times higher than the average MRGR of the other isolates (1.18±0.59 mm.d^{-1}) growing at the same temperature, with the only exception of the moderate eurypsychrophile ND5 (Subdivision *Pezizomycotina*) which showed a value of 6.81±0.19 mm.d^{-1}. How do they overcome the limiting condition imposed by temperature? From which adaptation or mechanisms do they take advantage to keep themselves in the game when competing with other more adapted fungi, at least from the growth rate point of view? How or why do they seem to avoid environment selection pressure imposed by temperature? Our results showed that *A. utricularis*, an intertidal macroalgae, was colonized simultaneously by a variety of cultivable fungi, most of them being moderate eurypsychrophiles. However, *A. utricularis* also shelter shelters a stenopsychrophile *(Mucoromycota* AUe4), an eurypsychrophile able to grow from 5 to 35 °C (*Penicillum* sp. AUe1) and several narrow eurypsychrophiles (*Penicilium* sp. and *Cladosporium* sp.). Further research could shed light on the complex physiological mechanisms involved in supporting this fungal diversity at low temperatures.

Based on the observation that only six of the thirteen macroalgae allowed the isolation of fungi, studies to evaluate the possible presence of antifungal activity in them were carried out. Organic extracts of *P. cartilagineum* presented fungal inhibition against all the isolates tested. This species is a broadly distributed red alga that contributes to the structure of algal-dominated coastal benthic ecosystems of the Western Antarctic Peninsula (Young et al. 2013). According to Hommersand et al. (2009), the *P. cartilagineum* present in Antarctica is a distinct species from those *P. cartilagineum* inhabiting other regions of the planet. This red alga has already proven to contain monoterpenes with cytotoxic activity against cervical cancer (Shilling et al. 2019), lung cancer, leukemia and colon cancer (Sabry et al. 2017) and insecticide and acaricide activities (San Martín et al. 1991). The production of such a battery of compounds would provide to the fungal isolates the capacity to overcome several biological challenges and would be one of the causes of its success as a coastal benthic ecosystem member.

The red algae *G. turquetii* was previously reported for its high content of mycosporine-like amino acids (MAAs) (Yuan and Athukorala 2011) and the production of lectins (haemagglutinins) with a potential biomedical use (Singh and Walia 2018). The results obtained in this work showed that HX extract of *G. turquetii* can significantly inhibit the growth of *Penicillum* sp. AUe8, *C. cladosporodes* AUi7, *A.psychrotrophicus* BC1, and *Pezizomycotina* ND5. As

far as we know, there are no previous reports about antifungal or antimicrobial activities of this macroalgae. EA and ME extract also showed a wider inhibition spectrum but with a smaller inhibition halo.

All the other macroalgae extracts (*A. mirabilis*, *D. anceps*, *D. Antarctica*, *D. menziesii*, *G. confluens* and *H. grandifolius*) presented inhibition zones when tested against some of the fungal isolates.

The EA extract of *H. grandifolius* showed activity only against *Phialocephala sp.* AM1. An ethyl acetate extract of *H. grandifolius* was previously reported as having antifungal activity against clinical isolates of *Candida albicans*, *C. parapsilosis*, *C. glabrata*, *C. lipolytica*, and *C. famata*, some of them being fluconazole-resistant microorganisms (Martins et al. 2018). No other bioactivity was found on the current bibliography of this macroalgae.

In the case of *D. antarctica* and *G. confluens*, Sevak (2012) reported the toxic activity of their fatty acid against diatoms, as a defense characteristic in coastal zones of Antarctica. Pacheco et al. (2018) reported some inhibition in the growth of human breast cancer cells also with a mix of *G. confluens* fatty acids. Finally, Souza et al. (2010) also reported the presence of lectins (haemagglutinins) in *G. confluens*. No antifungal activity has been reported so far. *D. menziesii* and *D. anceps* were reported as having some anti-inflammatory activity (at a cytotoxic level). Also, *D. menziesii* produces plastoquinones, which have been suggested to present cytotoxic activity against leukemia cells and *D. anceps* presents antibacterial and antifouling activity against diatom (Moles et al. 2014). No reports on the bioactive compound or inhibitory activities against fungi, bacteria or other microorganisms or cell cultures could be found on *D. antarctica* and *A. mirabilis.*

The results of *P. cartilagineum* on all the fungal isolates, *G. turquetii* on *Penicillium* sp. AU38 and *C. cladosporoides* AUi7 and its methanol extract on some of the other isolates, as well as the results of the rest of the macroalgae tested where small inhibition zones were present, are quite promising, and except for the above explained for *H. grandifolius*, represent the first report of the antifungal activity of these macroalgae.

Extracts from macroalgae from which no fungi development was observed proved to inhibit the growth of several of the isolates tested. However, they did not inhibit all the isolates, despite the absolute absence of fungal growth on the macroalgae. This observation would suggest that the macroalgae displayed antifungal mechanisms or molecules different from those extracted and evaluated in this work. Another possibility is that the extraction procedure was not efficient enough to recover/emulate the antifungal activity, due to molecules stability, required amounts or complementarity of mechanisms. Several reports refer to the need for synergy among mechanisms to inhibit fungal growth (Butassi et al 2015, Cui et al 2015).

This research raises the question of whether the active metabolites were produced by the algae itself or by endophytic organisms living in symbiosis with the algae and being part of the holobiont. This idea has been previously proposed and evaluated. In a study on the antifungal activity of a polycyclic macrolide (Lobophorolide) obtained from *L variegate*, Kubanek et al. (2003) found the structural similarity between the antibiotic and some bacterial metabolites, suggesting that this compound could have been produced by a symbiont bacteria (unidentified) belonging to the holobiont. Further experiments would clarify this mechanism.

The present study constitutes a limited screening for antifungal activity evaluation, considering that the amount of each alga used was respectful of the sampling regulation which guides scientific activity in Antarctica, managed in this case by the Argentinean Antarctic Environmental Protection Program. The result of the antifungal activity of the macroalgae extracts from Potter Cove, suggests that some of the macroalgae from Antarctica are a promising source for the isolation and characterization of compounds with bioactive potential. Further investigation and experimentation based on these results are being undertaken in order to fractionize and isolate the components of the more promising macroalgae and to elucidate the participation of each component of them in the antifungal activity. In a future step, evaluations will be focused on biocide activity against pathogenic fungi of clinical and agronomic importance.

CONCLUSION

The culturable fungal diversity recovered from macroalgae sampled in Potter Cove, Antarctica, proved that they are the shelter and source of a vast amount of fungi with different growth rates in a wide range of temperatures. In this work in particular, the presence of fungal isolates with no possible identification using the standard molecular tools contributes to the idea that Antarctica is the source of several new fungal taxa that, beyond their contribution to knowledge on the Antarctic microbial biodiversity, involve a significant bioprospecting and biotechnological potential. Moreover, some of the macroalgae evaluated in this work showed fungal growth inhibition capabilities, evidencing the presence of interesting defense mechanisms to survival in the wild environment and also representing a promising source of compounds to be evaluated in the future. These results open the way to research and understand the fungi/macroalgae relation in this particular marine environment and their contribution to the organic matter cycling in the Potter Cove coastal ecosystem, a model for studies related to global warming worldwide.

