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MICROBIOLOGY

Resistance to adverse conditions and characterization of *Cladosporium* species from marine and terrestrial Antarctic samples

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Abstract: Microbial adaptations to extreme environments can lead to biotechnological applications. This study aimed to evaluate the resistance of Antarctic *Cladosporium* to adverse conditions (temperature, salinity, UV radiation, and nutrients) and refine their taxonomy. Sequencing and phylogenetic analysis using ITS-*act* markers resulted in a more accurate taxonomic identification, revealing the presence of five different species, belonging to the complexes *C. cladosporioides* and *C. sphaerospermum*. The growth at different temperatures indicates that the soil isolates LAMAI 564 and 1800 (phylogenetically closely related) and LAMAI 2541 are psychrophilic, while the other isolates are psychrotolerant. The fungi isolated from the saline samples LAMAI 595, 616, and 1369 showed better growth results at higher salinity (15%). The fungi most resistant to UV radiation were isolated from terrestrial and marine samples (LAMAI 595, 616, 1800, and 564). LAMAI 595 and 616 (phylogenetically closely related and isolated from the same kind of sample) showed the capacity of nutritional versatility, growing well in both rich and poor-nutrient media. The fungus LAMAI 595 was the most promising for biotechnological application, exceeding the other isolates in the harsh conditions studied. The resistance of the Antarctic *Cladosporium* to adverse conditions opens new perspectives in the field of applied microbiology of extremophiles.

Key words: extremophile, taxonomy, biomolecules, biotechnology.

INTRODUCTION

The Antarctic ecosystem is considered one of the most extreme environments, characterized by low temperatures, which can reach -40 °C (Tindall 2004), high incidence of UV rays, freezing and thawing cycles, high salinity, low amount of available nutrients, and long periods of darkness (Wentzel et al. 2019, Duarte et al. 2018a, Singh et al. 2011). Despite these extreme climate and ecological conditions, this ecosystem supports a variety of organisms. Bacteria, fungi, and microalgae are among the main microorganisms found in Antarctica (Turner et al. 2009).

The microorganisms that inhabit the Antarctic environment have adaptations that allow them to thrive, despite the unfavorable conditions to which they are exposed (Duarte et al. 2018a, Ramasamy et al. 2023). At lower temperatures, the plasma membrane becomes more rigid and less fluid. To overcome this problem, these microorganisms present differences in their membranes that allow them to retain their fluidity, such as a greater proportion of unsaturated lipids, with a shorter chain (Loperena et al. 2012). The presence of antifreeze proteins and enzymes that have their maximum enzymatic activity at lower temperatures has also been observed (Wentzel et al. 2019). Some microorganisms have pigments, which grant protection against

ultraviolet radiation (Kostadinova et al. 2009, Sajjad et al. 2020).

The estimate of the number of fungal species in nature is between 2 and 11 million (Phukhamsakda et al. 2022), many of which are considered possible sources of natural resources (biomolecules) that can be explored and transformed into a series of viable products. Fungi are known to be the main organic matter decomposers in the environment where they are found and, some of them can cause disease in humans, plants, and animals (Naranjo-Ortiz & Gabaldón 2019). The correct identification, at the species level, is of paramount importance for improving studies related to these organisms, both for the generation of basic knowledge in ecology and taxonomy and for the possible discovery of new biotechnological applications (Raja et al. 2017).

Among the many fungal genera known, *Cladosporium* stands out for being a cosmopolitan and generalist group, found in several ecosystems, being easily dispersed, with the capacity to colonize and adapt to new habitats (Bensch et al. 2012). Several morphophysiological characteristics and adaptations allow species belonging to this group to survive in environments and conditions considered extreme for most living creatures (Loperana et al. 2012). Many of these properties can be explored due to their high potential for new biotechnological applications (Raja et al. 2017, Duarte et al. 2018a, Varrella et al. 2021, Kita et al. 2022, Farias et al. 2022).

The genus *Cladosporium* belongs to the phylum Ascomycota and was first described in 1816, by Link, who defined *Cladosporium herbarum* as the type species (Crous et al. 2007). For a long time, this genus was recognized as one of the largest and most heterogeneous groups of fungi, comprising more than 772 species. More recently, less than 200 species have been recognized as being true *Cladosporium* (Bensch et al. 2015), which are distributed into three large species complexes, distinguished mainly based on their morphology: *Cladosporium herbarum*, *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* (Bensch et al. 2018). The large majority of *Cladosporium* species are saprobic, but there are also endophytic species, plant and fungus parasites, and species that are pathogenic to animals, including humans (Iturrieta-González et al. 2021).

Several *Cladosporium* species have been isolated and identified in Antarctica. It is not uncommon to find strains that have probably been isolated from propagules in a dormant state (Ruisi et al. 2007), especially since *Cladosporium* is well known for presenting conidia that are easily dispersed (Kurkela 1997). *Cladosporium* representatives have also been found and isolated from several lakes in Antarctica (Brunati et al. 2009), and porous sandstone rocks from places such as the McMurdo Dry Valleys (Onofri et al. 2000), associated with other organisms (Rosa et al. 2020a), isolated from snow and air (Rosa et al. 2020b, Rosa et al. 2021), and even some of them possibly associated with anthropogenic activities in the region (Ogaki et al. 2020).

Fungi from the genus *Cladosporium,* found in the Antarctic environment, have been reported as being able to produce cold-adapted enzymes (Duarte et al. 2018b). Widely used in the industrial scope, cold-adapted enzymes present high activity while operating at low and moderate temperatures, which translates into energy savings. Furthermore, these enzymes are also stable at higher temperatures and require a lower activation energy (Wentzel et al. 2019, Yusof et al. 2021, Morozova et al. 2022).

The pigment melanin is another adaptation found and explored in the genus *Cladosporium*. These pigments are an important adaptive factor for survival, acting as a defense against

numerous environmental stress conditions, and being able to absorb ultraviolet radiation (Gessler et al. 2014). Melanin pigments have radioprotective properties, and several species of fungi, including members of the *Cladosporium* genus, have been found in the vicinity of the destroyed Chernobyl reactor (Shunk et al. 2020). Some of these fungi showed radiotropism, and from this characteristic it has been theorized that melanin pigments are capable of assisting in the integration of the surrounding radioactive energy into metabolic processes, thereby transforming radioactive energy into chemical energy (Dadachova & Casadevall 2008). Since then, it has been discovered that these melanized pigments have semiconductor properties, which is of special interest in the field of bioelectronics (Gessler et al. 2014).

Considering that the adaptive strategies of fungi have enabled the colonization of different niches and can result in the synthesis of innovative biomolecules, and since phylogenetic diversity may not be directly related to functional diversity, this study aimed to characterize and investigate the resistance of *Cladosporium* species isolated from Antarctic marine and terrestrial environments to adverse conditions.

MATERIALS AND METHODS

Microorganisms

A total of eight fungi belonging to the genus *Cladosporium*, isolated from marine and terrestrial Antarctic samples, were used in this study (Table I). Besides these eight fungi, the fungus *Cladosporium cladosporioides* LAMAI 446, isolated from a marine invertebrate on the Brazilian coast (da Silva et al. 2008), was used as a comparative model (tropical environment) in the experiments related to the growth under adverse conditions. The fungal isolates were preserved by two distinct methods: cryopreservation (-80 °C) and Castellani (4 °C), using cryotubes with 10% glycerol and water, respectively. All fungi are currently maintained in the research collection of the Laboratory of

Table I. Data related to the sites at King George Island (Antarctic Peninsula), the samples of origin, and *Cladosporium* codes.

Original code	LAMAI code	Site	Geographic coordinates	Sample	Reference
ITAE4	595	Arctowski	62°08' S 58°27' W	Ornithogenic soil	(Duarte et al. 2018b)
S3	564	Punta plaza	62°05' S 58°24' W	Marine invertebrate *Salpa* sp.	(Duarte et al. 2018b)
ITAE3	616	Arctowski	62°08' S 58°27' W	Ornithogenic soil	(Duarte et al. 2018b)
MB9	598	Punta plaza	62°05' S 58°24' W	Wood	(Duarte et al. 2018b)
11P-3.10 II-15C	1800	Punta plaza	62°05.363' S 58°24.691' W	*Deschampsia antarctica* Root-associated soil	(Wentzel et al. 2019)
4D-3C1II	1345	Punta Ullman	62°05.015' S 58°20.987' W	Marine sediment	(Wentzel et al. 2019)
4B-1C115IIII	1369	Punta Ullman	62° 05.015' S 58°20.987' W	Marine sediment	(Wentzel et al. 2019)
B0.346	2541	Collins glacier (Fildes Peninsula)	62° 9.821'S 58° 55.373'W	Glacier retreat soil	(Santos et al. 2020)

Environmental and Industrial Mycology (LAMAI), which is associated with the microbial biobank Central of Microbial Resources (CRM-UNESP) of the São Paulo State University (UNESP, Brazil).

Molecular taxonomy

The Antarctic-derived fungi used in this study had been previously identified as *Cladosporium* sp. using the ITS barcode marker as described by Duarte et al. (2018b), Wentzel et al. (2019) and Santos et al. (2020). In the present study, the taxonomic characterization of these strains was refined using the secondary marker (partial *act* gene) concatenated with ITS, as described below.

DNA extraction followed the method adapted from Moller et al. (1992) and Gerardo et al. (2004). The gene *act* was amplified using the primers ACT-512F (5'-ATGTGCAAGGCCGGTTTCGC-3') and ACT-783R (5'-TACGAGTCCTTCTGGCCCAT-3'). The PCR reactions were optimized for each isolate, as reported by Carbone & Kohn (1999) and Bensch et al. (2012). The amplicons were purified using the PureLink™ PCR Purification Kit (Invitrogen by Thermo Fisher Scientific), following the manufacturer's protocol. The samples were quantified using NanoDrop® (Thermo Scientific).

The amplified and purified *act* gene fragment of each isolate was inserted into the pGEM-T Easy vector (Promega, Madison, USA), according to the manufacturer's protocol. The vector with the insert was then inserted into the competent *E. coli* JM109 cells provided in the kit, by transformation via heat shock. These competent cells were combined with the vector by staying at 42 °C for 50 seconds, followed by 2 min on ice. Subsequently, they were cultivated in Luria Bertani (LB) medium supplemented with ampicillin (100 µg/mL), X-gal (40 µg/mL) and IPTG (0.5 mM), and incubated at 37 °C for 16-18 h, for the selection of possible clones containing the fragment of interest. The presence of the fragment in the clones containing the insert (white colonies) was confirmed by colony PCR, followed by agarose gel electrophoresis. The colonies containing the insert were then cultivated in LB medium for 16-18 h at 37 °C and 150 rpm and preserved in LB medium containing 40% glycerol, at -80 °C. The cloning vectors were extracted using the GeneJET Plasmid Miniprep KIT (Thermo Fisher). Agarose gel electrophoresis was performed to confirm that the plasmid was successfully extracted. The extracted vectors were sequenced by the Sanger Method by Macrogen Inc. (South Korea) using the oligonucleotides M13 (M13F – GTAAACGACGGCCAGT and M13R – CAGGAAACAGCTATGAC).

The *act* sequences generated were assembled into contigs with ITS sequences (ITS-*act)* using BioEdit v.7.0.5.3 (Hall 1999). The concatenated sequences were aligned with ITS-*act* sequences from *Cladosporium* species (Bensch et al. 2018) using ClustalX v.2.1 (Thompson et al. 1997), manually edited in Bioedit v.7.0.5.3, and the evolutionary analyses were conducted in MEGA X (Kumar et al. 2018). The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model (Kimura 1980). The bootstrap consensus tree inferred from 1000 replicates was obtained to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). The branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Initial tree(s) for the heuristic search were obtained applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (3 categories

(+G, parameter = 0.3238)). The rate variation model allowed some sites to be evolutionarily invariable ([+I], 28.69% sites). This analysis involved 58 nucleotide sequences. There was a total of 3322 positions in the final dataset. The sequences are deposited in GenBank under the following accession numbers: OR044437 to OR044444.