Acknowledgments

This work was supported by the Instituto Antártico Argentino/Dirección Nacional del Antártico (IAA/DNA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica and Universidad de Buenos Aires. We want to thank the scientific, logistic and military crew, in particular / especially the divers, of Carlini Station, for their hospitality and support, Marta Sierra and Cecilia Ferreiro for their help with the maps and English language editing, respectively.

REFERENCES

Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA, 2006. Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol Biochem 38(10):3057-3064

Bodet C3, Jorgensen JH, Drutz DJ, 1985. Simplified bioassay method for measurement of flucytosine or ketoconazole. J Clin Microbiol 22(2):157-160.

Braeckman U, Pasotti F, Vázquez S, Zacher K, Hoffmann R, Elvert M, Marchant H, Buckner C, Quartino ML, Mác Cormack W, Soetaert K, Wenzhöfer F, Vanreusel A, 2019. Degradation of macroalgal detritus in shallow coastal Antarctic sediments. Limnol Oceanogr 64:1–19.

Brancato FP, Golding NS, 1953. The diameter of the mold colony as a reliable measure of growth. Mycologia 45(6):848-864.

Butassi E, Svetaz LA, Ivancovich JJ, Feresin GE, Tapia A, Zacchino SA, 2015. Synergistic mutual potentiation of antifungal activity of *Zuccagnia punctata* Cav. and *Larrea nitida* Cav. extracts in clinical isolates of *Candida albicans* and *Candida glabrata*. Phytomedicine 22(6):666-678.

Cui J, Ren B, Tong Y, Dai H, Zhang L, 2015. Synergistic combinations of antifungals and anti-virulence agents to fight against *Candida albicans*. Virulence 6(4):362-371.

Dayton PK, 1985. Ecology of kelp communities. Annu Rev Ecol Systemat 16(1):215-245.

Deming W, 2019. Extremophiles: cold environments. In Schmidt (Ed) Encyclopedia of Microbiology, 4rd edn. Academic Press, London, pp 228-238 .

Ding Z, Li L, Che Q, Li D, Gu Q, Zhu T, 2016. Richness and bioactivity of culturable soil fungi from the Fildes Peninsula, Antarctica. Extremophiles 20(4):425-435.

452 Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T, 2013. The seaweed holobiont: understanding
453 seaweed–bacteria interactions. FEMS Microbiol Rev 37(3):462-476.
454 Feller G, Gerday C, 2003. Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1(3):200-
455 208.
456 Furbino LE, Godinho VM, Santiago IF, Pellizari FM, Alves TM, Zani CL, Policarpo AS, Romanha AJ,
457 Carvalho AGO, Gil LHVG, Rosa CA, Minnis AM, Rosa LH, 2014. Diversity patterns, ecology and biological
458 activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. Microb
459 Ecol 67(4):775-787.
460 Furbino LE, Pellizzari FM, Neto PC, Rosa CA, Rosa LH, 2018. Isolation of fungi associated with macroalgae
461 from maritime Antarctica and their production of agarolytic and carrageenolytic activities. Polar Biol 41(3):527-
462 535.
463 Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya NS, Pupo D, Alves TMA, Policarpo ASJ,
464 Romanha AJ, Zani CL, Cantrell CL, Rosa CA, Rosa LH, 2013. Diversity and bioprospecting of fungal
465 communities associated with endemic and cold-adapted macroalgae in Antarctica. ISME J 7(7):1434.
466 Gonçalves VN, Vaz AB, Rosa CA, Rosa LH, 2012. Diversity and distribution of fungal communities in lakes of
467 Antarctica. FEMS Microbiol Ecol 82(2):459-471.
468 Hasan S, Ansari MI, Ahmad A, Mishra M, 2015. Major bioactive metabolites from marine fungi: A Review.
469 Bioinformation 11(4):176.
470 Hayes MA, 2012. The Geomyces fungi: ecology and distribution. Bioscience 62(9):819-823.
471 Hendricks KE, Christman MC, Roberts PD, 2017. A statistical evaluation of methods of in-vitro growth
472 assessment for Phyllosticta citricarpa: average colony diameter vs. area. PloS one 12(1).
473 Hepperle D, 2011. DNA Dragon 1.4. 1–DNA Sequence Contig Assembler Software. Available at: www. dna-
474 dragon.com. Accessed 25 November 2010.
475 Hommersand MH, Moe RL, Amsler CD, Fredericq S, 2009. Notes on the systematics and biogeographical
476 relationships of Antarctic and sub-Antarctic Rhodophyta with descriptions of four new genera and five new
477 species. Bot Mar 52(6):509-534.
478 Kochkina GA, Ivanushkina NE, Lupachev AV, Starodumova IP, Vasilenko OV, Ozerskaya SM, 2019. Diversity
479 of mycelial fungi in natural and human-affected Antarctic soils. Polar Biol 42(1):47-64.

Krock B, Schloss IR, Trefault N, Tillmann U, Hernando M, Deregibus D, Antoni J, Almandoz GO, Hoppenrath M, 2020. Detection of the phycotoxin pectenotoxin-2 in waters around King George Island, Antarctica. Polar Biol 43(3):263-277.

Krug JC, 2004. Moist chambers for the development of fungi. In Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: standard methods for inventory and monitoring. Elsevier Academic Press, San Diego, pp 589-593.

Kubanek J, Jensen PR, Keifer PA, Sullards MC, Collins DO, Fenical W, 2003. Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. Proc Natl Acad Sci USA 100(12):6916-6921.

Laszlo JA, Silman RW, 1993. Cellular automata simulations of fungal growth on solid substrates. Biotechnol Adv 11(3):621-633.

Loque CP, Medeiros AO, Pellizzari FM, Oliveira EC, Rosa CA, Rosa LH, 2010. Fungal community associated with marine macroalgae from Antarctica. Polar Biol 33(5):641-648.

Ma R, Huang H, Bai Y, Luo H, Fan Y, Yao B, 2018. Insight into the cold adaptation and hemicellulose utilization of *Cladosporium neopsychrotolerans* from genome analysis and biochemical characterization. Sci Rep 8(1):1-14.

Martins RM, Nedel F, Guimarães V, da Silva AF, Colepicolo P, de Pereira CM, Lund RG, 2018. Macroalgae extracts from Antarctica have antimicrobial and anticancer potential. Front Microb 9,412.

Martorell MM, Ruberto LAM, Fernández PM, De Figueroa LIC, Mac Cormack WP, 2019. Biodiversity and enzymes bioprospection of Antarctic filamentous fungi. Antarc Sci 31(1):3-12.

Moles J, Torrent A, Alcaraz MJ, Ruhí R, Avila C, 2014. Anti-inflammatory activity in selected Antarctic benthic organisms. Front Mar Sci 1,24.