Growth under adverse conditions

Temperature

The methodology used to define the best growth temperatures (optimal temperature) for each fungus was adapted from Da Silva Junior et al. (2018). From a monosporic culture grown for 5 days at 15°C in malt extract 2% (MA2%: 20g/L of malt extract and 20g/L of agar) (KASVI), cylinders (5 mm in diameter) were removed and deposited in the center of Petri dishes (1 cylinder per plate) containing the MA2% culture medium. Subsequently, the plates were incubated at different temperatures (5, 10, 15, 20, 25, and 30°C) in a Bio-Oxygen Incubator (BOD). After 10 days of growth, the diameter of each fungus was measured in millimeters, using the software ImageJ. The experiment was conducted in quadruplicate.

Culture media

The methodology for measuring the growth of each isolate in different culture media was adapted from Da Silva Junior (2018). From a monosporic culture grown for 5 days at the optimum growth temperature in MA2% medium, cylinders (5 mm in diameter) were removed and deposited in the center of Petri dishes containing the following media (KASVI): Potato Dextrose Agar (PDA), PDA1%, MA2%, and Synthetic Nutrient-poor Agar (SNA: 1g/L of KH_2PO_4; 1g/L of KNO_3; 0.5g/L of $MgSO_4.7H_2O$; 0.5g/L of KCl; 0.2g/L of glucose; 0.2g/L of sucrose and 20g/L of agar). Subsequently, the plates were incubated in a BOD, at the optimum temperature for each isolate. After 10 days of growth, the diameter of each fungus was measured in millimeters, using the software ImageJ. The experiment was conducted in quadruplicate.

Salinity

The methodology used to measure the growth of each isolate under osmotic stress was adapted from Onofri et al. (2007). From a monosporic culture grown for 5 days at the optimum temperature in MA2% medium, cylinders (5 mm in diameter) were removed and deposited in the center of Petri dishes containing MA2% medium, supplemented with different concentrations of NaCl (5, 10, 15, 20, and 25% w/v). Subsequently, the plates were incubated in a BOD at the optimum temperature for each isolate. After 10 days of growth, the diameter of each fungus was measured in millimeters, using the software ImageJ. The experiment was conducted in quadruplicate.

UV Radiation

For the evaluation of the ultraviolet (UV) resistance of *Cladosporium* isolates, a laminar flow cabinet was used. The cabinet contained different low-pressure Hg lamps, specific for each type of radiation tested: ultraviolet B (15W, 305nm, 2.54 W/m^2, G15T8E, Ushio) and ultraviolet C (15W, 254 nm, 3.39 W/m^2, G13T8, Osram). Petri dishes with MA2% medium were inoculated with 0.1 ml of 0.9% saline solution, containing 10^6 spores/ml, in triplicate, and were exposed for different periods to the irradiation of each lamp: UVB (90, 100, 110, 120, and 130 min) and UVC (10, 15, 20, 25, and 30 min). A screening was previously performed to determine these exposure times. After the exposure period, all plates were incubated in a BOD, at the optimum growth temperature for each isolate.

The growth of the isolates was determined by counting Colony Forming Units (CFU). This methodology was adapted from Selbmann et al. (2011). Antarctic *Cladosporium* isolates grown under the same conditions as the irradiated samples but without exposure to UV were used as controls. Furthermore, the tropical marine fungus *C. cladosporioide* LAMAI 446 was also used as a control (non-Antarctic fungus).

Statistical analysis

The statistical analysis was executed using the software XLSTAT. For the growth under different temperatures, the Kruskal-Wallis statistical test was employed (P<0.001). For the growth on different culture media, salinity, and UV radiation, One-Way ANOVA was used and the statistical analysis was performed using Duncan's method (P<0.05).

RESULTS

Molecular Taxonomy

Based on the ITS-*act* sequences and phylogenetic analysis (Figure 1), the eight *Cladosporium* strains from Antarctic origin are distributed within two of the three complexes: *C. cladosporioides* (LAMAI 564, 595, 598, 616, 1345, 1369 and 1800) and *C. sphaerospermum* (LAMAI 2541).

Considering the molecular analyses of representatives of the *C. cladosporioides* complex, the ITS-*act* sequences of the isolates LAMAI 564 and 1800 were identical and grouped