Newsham KK, Upson R, Read DJ, 2009. Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecol 2(1):10-20.

Ogaki MB, Coelho LC, Vieira R, Neto AA, Zani CL, Alves TM, Policarpo ASJ, Murta SMF, Barbosa EC, Oliveira JG, Ceravolo IP, Pereira PO, Cota BB, Viana RO, Alvez VS, Rosa LH, 2020. Cultivable fungi present in deep-sea sediments of Antarctica: taxonomy, diversity, and bioprospecting of bioactive compounds. Extremophiles 24(2):227-238.

Ogaki MB, de Paula MT, Ruas D, Pellizzari FM, García-Laviña CX, Rosa LH, 2019. Marine fungi associated with Antarctic macroalgae. In Rosa LH (ed) The ecological role of micro-organisms in the Antarctic environment, Springer, 1st edn. Springer, Basel, pp 239-255.

Onofri S, Selbmann L, De Hoog GS, Grube M, Barreca D, Ruisi S, Zucconi L, 2007. Evolution and adaptation of fungi at boundaries of life. Adv Space Res 40(11):1657-1664

Onofri S, Selbmann L, Zucconi L, Pagano S, 2004. Antarctic microfungi as models for exobiology. Planet Space Sci 52(1):229-237.

Pacheco BS, dos Santos MAZ, Schultze E, Martins RM, Lund RG, Seixas FK, Colepicolo P, Collares T, Favero Reisdorfer P, De Pereira CMP, 2018. Cytotoxic activity of fatty acids from antarctic macroalgae on the growth of human breast cancer cells. Front Bioeng Biotech 6:185.

Park MS, Oh S, Fong JJ, Houbraken J, Lim JW, 2018. The diversity and ecological roles of *Penicillium* in intertidal zones. Sci Rep 9, 13540.

Quartino ML, Boraso de Zaixso AL, 2008. Summer macroalgal biomass in Potter Cove, South Shetland Islands, Antarctica: its production and flux to the ecosystem. Polar Biol 31(3):281-294.

Quartino ML, Deregibus D, Campana GL, Latorre GEJ, Momo FR, 2013. Evidence of macroalgal colonization on newly ice-free areas following glacial retreat in Potter Cove (South Shetland Islands), Antarctica. PLoS One 8(3)

Quartino ML, Zaixso HE, Boraso de Zaixso AL, 2005. Biological and environmental characterization of marine macroalgal assemblages in Potter Cove, South Shetland Islands, Antarctica. Bot Mar *48*(3):187-197.

Raja HA, Miller AN, Pearce CJ, Oberlies NH, 2017. Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod 80(3):756-770.

Rateb ME, Ebel R, 2011. Secondary metabolites of fungi from marine habitats. Nat Prod Rep 28(2):290-344.

Rosa LH, 2019. Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications. Springer, Basel.

Rosa LH, Vaz AB, Caligiorne RB, Campolina S, Rosa CA, 2009. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv (Poaceae). Polar Biol 32:161–167.

Sabry OM, Goeger DE, Valeriote FA, Gerwick WH, 2017. Cytotoxic halogenated monoterpenes from *Plocamium cartilagineum*. Nat Prod Res 31(3):261-267.

San-Martin A, Negrete R, Rovirosa J, 1991. Insecticide and acaricide activities of polyhalogenated monoterpenes from Chilean *Plocamium cartilagineum*. Phytochemistry 30(7):2165-2169.

Santiago IF, Soares MA, Rosa CA, Rosa LH, 2015. Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. Extremophiles 19(6):1087-1097.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, 2012. Fungal Barcoding Consortium. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA 109(16):6241-6246.

Sevak HP, Amsler CD, McClintock JB, Maschek JA, Amsler MO, Aumack CF, Peters KJ, Baker BJ, 2012. Algicidal activity and potential antifouling defenses in macroalgae from the western Antarctic Peninsula including probable synergistic effects of multiple compounds. Bot Mar 55(3):311-315.

Shilling AJ, von Salm JL, Sanchez AR, Kee Y, Amsler CD, McClintock JB, Baker BJ, 2019. Anverenes B–E, New Polyhalogenated Monoterpenes from the Antarctic Red Alga *Plocamium cartilagineum*. Mar drugs 17(4):230.

Shobier AH, Ghani SAA, Barakat KM, 2016. GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. Egypt J Aquat Res *42*(3):289-299.

Singh RS, Walia AK, 2018. Lectins from red algae and their biomedical potential. J Appl Phycol 30(3): 1833-1858.

Souza BWS, Andrade FK, Teixeira DIA, Mansilla A, Freitas ALP, 2010. Haemagglutinin of the antarctic seaweed *Georgiella confluens* (Reinsch) Kylin: isolation and partial characterization. Polar Biol 33(10):1311-1318.

Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W, 2012. Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors. J Phycol 48(6):1433-1447.

Stchigel AM, Josep CANO, Mac Cormack W, Guarro J, 2001. *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. Mycol Res 105(3):377-382.

Stchigel AM, Rrodriguéz-Andrade E, Mac Cormack WP, Cano-Lira JF, Guarro J, 2017. *Cadophora antarctica* Rodr-Andrade, Stchigel, Mac Cormack & Cano, sp. nov. Fungal Planet 627–20,P287.

Stengel DB, Connan S, 2015. Natural products from marine algae. Methods in Molecular Biology. Humana Press, New York.

Vallet M, Strittmatter M, Murúa P, Lacoste S, Dupont J, Hubas C, Genta-Jouve G, Gachón CMM, Kim GH, Prado S, 2018. Chemically-mediated interactions between macroalgae, their fungal endophytes, and protistan pathogens. Front Microbiol 9,3161.

Wichard T, Beemelmanns C, 2018. Role of chemical mediators in aquatic interactions across the prokaryote–eukaryote boundary. J Chem Ecol 44(11):1008-1021.

Wichard T, Charrier B, Mineur F, Bothwell JH, Clerck OD, Coates JC, 2015. The green seaweed *Ulva*: a model system to study morphogenesis. Front Plant Sci 6,72.

Wiencke C, Amsler CD, 2012. Seaweeds and their communities in polar regions. In Seaweed Biology, Springer, Berlin, pp 265-291.

Wiencke C, Clayton MN, 2002. Antarctic seaweeds. In: Wägele JW (ed) Synopsis of the Antarctic benthos, vol 9. A.R.G. Ganter Verlag KG Ruggell, Lichtenstein pp[2]1-239.

Wiencke C, Clayton MN, Gómez I, Iken K, Lüder UH, Amsler CD, Karsten U, Hanelt D, Bischof K, Dunton K, 2007. Life strategy, ecophysiology and ecology of seaweeds in polar waters. Rev Environ Sci Biotechnol 6(1-3):95-126.