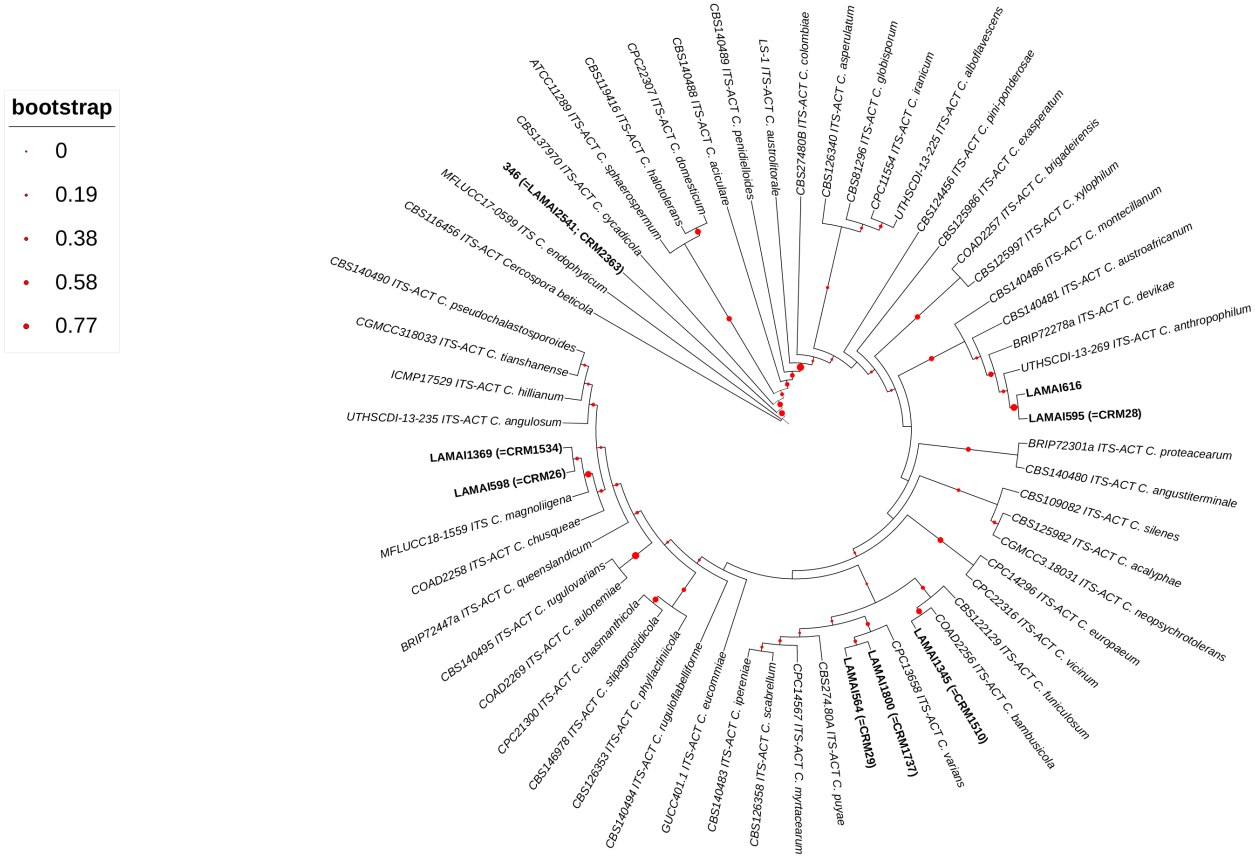

Figure 1. Phylogenetic analysis of *Cladosporium* ITS-*act* sequences. The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model. The percentage of trees in which the associated taxa cluster (bootstrap of 1000 replicates) is shown next to the branches.

with the ITS-*act* sequences of the type strain *C. varians*. Isolate LAMAI 598 formed a clade together with isolate LAMAI 1369 and with the type strain of the species, *C. magnoliigena*. It should be noted that, in the phylogenetic tree generated with the ITS marker, isolate LAMAI 1369 formed a cluster with the species *C. alboflavescens* (data not shown). According to the result of the analysis of the ITS-*act* concatenated markers, the isolates LAMAI 595 and 616 grouped together and formed a cluster with the type strain of the species *C. anthropophilum*. Data derived from the phylogenetic analysis of isolate LAMAI 595 from the tree generated with the ITS marker revealed that it formed a clade with isolate LAMAI 616 and three different species of the studied genus: *C. bambusicola*; *C. devikae* and *C. anthropophilum* (data not shown). The ITS-*act* markers grouped the isolate LAMAI 1345 with the species *C. bambusicola*.

The fungus LAMAI 2541, the only isolate that belongs to a different complex (*C. sphaerospermum*), clustered separately from other Antarctic *Cladosporium* species, and it was closer to the type strains of the species *C. endophyticum*. It should be noted that, for the species *C. endophyticum*, only the sequence of the ITS gene was included in the ITS-*act* tree, since there are no sequences of the *act* gene for this species in public databases. In the phylogenetic tree using the ITS marker (data not shown) this isolate was closer to the species *C. halotolerans*.

Growth under adverse conditions

Regarding temperature, all *Cladosporium* isolates showed statistically significant differences (Table II), which indicates that this variable is important for the growth of the studied fungi. The most frequent optimal growth temperatures were 20 and 25 °C. Five of the eight Antarctic isolates (LAMAI 595, 598, 616, 1345, and 1369) grew best at these temperatures. Isolates LAMAI 564 and 2541 showed the best growth at 15 °C and 20 °C, with no significant differences between these two temperatures. For the isolate LAMAI 1800, the best growth temperature was 15 °C. The fungus from the tropical region *C. cladosporioides* LAMAI 446, used as a basis for comparison, showed the best growth at 25 °C. In general, this isolate showed a lower mycelial growth at the temperatures at which it was able to grow (from 10 °C to 30 °C) in comparison with the Antarctic *Cladosporium*. The Antarctic isolates LAMAI 564

Table II. Fungal growth (mm) at different temperatures. Means followed by distinct letters on the line differ by the non-parametric Kruskal-Wallis test (P<0.001).

LAMAI code	Temperature (°C)					
	5	10	15	20	25	30
446*	0.0^{f}	9.06±2.1^{e}	13.43±0.4^{d}	16.34±0.5^{b}	21.27±4.1^{a}	10.43±0.7^{c}
564	0.0^{c}	15.87±0.5^{b}	21.46±4.2^{a}	20.89±1.5^{a}	0.0^{c}	0.0^{c}
595	8.51±0.3^{e}	18.77±0.2^{c}	29.67±1.3^{b}	42.22±2.6^{a}	42.10±0.8^{a}	15.15±0.7^{d}
598	0.0^{f}	9.02±0.7^{e}	16.45±0.5^{d}	23.41±0.9^{b}	29.67±2.3^{a}	21.38±1.7^{c}
616	9.74±0.7^{f}	22.83±1.2^{d}	30.41±1.5^{c}	35.24±0.5^{b}	40.61±2.2^{a}	13.46±1.6^{e}
1345	8.85±0.5^{d}	22.03±1.4^{c}	31.39±1.1^{b}	42.63±0.9^{a}	20.76±1.2^{c}	0.0^{e}
1369	9.43±0.7^{d}	21.01±2.5^{c}	26.9±1.7^{b}	28.78±1^{ab}	29.7±0.31^{a}	11.98±0.6^{d}
1800	0.0^{d}	11.2±1^{b}	16.21±0.8^{a}	9.3±0.9^{c}	0.0^{d}	0.0^{d}
2541	4.65±0.7^{c}	10.59±0.2^{b}	16.68±1.5^{a}	16.82±2.1^{a}	0.0^{d}	0.0^{d}

***tropical strain.**

and 1800 (grouped with *C. varians*) and LAMAI 2541 (related to *C. endophyticum*) did not grow above 20 °C. Isolates LAMAI 564 and 1800 and LAMAI 595 (grouped with *C. anthropophilum*) did not grow below 10 °C. At the highest temperature (30 °C), the Antarctic isolates showed no growth or low growth. The isolates LAMAI 564, 1800, and 2541 were classified as psychrophilic, while isolates LAMAI 446, 595, 616, 598, 1369, and 1345 were classified as psychrotolerant.

The results showed that the growth of the Antarctic isolates LAMAI 564, 595, 598, 616, 1345, 1369, and 2541 with the different nutrients (culture media) were statistically significant (Figure 2). Nevertheless, for the Antarctic isolate LAMAI 1800 (grouped with *C. varians*) and the tropical isolate *C. cladosporioides* LAMAI 446, no

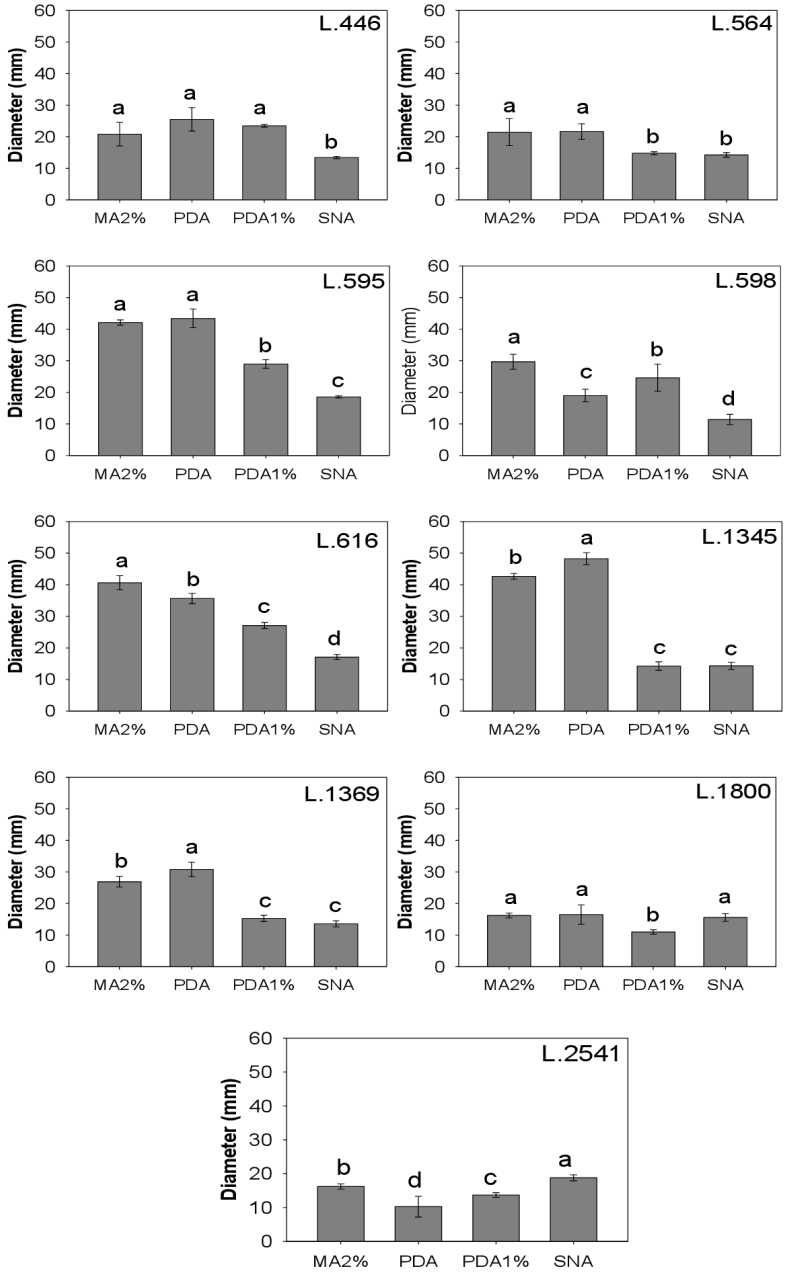

Figure 2. Diameter, in millimeters, of the *Cladosporium* isolates in the culture media MA2%, BDA, BDA1%, and SNA after 10 days of growth at the optimum temperature. Means followed by different letters differ by the Duncan's test (α = 5%).

significant variation in growth was observed in the media MA2% and PDA (P>0.05). The isolate LAMAI 1345 (grouped with *C. bambusicola*) showed the highest growth in the media MA2% (42.6 mm) and PDA (48.2 mm). In the medium PDA1%, only the tropical isolate *C. cladosporioides* LAMAI 446 was able to achieve the same growth obtained in MA2% and PDA. In general, in SNA medium all isolates tested showed the smallest growth rates (<20 mm in diameter) compared to the other media, except for isolate LAMAI 2541 (related to *C. endophyticum*), which showed the best growth in SNA medium (18.78 mm).

There was a statistical difference in the fungal growth at different NaCl concentrations (Table III). All *Cladosporium* from Antarctica and the tropical isolate showed the best growth at 5% NaCl. The highest growth was achieved by isolates LAMAI 595 and 616 (grouped with *C. anthropophilum*) (31.2 mm and 35 mm, respectively), which also presented growth, together with LAMAI 1369 (grouped with *C. magnoliigena*), and the tropical isolate *C. cladosporioides* LAMAI 446 at 10% and 15% NaCl. At 20 and 25% NaCl, none of the tested fungi were able to grow.

The results of UV radiation demonstrated that both UVB (Figure 3) and UVC (Figure 4) were important selective agents for the survival of the Antarctic *Cladosporium* fungi. Isolate LAMAI 595 (grouped with *C. anthropophilum*) showed the highest resistance against UVB radiation compared to the other isolates studied, in all tested exposure times (statistically significant, p<0.05) (Supplementary Material - Table SI). The Antarctic isolates which grouped with *C. varians* (LAMAI 564 and 1800) and the isolate which grouped with *C. anthropophilum* (LAMAI 616) also showed resistance to UVB at all exposure periods, but with a smaller number of surviving colonies (Table SI). After 130 min of exposure, isolate LAMAI 564 showed the highest number of surviving colonies (121.3 CFU/mL), followed by LAMAI 616 (115 CFU/mL), while the tropical fungus *C. cladosporioides* LAMAI 446 showed, at this same condition, 66.6 CFU/mL. Isolates LAMAI 1369 (grouped with *C. magnoliigena*), LAMAI 1345 (grouped with *C. bambusicola*), and LAMAI 2541 (related to *C. endophyticum*) survived from 90 to 110 min of exposure, but with a low number of surviving colonies.

Similarly to what was observed in the UVB treatment, isolate LAMAI 595 (grouped with *C. anthropophilum*) demonstrated significantly greater resistance to UVC radiation (5029.8 CFU/mL after 10 min of exposure) (Table SII). The

Table III. Growth of fungal isolates (mm) at different NaCl concentrations (5, 10, 15, 20, and 25 %). Means followed by distinct letters on the line differ from each other by the Duncan's test (α = 5%).