Young RM, Von Salm JL, Amsler MO, Lopez-Bautista J, Amsler CD, McClintock JB, Baker BJ, 2013. Site-specific variability in the chemical diversity of the Antarctic red alga *Plocamium cartilagineum.* Mar drugs 11(6):2126-2139.

Yuan YV, Athukorala Y, 2011. Red Algal Mycosporine-Like Amino Acids (MAAs) as Potential Cosmeceuticals. In Kim S (ed) Marine Cosmeceuticals: Trends and Prospects, CRC press, Boca Raton pp 143-166.

Zucconi L, Selbmann L, Buzzini P, Turchetti B, Guglielmin M, Frisvad JC, Onofri S, 2012. Searching for eukaryotic life preserved in Antarctic permafrost. Polar Biol 35(5):749-757.

Figure Captions

Fig. 2 Location of the sampling sites, Peñón Uno (1) in Potter Peninsula (62°14'49.9"S 58°40'54.5"W) and subtidal sites (2) of Potter Cove (62° 14´ S, 58° 40´W), in 25 de Mayo / King George Island, South Shetland Islands, within the Antarctic Peninsula in Antarctica.

Fig. 2 Macroalgae used for fungal study, from different areas and deeps of Potter Cove. **a-f** Phaeophyceae: (**a**) *Desmarestia anceps*, (**b**) *D. antarctica*, (**c**) *D. menziesii*, (**d**) *Adenocystis utricularis (*~ 5-8 cm) , (**e**) *Himantothallus grandifolius* (scale: 1.5m, each mark: 10cm), (**f**) *Ascoseira mirabilis* (~1.3 m); **g-m** Rhodophytas: (**g**) *Neuroglossum delesseriae*, (**h**) *Palmaria decipiens*, (**i**) *Gigartina skottsbergii*, (**j**) *Gymnogongrus turquetii*, (**k**) *Plocamium cartilagineum*, (**l**) *Georgiella confluens* and (**m**) *Ballia callitricha.* **(d)** and **(f)** intertidal pictures.

Fig. 3 Maximal radial growth rate ($mm.d^{-1}$) of representative isolates: (♦) *Pezizomycotina* ND5, (■) *Mucoromycota* AUe4, (▲) *A. psychrotrophicus* BC1, (●) *Penicillium* sp. ND3, (○) *C .cladosporoides* AUe7, (□) *Penicillium* sp. ND2, (Δ) *Penicillium* sp. GS3 and (◊) *Cladosporium* sp. PD2 at each tested temperature (5, 15, 25 and 35°C). Error bars represent 2xSD.

Fig. 4 Fungal growth inhibition of the macroalgae organic extracts. PC: *P. cartlagineum*, GT: *G. turquetii*, GC: *G. confluens*, HG: *H. grandifolius*, DA: *D. anceps*, DM: *D. menziesii*, DAnt: *D. Antarctica* and AM: *A. mirabilis*. HX: hexane, EA: Ethyl acetate, ME: methanol. * is the duplicate of the same organic extract.

Table 1 Molecular identification of the isolated fungi

Macroalgae host	Code	sequence accesion number	sequence lenght (bp)	cover (%)	Closest relative NCBI database	% identity	accesion number NCBI	Closes relative Unite database	% identity	ID number Unite (ENA)	Presumtive identification[A]
Adenocystis utricularis	AUE 1	406194	1053	98	*Penicillium polonicum* NRRL 995	98	AF033475	*Penicillium chrysogenum* Thom, 1910	99	KY218674	*Penicillium* sp. AUe1
	AUE 2	406202	721	100	*Cladosporium ossifragi* CBS:842.91	96	EF679381	*Cladosporium* Link, 1816	97	MN543925	*Cladosporium* sp. AUe2
	AUE 3	406203	1093	97	*Cladosporium ossifragi* CBS:842.91	95	EF679381	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries, 1952	95	KX664412	*Cladosporium* sp. AUe3
	AUE 4	406230	1008	99	*Mortierella stylospora* CBS 211.32	88	MH855291	*Mortierella stylospora* Dixon-Stew., 1932	87	MH866744	*Mucoromycota* AUe4
	AUE 5	406198	1004	98	*Penicillium camemberti* IF2SW-F1	99	KY218668	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. AUe5
	AUE 6	406196	1002	99	*Penicillium camemberti* IF2SW-F1	99	KY218668	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. AUe6
	AUE 7	406220	1091	97	*Cladosporium ossifragi* CBS:842.91	96	EF679381	*Cladosporium sphaerospermum* Penz., 1882	98	KC311475	*Cladosporium* sp. AUe7
	AUE 8[B]	375864	574	98	*Penicillium charlesii* CBS 304.48	99	MH867906	*Penicillium dierckxii* Biourge, 1923	99	JQ437599	*Penicillium* sp. AUe8
	AUE 9	406204	1064	97	*Cladosporium ossifragi* CBS:842.91	96	EF679381	*Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cim., 2007	98	LC414352	*Cladosporium* sp. AUe9
	AUE 10	406221	1098	99	*Cladosporium ossifragi* CBS:842.91	95	EF679381	*Cladosporium sphaerospermum* Penz., 1882	99	KC311475	*Cladosporium sphaerospermum* AUe10
	AUE 11	406222	1094	97	*Cladosporium ossifragi* CBS:842.91	99	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUe11
	AUE 12	406223	1028	96	*Cladosporium ossifragi* CBS:842.91	99	EF679381	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries, 1952	99	KX664412	*Cladosporium cladosporoides* AUi12
	AUE 13	406205	1088	95	*Cladosporium ossifragi* CBS:842.91	98	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUe13
	AUE 14	406196	1088	99	*Cladosporium sphaerospermum* D_D48	99	KC311475	*Cladosporium sphaerospermum* Penz., 1882	99	KC311475	*Cladosporium sphaerospermum* AUe14
	AUI 1	406225	1096	96	*Cladosporium ossifragi* CBS:842.91	99	EF679381	Cladosporium Link, 1816	99	MN543925	*Cladosporium* sp. AUi1
	AUI 2	406206	892	99	*Cladosporium ossifragi* CBS:842.91	98	EF679381	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries, 1952	99	KX664412	*Cladosporium cladosporoides* AUi2
	AUI 3	406226	752	99	*Cladosporium ossifragi* CBS:842.91	95	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUi3
	AUI 4	406207	1054	98	*Cladosporium ossifragi* CBS:842.91	96	EF679381	*Cladosporium sphaerospermum* Penz., 1882	99	KC311475	*Cladosporium sphaerospermum* AUi4
	AUI 5	406208	882	99	*Cladosporium ossifragi* CBS:842.91	98	EF679381	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries, 1952	99	KX664412	*Cladosporium cladosporoides* AUi5
	AUI 6	406209	875	100	*Cladosporium ossifragi* CBS:842.91	98	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUi6

	AUI 7	406210	896	99	*Cladosporium ossifragi* CBS:842.91	98	EF679381	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries, 1952	99	KX664412	*Cladosporium cladosporoides* AUi7
	AUI 8	406211	899	99	*Cladosporium ossifragi* CBS:842.91	95	EF679381	*Cladosporium sphaerospermum* Penz., 1882	98	KC311475	*Cladosporium* sp. AUi8
	AUI 9	406227	1091	96	*Cladosporium ossifragi* CBS:842.91	99	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUi9
	AUI 10	406228	1027	96	*Cladosporium ossifragi* CBS:842.91	99	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUi10
	AUI 11	406212	932	100	*Cladosporium ossifragi* CBS:842.91	96	EF679381	*Cladosporium* Link, 1816	99	MN543925	*Cladosporium* sp. AUi11
Ascoseira mirabilis	AM 1	406200	970	94	*Mycochaetophora gentianae*	98	AB434661	*Phialocephala* W.B. Kendr., 1961	99	AB752275	*Phialocephala* sp. AM1
	AM 2	406199	1095	98	*Mycochaetophora gentianae*	97	AB434661	*Phialocephala* W.B. Kendr., 1961	99	AB752273	*Phialocephala* sp. AM2
	AM 4	406201	875	99	*Mycochaetophora gentianae*	96	AB434661	*Cadophora malorum* (Kidd & Beaumont) W. Gams, 2000	97	MF494620	*Cadophora* sp. AM4
Ballia calliatrichia	BC 1	406185	1062	98	*Antarctomyces psychrotrophicus* CBS 100573	99	MH874317	*Antarctomyces psychrotrophicus* Stchigel & Guarro, 2001	99	MH874317	*Antarctomyces psychrotrophicus* BC1
	BC 2	406190	724	98	*Penicillium italicum* CBS 339.48	99	JF772180	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. BC2
Gigartina skottsbergii	GS 1	406188	1052	98	*Cladosporium ossifragi* CBS:842.91	99	EF679381	*Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cim., 2007	99	LC414352	*Cladosporium halotolerans* GS1
	GS 2	406213	1060	99	*Penicillium aurantiogriseum* NRRL 971	96	AF033476	*Penicillium dipodomyicola* (Frisvad, Filt. & Wicklow) Frisvad, 2000	96	KY218680	*Penicilium* sp. GS2
	GS 2.2	406214	958	99	*Mycochaetophora gentianae*	97	AB434661	*Cadophora malorum* (Kidd & Beaumont) W. Gams, 2000	98	MF494620	*Cadophora* sp. GS2.2b
	GS 2.3	406215	1011	98	*Penicillium aurantiogriseum* NRRL 971	97	AF033476	*Penicillium rubens* Biourge, 1923	98	LT558863	*Penicillium* sp. GS2.3
	GS 3	406216	1008	99	*Penicillium* sp. MG-2017a	98	LT898167	*Penicillium dipodomyicola* (Frisvad, Filt. & Wicklow) Frisvad, 2000	99	KY218680	*Penicillium* sp. GS3
	GS 4	406186	1049	98	*Pseudogymnoascus pannorum* var. asperulatus CBS 124.77	98	MH861038	*Pseudogymnoascus pannorum* (Link) Minnis & D.L. Lindner, 2013	99	KX664356	*Pseudogymnoascus pannorum* GS4
	GS 5	406224	996	100	*Penicillium egyptiacum* NRRL 2090	98	AF033467	*Penicillium rubens* Biourge, 1923	99	LT558863	*Penicillium* sp. GS5
	GS 6	406189	1034	98	*Cladosporium ossifragi* CBS:842.91	96	EF679381	*Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cim., 2007	99	LC414352	*Cladosporium halotolerans* GS6
	GS 23[B]	375863	570	96	*Penicillium chrysogenum* CBS306.48	99	JF922035	*Penicillium chrysogenum* Thom, 1910	99	KY218674	*Penicillium* sp. GS23
	GS 24	406217	1068	98	*Penicillium polonicum* NRRL 995	98	AF033475	*Penicillium echinulatum* Raper & Thom ex Fassat., 1976	97	MH856364	*Penicillium* sp. GS24
Neuroglossum delesseriae	ND 1	406193	1055	99	*Penicillium polonicum* NRRL 995	99	AF033475	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. ND1
	ND 2	406192	1059	98	*Penicillium polonicum* NRRL 995	98	AF033475	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. ND2
	ND 3	406187	1043	98	*Penicillium polonicum* NRRL 995	99	AF033475	*Penicillium chrysogenum* Thom, 1910	99	KY218674	*Penicillium* sp. ND3
	ND 4	406218	1107	96	*Cladosporium ossifragi* CBS:842.91	97	EF679381	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries, 1952	97	KX664412	*Cladosporium* sp. ND4
	ND 5	406195	1089	82	*Otidea subterranea* RH69 18S	85	FJ404767	Environmental Fungi	99	KC966218.1	*Pezizomycotina* ND5

	ND 6	406229	946	99	*Penicillium polonicum* NRRL 995	99	AF033475	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. ND6
Palmaria decipiens	PD 1	406191	1058	99	*Penicillium polonicum* NRRL 995	99	AF033475	*Penicillium camemberti* Thom, 1906	99	KY218668	*Penicillium* sp. PD1
	PD 2	406219	1056	99	*Cladosporium ossifragi* CBS:842.91	95	EF679381	*Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cim., 2007	96	LC414352	*Cladosporium* sp. PD2

[A]Presumptive identification corresponds to the database identification with higher percentage of identity and coverage (data not shown). [B]These isolates were identified using NL1-NL4 primers.