Isolate	NaCl concentration (%)				
	5	10	15	20	25
L.446*	27.83±0.9^{a}	19.77±1.3^{b}	8.37±0.7^{c}	0.0^{d}	0.0^{d}
L.564	13.18±1^{a}	8.82±0.5^{b}	0.0^{c}	0.0^{c}	0.0^{c}
L.595	31.24±1^{a}	17.13±1.1^{b}	11.40±1.5^{c}	0.0^{d}	0.0^{d}
L.598	18.60±0.6^{a}	0.0^{b}	0,0^{b}	0.0^{b}	0.0^{b}
L.616	35.04±2.9^{a}	24.85±1.3^{b}	12.13±1.3^{c}	0.0^{d}	0.0^{d}
L.1345	7.36±0.5^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}
L.1369	17.69±0.4^{a}	15.58±0.8^{b}	14.66±0.5^{c}	0.0^{d}	0.0^{d}
L.1800	8.79±2.2^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}
L.2541	8.11±1^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}

***tropical strain.**

isolates L564 and 1800 (grouped with *C. varians*), LAMAI 616 (grouped with *C. anthropophilum*), and LAMAI 1345 (grouped with *C. bambusicola*) showed UVC resistance at all exposure times. After 30 min of exposure, isolate LAMAI 564 showed the highest number of surviving colonies (104 CFU/mL), followed by LAMAI 1345 (64 CFU/mL), LAMAI 616 (41.6 CFU/mL) and LAMAI 1800 (28 CFU/mL). The tropical marine fungus *C. cladosporioides* LAMAI 446 showed similar growth to the Antarctic fungi after all times of exposure to UVC, with 83.3 CFU/mL of surviving colonies after 30 min of exposure. Isolate LAMAI 2541 (related to *C. endophyticum*) did not withstand exposure times longer than 10 min and, even with only 10 min of exposure, it showed a low number of surviving colonies. It can be seen that all isolates showed significantly less resistance against UVB and UVC radiation with increased exposure times (Table SI and SII).

DISCUSSION

Molecular Taxonomy

The Antarctic environment possesses an undiscovered amount of microbiological diversity, from which *Cladosporium* represents an important portion (Rosa et al. 2020b, Rosa et al. 2021, Menezes et al. 2022). The results of molecular and phylogenetic analyses using ITS-*act* concatenated markers showed that the studied Antarctic fungi represent at least five different species. Sequence similarity between the isolates LAMAI 564 (recovered from a sample of the marine organism *Salpa* sp.) and LAMAI 1800 (isolated from *D. antarctica* root-associated soil) and the type strain of *C. varians* was high. The species *C. varians* has been isolated from dead leaves and is a saprobic or endophytic fungus. Representatives of this species have already been reported in different regions of the world (Bensch et al. 2010). *Cladosporium* species associated with other organisms are commonly found in the Antarctic environment (Tosi et al. 2002, Rosa et al. 2020a). Although these fungi have been isolated from different environments, their morphological characteristics were similar (greyish olive color in MA2% medium – data not shown), as well as the growth parameters at different temperatures, culture media, and resistance to UVB and UVC radiation. These results indicate that these two isolates may belong to the same species.

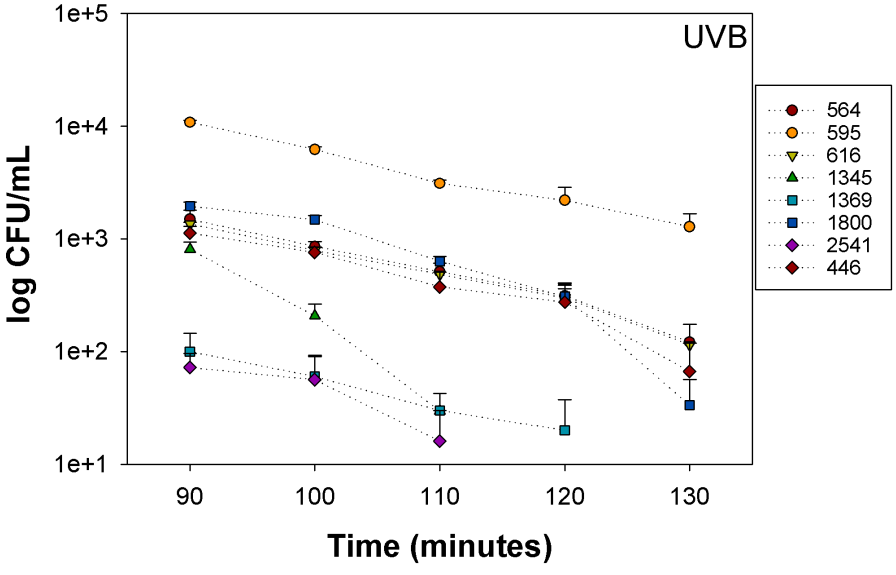

Figure 3. Colony Forming Units as a function of time for each *Cladosporium* isolate when subjected to UVB radiation.

The fungi LAMAI 595 and 616 showed a close phylogenetic position and grouped with the type strain of *C. anthropophilum*. This species is known as a common saprobic fungus and also represents a clinically relevant fungus (Sandoval-Denis et al. 2016). A representative of the species *C. anthropophilum* was isolated from a seed coat of *Pinus armandii* (Tibpromma et al. 2019). LAMAI 595 and 616 were isolated from the same Antarctic substrate (ornithogenic soil) and have similar morphological characteristics (brownish olive color in MA2% medium - data not shown). The results of the experiments of growth parameters at different temperatures, salinity, and culture media also revealed similar patterns, indicating that these two isolates may belong to the same species.

Isolates LAMAI 598 (recovered from a wood sample) and LAMAI 1369 (isolated from marine sediments) also showed a close relationship, although with a greater evolutionary distance. These fungi grouped closely to the type strain of *C. magnoliigena*, reported as a saprobe and isolated from rotting cones of the land plant *Magnolia grandiflora* in the Yunnan Province, China (Jayasiri et al. 2019). Isolates LAMAI 598 and 1369 showed differences in the experimental tests, including morphological characteristics (LAMAI 598 showed a yellowish grey color, while LAMAI 1369 showed a darker green color with tones of yellow on the reverse in MA2% medium - data not shown), tolerance to NaCl concentrations, growth under different temperatures, and resistance against UV radiation.