Table 2 Growth temperatures of the fungal isolates

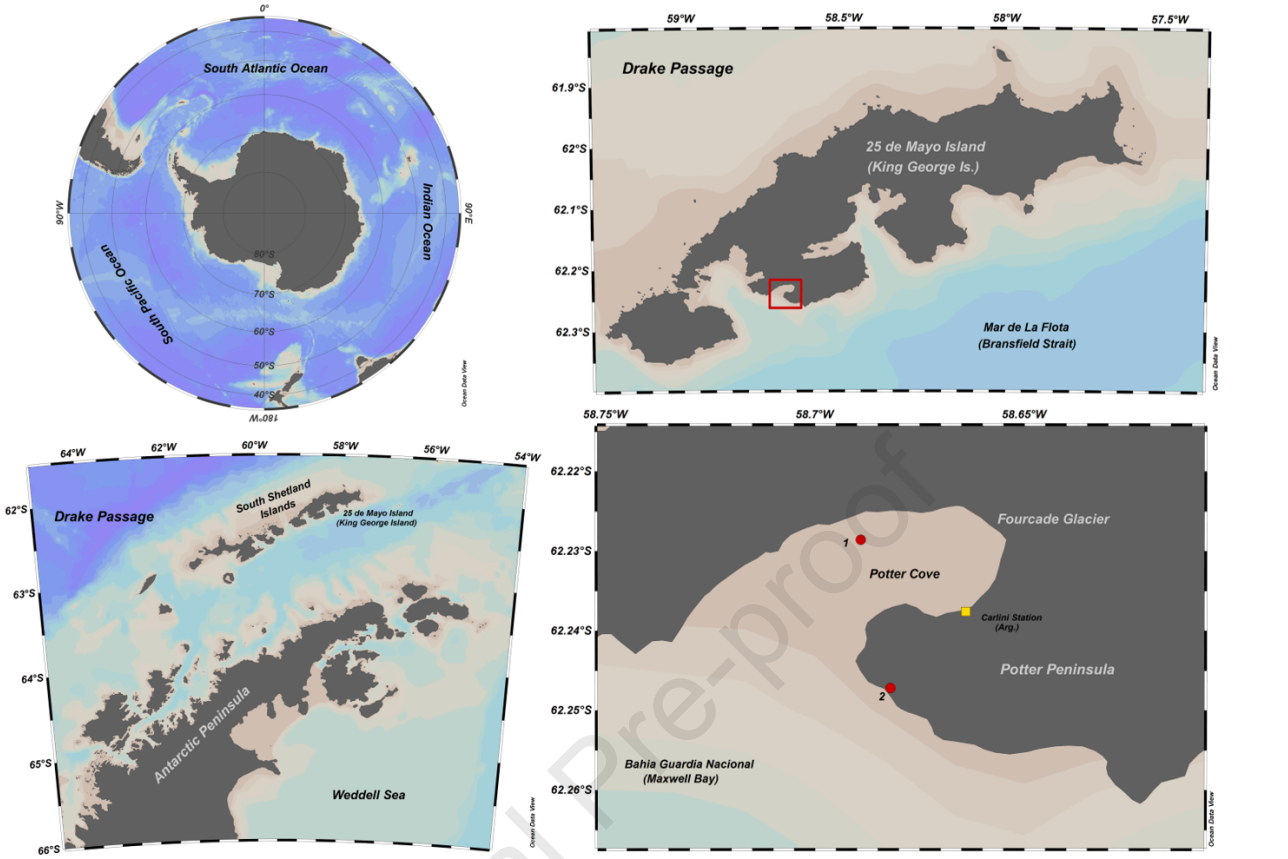
Macroalgae host	identificacion	Colony diameter (mm) at different temperatures at 4 and 25 days of incubation								classification	Higher growth temperature
		5°C		15°C		25°C		35°C			
		4,00	25,00	4,00	25,00	4,00	25,00	4,00	25,00		
Ascoseira mirabilis	*Phialocephala* sp. AM1	0.00	9.50	4.00	39.50	10.13	46.67	-	-	moderate eurypsycrophilic	25°C
	Phialocephala sp. AM2	0.00	11.17	3.67	40.50	10.03	47.00	-	-	moderate eurypsycrophilic	25°C
	Cadophora sp. AM4	0.00	16.00	5.67	38.67	10.07	46.75	-	-	moderate eurypsycrophilic	25°C
Adenocystis utricularis	*Penicillium* sp. AUe1	1.00	25.00	6.00	80.00	22.33	80.00	4.00	15.00	eurypsycrophilic	25°C
	C. sphaerospermum AUe10	0.33	0.33	4.33	48.33	6.00	72.00	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUe11	1.00	15.67	10.33	87.33	19.67	90.00	-	-	moderate eurypsycrophilic	25°C
	C. cladosporoides AUi12	0.33	12.67	10.00	81.00	11.33	75.67	-	-	moderate eurypsycrophilic	15°C
	Cladosporium sp. AUe13	0.50	5.00	12.67	64.00	13.83	74.33	-	-	moderate eurypsycrophilic	25°C
	C. sphaerospermum AUe14	0.67	2.00	4.33	50.00	7.00	65.50	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUe2	0.00	30.00	11.67	81.33	10.00	89.50	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUe3	0.00	4.00	14.00	65.00	14.33	70.00	-	-	moderate eurypsycrophilic	25°C
	Mucoromycota isolate AUe4	0.00	78.33	0.00	94.00	-	-	-	-	stenopsycrophilic	15°C
	Penicillium sp. AUe5	0.33	32.00	0.00	77.67	17.00	52.00	-	-	moderate eurypsycrophilic	15°C
	Penicillium sp. AUe6	1.67	29.67	13.00	71.00	15.00	75.00	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUE7	-	-	2.33	34.33	9.67	61.00	-	-	narrow eurypsycrophilic	25°C
	Penicillium sp. AUe8	-	-	6.40	21.67	10.33	38.00	-	-	narrow eurypsycrophilic	25°C
	Cladosporium sp. AUe9	-	-	3.67	31.67	5.00	54.00	-	-	narrow eurypsycrophilic	25°C
	Cladosporium sp. AUi1	0.00	19.00	9.33	81.00	10.00	87.50	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUi10	0.00	19.33	11.67	80.00	7.66	80.00	-	-	moderate eurypsycrophilic	15 - 25°C
	Cladosporium sp. AUi11	-	-	7.33	46.00	8.83	55.67	-	-	narrow eurypsycrophilic	25°C
	C. cladosporoides AUi2	0.33	1.67	5.33	52.00	5.33	72.50	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUI3	-	-	7.67	44.33	10.50	63.33	-	-	narrow eurypsycrophilic	25°C
	C. sphaerospermum AUi4	0.00	15.25	11.33	82.00	11.00	89.00	-	-	moderate eurypsycrophilic	25°C
	C. cladosporoides AUi5	0.00	16.00	10.67	85.33	8.00	64.00	-	-	moderate eurypsycrophilic	15°C
	Cladosporium sp. AUi6	0.33	6.67	14.00	83.67	14.17	85.00	-	-	moderate eurypsycrophilic	15 - 25°C
	C. cladosporoides AUi7	0.00	24.50	8.33	84.33	18.33	88.00	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUi8	0.00	2.00	4.33	56.67	6.33	65.00	-	-	moderate eurypsycrophilic	25°C
	Cladosporium sp. AUI9	0.00	17.67	10.00	86.33	9.00	85.00	-	-	moderate eurypsycrophilic	15 - 25°C
Ballia calliatrichia	*A. psychrotrophicus* BC1	3.67	45.67	11.67	33.00	15.33	59.33	-	-	moderate eurypsycrophilic	25°C
	Penicillium sp. BC2	3.00	17.67	10.67	53.00	17.00	42.33	-	-	moderate eurypsycrophilic	15°C