Isolate LAMAI 1345 (recovered from a marine sediment sample) was distant from the other Antarctic isolates and formed a clade together with the recently discovered *Cladosporium* species *C. bambusicola*, isolated from decomposing bamboo leaves in a fragment of the Atlantic Forest, in "Parque Estadual da Serra do Brigadeiro", Minas Gerais (Costa et al. 2022). Despite being a fungus isolated from a marine sample, LAMAI 1345 showed a very low tolerance to higher salt concentrations, being able to grow only at 5% NaCl.

Isolate LAMAI 2541 (recovered from a retreat soil sample from the Collins Glacier) was the only one that grouped with a *Cladosporium* species belonging to the *C. sphaerospermum* complex, related to *C. endophyticum*, an

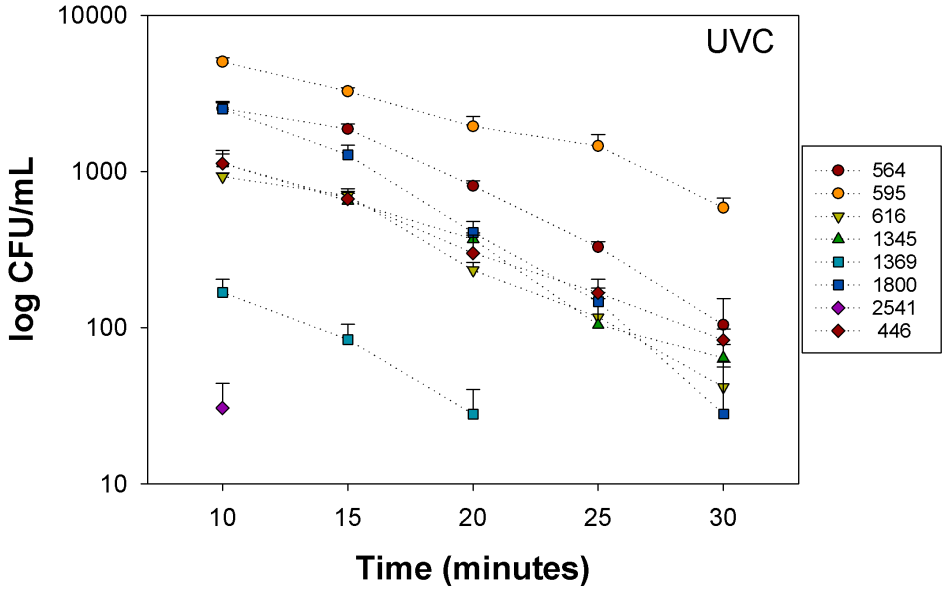

Figure 4. Colony Forming Units as a function of time for each *Cladosporium* isolate when subjected to UVC radiation.

endophytic fungus reported by Tibpromma et al. (2018), isolated from healthy leaves of *Pandanus* sp., a terrestrial plant, in Thailand. In addition to being phylogenetically more distant from other Antarctic *Cladosporium*, this isolate presented the most distinct morphological characteristics (yellowish-white color in MA2% medium - data not shown) and showed different results in the experimental tests.

Growth under adverse conditions

The optimal growth temperature of *Cladosporium* isolates was between 15 and 25 °C. This temperature variation is also consistent with data from other studies with the genus *Cladosporium*, since this group is active at low temperatures (Onofri et al. 2000, Ogórek et al. 2012), due to the production of a large number of spores that are easily dispersed by the wind, covering great distances (Ttarvevl 1970, Kurkela 1997, Bensch et al. 2012). Therefore, it is assumed they might have arrived in the Antarctic ecosystem this way, and over the years they have adapted to survive in this environment.

The seas around the Antarctic Peninsula, in general, have lower temperatures than the land portion, with temperatures often below -20 °C. Marine organisms have evolved to withstand the extreme temperatures of this habitat (Thomas & Dieckmann 2002). Both marine-derived isolates LAMAI 1345 (grouped with *C. bambusicola*) and LAMAI 1369 (grouped with *C. magnoliigena*) were able to grow at 5 °C. These two fungi were isolated from marine sediment samples collected in Punta Ulman at a depth of 20.3 m and an average temperature of 0.3 °C (Wentzel et al. 2019). On the other hand, the fungus LAMAI 564 (grouped with *C. varians*), isolated from the marine organism *Salpa* sp., did not grow below 10 °C. However, the marine specimen was collected from shallow waters on the coast of Antarctica, where temperatures are less cold than at a 20 m depth. Even though the tropical fungus *C. cladosporioides* LAMAI 446 did not originate from Antarctica, it was able to grow at 10 °C. This fungus was isolated from the marine cnidarian *Palythoa caribaeorum*, collected at Praia Preta (São Sebastião), on the north coast of the State of São Paulo (da Silva et al., 2008), where the average temperatures (24 °C) are higher than in Antarctica (Rodrigues & Bulhões 2017). These results highlight the plasticity of *Cladosporium* in response to the environment.

Considering the results of growth in different culture media and the samples from which the *Cladosporium* spp. were isolated, some correlations can be indicated. The isolates recovered from ornithogenic soil samples and grouped with *C. anthropophilum* (LAMAI 595 and 616) showed good growth in all tested media, including the poorest nutrient media SNA and PDA1%. This result indicates that these fungi are capable of metabolizing a wide range of nutrients and growing even in the poorest conditions, taking full advantage of any source of energy and carbon available. This adaptation may have arisen due to the environment in which they were found (ornithogenic soil), which is enriched by guano, the main excreta of penguins and rich in nutrients (Simas et al. 2007). Additionally, isolate LAMAI 2541 (related to *C. endophyticum*), recovered from the Collins Glacier retreat soil, showed the best growth in the poorest medium (SNA). Except for ornithogenic soils, other soils found in the Antarctic Peninsula are generally very poor in nutrients (Simas et al. 2007).