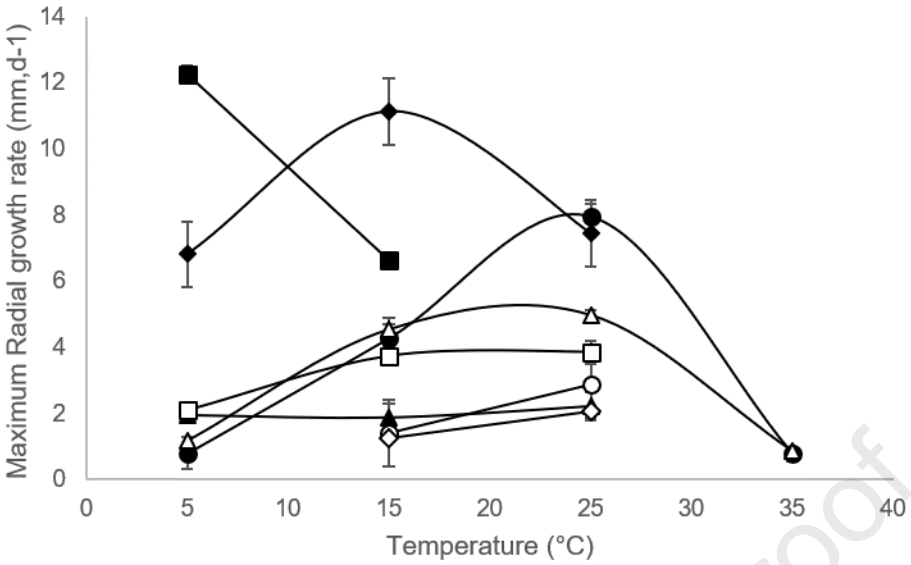
Gigartina skottsbergii	*C. halotolerans* GS1	1.00	3.00	6.67	31.33	13.33	54.00	-	-	moderate eurypsycrophilic	25°C
	Penicilium sp. GS2	0.33	10.33	10.00	42.00	18.33	68.67	2.00	11.00	eurypsycrophilic	25°C
	Cadophora sp. GS2.2b	0.00	9.83	6.00	46.33	10.1	41.25	-	-	moderate eurypsycrophilic	15°C
	Penicillium sp. GS2.3	0.00	7.83	6.67	47.67	7.33	59.00	-	-	moderate eurypsycrophilic	25°C
	Penicillium sp. GS23	3.33	14.67	16.00	57.33	18.33	47.33	9.70	13.00	eurypsycrophilic	15°C
	Penicillium sp. GS24	4.67	20.00	23.33	45.00	17.00	64.00	-	-	moderate eurypsycrophilic	25°C
	Penicillium sp. GS3	5.33	18.00	5.33	50.00	20.33	78.67	2.33	10.33	eurypsycrophilic	25°C
	P. pannorum GS4	1.00	15.66	5.33	29.67	7.83	22.67	-	-	moderate eurypsycrophilic	15°C
	Penicillium sp. GS5	1.33	10.00	10.67	44.33	18.33	39.33	1.33	11.00	eurypsycrophilic	15°C
	C. halotolerans GS6	1.17	3.00	6.33	32.67	30.67	65.67	-	-	moderate eurypsycrophilic	25°C
Neuroglossum delesseriae	*Penicillium* sp. ND1	1.70	28.00	11.33	47.00	15.00	70.67	-	-	moderate eurypsycrophilic	25°C
	Penicillium sp. ND2	2.66	28.00	13.33	59.67	15.67	79.33	-	-	moderate eurypsycrophilic	25°C
	Penicillium sp. ND3	1.33	7.33	14.67	67.00	28.00	82.00	4.33	8.00	eurypsycrophilic	25°C
	Cladosporium sp. ND4	2.66	20.33	5.00	67.00	6.33	24.33	-	-	moderate eurypsycrophilic	15°C
	Pezizomycotina isolate ND5	3.00	94.00	30.67	94.00	26.67	88.00	-	-	moderate eurypsycrophilic	15°C
	Penicillium sp. ND6	2.67	15.33	12.67	50.00	15.67	66.67	-	-	moderate eurypsycrophilic	25°C
Palmaria decipiens	*Penicillium* sp. PD1	1.00	15.67	14.70	71.67	17.00	67.00	-	-	moderate eurypsycrophilic	15°C
	Cladosporium sp. PD2	-	-	3.00	25.67	8.33	45.33	-	-	narrow eurypsycrophilic	25°C

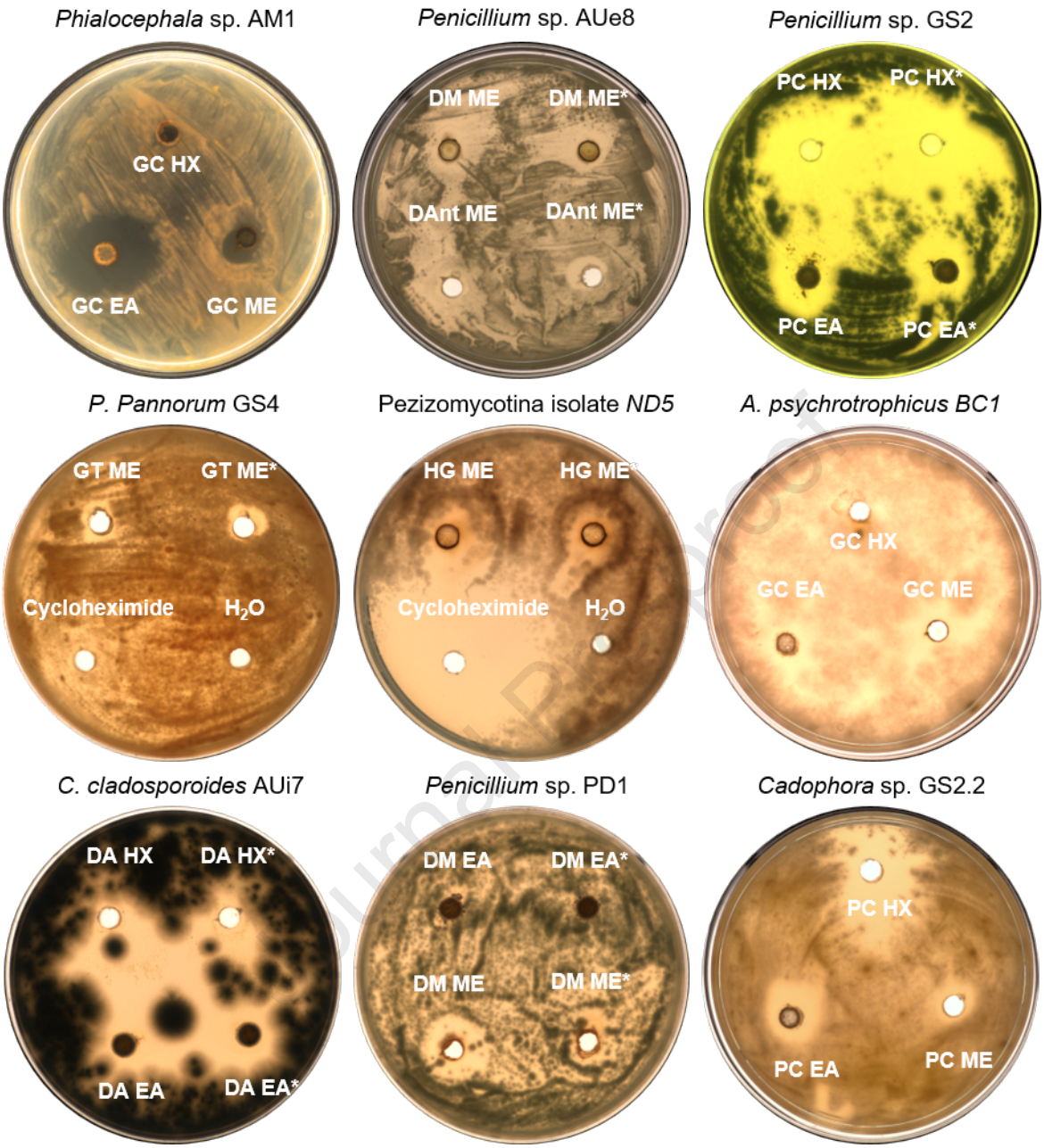
Table 3 Fungal growth inhibition of the macroalgae organic extracts

Macroalgae species	Extract	Inhibition zone ratio (mm)								
		Penicillium sp. PD1	*Penicillium* sp. GS2	*Penicillium* sp. AUe8	*C. cladosporoides* AUi7	*Phialocephala* sp. AM1	*Cadophora* sp. GS2.2b	*A.psychrotrophicus* BC1	Pezizomycotina isolate ND5	*P. pannorum* GS4
Ascoseira mirabilis	hexane	-	-	-	-	**4 ± 1.06**	-	-	-	-
	ethyl acetate	-	-	-	**3.38 ± 1.24**	1.63 ± 0.53	1.5 ± 0.71	**1.5 ± 0.35**	**3.5 ± 1.06**	1.13 ± 0.18
	methanol	-	**2.38 ± 0.88**	**3 ± 0.71**	-	**2.5 ± 1.06**	-	-	**3.38 ± 1.59**	3.38 ± 1.24
Desmarestia antarctica	hexane	-	-	-	-	-	-	-	-	-
	ethyl acetate	-	**1.38 ± 0.53**	**2.88 ± 0.18**	**5.25 ± 1.06**	**2.13 ± 0.18**	1.5 ± 0.71	**1.63 ± 0.18**	-	**2 ± 0.35**
	methanol	1.38 ± 0.18	**2.63 ± 0.18**	**3.19 ± 1.78**	-	**1.5 ± 0.35**	-	**1.5 ± 0.71**	-	1.75 ± 1.06
Desmarestia anceps	hexane	-	-	-	-	-	-	-	-	-
	ethyl acetate	-	-	-	**2.5 ± 0.71**	**6.38 ± 0.88**	1.13 ± 0.18	-	-	-
	methanol	**6.88 ± 0.32**	**2.5**	**2.13 ± 0.88**	**3.63 ± 0.88**	**2 ± 0.71**	2.63 ± 0.18	-	-	2.5
Desmarestia menziesii	hexane	-	-	-	-	-	-	-	-	-
	ethyl acetate	-	**2 ± 1.41**	-	**3.44 ± 0.66**	1.5 ± 0.71	3.63 ± 2.30	**3.13 ± 0.53**	-	**3.13 ± 1.59**
	methanol	**4.5 ± 1.59**	**2 ± 1.41**	**5 ± 3.07**	**3.63 ± 0.18**	**2.25 ± 0.71**	2.38 ± 0.88	**2.75 ± 0.48**	-	2.13 ± 0.53
Georgiella confluens	hexane	-	-	-	-	**0.75 ± 0.35**	-	-	-	-
	ethyl acetate	-	-	-	-	**6.5 ± 4.60**	1.88 ± 0.88	**3.25 ± 1.06**	-	-
	methanol	-	**2**	-	-	**1.38 ± 0.53**	-	**2.75 ± 2.47**	-	-
Gymnogongrus turquetii	hexane	-	-	**10.5 ± 2.47**	**17 ± 0.71**	**1**	-	**7.75**	**5.06 ± 3.85**	-
	ethyl acetate	-	-	**1.88 ± 0.18**	**6.13 ± 3.01**	1	**2.75 ± 0.35**	-	-	**6.13 ± 0.18**
	methanol	**4.5 ± 1.70**	**3.5 ± 1.41**	**2.5 ± 1.06**	**4 ± 0.12**	**2 ± 1.41**	2.38 ± 0.53	-	-	2.88 ± 0.18
Himantothallus grandifolius	hexane	-	-	-	-	-	-	**2.25 ± 0.35**	-	-
	ethyl acetate	-	-	-	-	1.25 ± 0.35	-	-	-	-
	methanol	-	-	**4.13 ± 0.18**	-	-	-	-	**3.88 ± 0.18**	-
Plocanium cartlagineum	hexane	**11.75 ± 2.47**	**10.75 ± 1.06**	**10.63 ± 2.65**	**11.75**	**7.25 ± 1.77**	**8.13 ± 3**	**10.5 ± 4.60**	**12.88 ± 3**	**13.25 ± 1.77**
	ethyl acetate	**4.13 ± 0.18**	**5.13 ± 0.18**	**5.38 ± 0.18**	**6.13 ± 0.53**	**8.38 ± 1.24**	**4.75 ± 0.71**	**3.5 ± 1.41**	**6.63 ± 1.59**	**9.38 ± 1.24**
	methanol	**5.5 ± 1.06**	**8.25**	**1.88 ± 0.18**	**5.56 ± 1.36**	**4.75**	**3.63 ± 1.24**	**5.13 ± 1.60**	**9.88 ± 1.24**	5.75 ± 0.35
Controls										
Cycloheximide[2]		5.5 ± 0.58	8 ± 2.31	10	11.25 ± 2.5	13.5 ± 1.29	13.5 ± 0.58	4.75 ± 6.18	19.25 ± 2.63	11.75 ± 4.27
Hexane		-	-	-	-	-	-	4.5 ± 6.35	-	-
Ethyl Acetate		1.25 ± 0.5	-	-	-	1.25 ± 0.5	1	-	-	1

Methanol	1.75 ± 0.5	-	-	-	1	2.75 ± 1.71	-	-	4.25 ± 0.96






Phialocephala sp. AM1
GC HX
GC EA
GC ME
Penicillium sp. AUe8
DM ME
DM ME*
DAnt ME
DAnt ME*
Penicillium sp. GS2
PC HX
PC HX*
PC EA
PC EA*
P. Pannorum GS4
GT ME
GT ME*
Cycloheximide
H_2O
Pezizomycotina isolate *ND5*
HG ME
HG ME*
Cycloheximide
H_2O
A. psychrotrophicus BC1
GC HX
GC EA
GC ME
C. cladosporoides AUi7
DA HX
DA HX*
DA EA
DA EA*
Penicillium sp. PD1
DM EA
DM EA*
DM ME
DM ME*
Cadophora sp. GS2.2
PC HX
PC EA
PC ME

- Several Antarctic macroalgae are habitat for marine and cosmopolitan fungi.
- Some macroalgae present antifungal activities with biotechnological potential.
- Isolated fungi showed a different spectrum of growth temperatures.