The fungus LAMAI 1345 (grouped with *C. bambusicola*), isolated from marine sediment, showed expressive growth in the media MA2% and PDA. Nonetheless, marine sediments, in general, are nutrient-poor places (Gonçalves et. al. 2013). This fungus was isolated from marine sediment collected at Punta Ulman in Admiralty Bay, a sample with a very low amount of carbon

(Wentzel et al. 2019). Microorganisms from the Antarctic environment need to overcome the scarcity of available nutrients (Singh et al. 2011), making this aspect an important selective factor in this habitat. Considering the results of growth in different nutrient media and the origin of the isolate LAMAI 1345, this could be a fungus well adapted to metabolize simpler nutrients more efficiently. It should be noted that the marine sediment may not be the natural reservoir of this fungus, which may have arrived there in diverse ways and remained inert as a spore or propagule.

Many fungi, including *Cladosporium* species, have been isolated from saline sites in Antarctica (Brunati et al. 2009). The isolates recovered from ornithogenic soil samples and grouped with *C. anthropophilum* (LAMAI 595 and I 616) showed the best growth performance at 5% NaCl and the capability to develop well at up to 15% NaCl. The ornithogenic soil has significantly higher salt concentrations than other soils, due to the accumulation of guano, derived from the activity of penguins that choose the coastal parts of the Antarctic Peninsula to make their nests (Simas et al. 2007, Wing et al. 2021). This may explain the tolerance of these isolates to higher NaCl concentrations.

The isolate LAMAI 1369 (grouped with *C. magnoliigena*), which showed good growth at 5 to 15% NaCl, was recovered from marine sediment (Punta Ulman, Admiralty Bay). Possibly, this isolate is more adapted to the high levels of salinity, since the seas surrounding Antarctica have salt concentrations that can vary between 35 and 150 ppm (Thomas & Dieckmann 2002). On the other hand, the fungus LAMAI 1345 (grouped with *C. bambusicola*), also isolated from marine sediment (Punta Ulman, Admiralty Bay), showed a low tolerance to high salinity, growing little at the lowest salt concentration tested. The low salinity tolerance of this isolate reinforces the hypothesis that it does not belong to the marine environment, having arrived there in different possible ways, since fungal spores can travel long distances (Wang et al. 2021). The tropical fungus *C. cladosporioides* LAMAI 446, isolated from a marine sample, was also able to grow well at up to 15% NaCl.

Adaptations to solar radiation are an important selective factor in the Antarctic environment (Ruisi et al. 2007). In the present study, a longer exposure time resulted in a smaller number of surviving colonies for all isolates subjected to UVB and UVC radiation. Similar results have been observed by Onofri et al. (2007) and Monyethabeng & Krugel (2016). UVC is efficient in generating pyrimidine dimers that react and damage the DNA, while UVB is more efficient in generating oxygen species that also react and damage the DNA, but to a lesser degree (Pulschen et al. 2015, Reis-Mansur et al. 2019). Other substances, like melanin and carotenoids, are also observed in microorganisms resistant to ultraviolet radiation (Lin & Xu 2020). Melanins protect the organisms mainly from direct damage to the DNA, while carotenoids in fungal cells are associated with reducing the formation of reactive oxygen species (Wong et al. 2019).

These differences may explain the variation that occurred among the isolates, in both treatments with UVB and UVC radiation. The fungus LAMAI 595 (grouped with *C. anthropophilum*) was isolated from ornithogenic soil, and was the most prominent in terms of resistance, demonstrating a photoprotective capacity superior to that of the other fungi. On the other hand, isolate LAMAI 598 (grouped with *C. magnoliigena*) was poorly equipped to deal with the solar radiation of the Antarctic environment. It is possible that isolate LAMAI 598 has been recently introduced into the Antarctic environment, as a result of the presence of people there, since it has been known that

anthropogenic activity in Antarctica might affect fungal diversity and composition (Ogaki et al. 2020). Wood, the substrate from which this fungus was isolated, is not found naturally in Antarctica, and the main sources of this material only exist due to human activity (Bolter et al. 2002). Furthermore, isolate LAMAI 598 was also unable to grow at the lowest temperature tested (5 °C). Temperature and solar radiation are two important selection factors in the Antarctic environment (Thomas & Dieckmann 2002).

The low UV resistance of isolate LAMAI 2541 (related to *C. endophyticum*) recovered from the Collins Glacier retreat soil appears to be related to the sampling site. This fungus was isolated from a soil sample collected at 0 meters from the glacier (a recently exposed part). Sulzbach et al. (2021) reported the retreat of the Collins Glacier over the last 30 years and noted that this trend may be associated with the increase in Antarctic air temperature, among other factors. It is assumed that, for a long time, isolate LAMAI 2541 was protected from solar radiation due to the vast mass of ice, which possibly covered this site and, consequently, protected the fungus from the harmful actions of UV radiation.

The low resistance of isolates LAMAI 1345 (grouped with *C. bambusicola*) and LAMAI 1369 (grouped with *C. magnoliigena*) may also be related to the habitat of origin. Both fungi were isolated from marine sediment collected at a depth of 20 m (Wentzel et al. 2019). The water column, as well as the freezing of the Antarctic seas for a long period, protects against solar radiation, preventing much of the UV light from reaching the sediments (Thomas & Dieckmann, 2002). However, isolate LAMAI 1345 showed a much higher resistance compared to isolate LAMAI 1369. This result can be explained by the phenotypic characteristics of LAMAI 1369 (the possible absence of melanin). On the other hand, the fungus LAMAI 564, isolated from the marine organism *Salpa* sp. that had been collected from shallow waters, was one of the most resistant to UV radiation. *Salpa* are planktonic tunicates that feed mainly on zooplankton (Huntley et al. 1989). In Antarctica, this zooplankton is found close to the surface, in the water column (Phleger et al. 1998), where UV radiation exerts a greater influence than on seafloor sediments.

The tropical *C. cladosporioides* LAMAI 446 isolated from a marine invertebrate also showed good resistance against UVB and UVC radiation, being more resistant than some of the Antarctic fungi. This dark-colored (olive brown) fungus, which might indicate the presence of melanin (data not shown), was isolated from a sample collected in shallow water, more exposed to UV.

In general, the results related to growth and resistance to adverse conditions were consistent with the phylogenetic position and origin (sampling sites) of the Antarctic *Cladosporium*. The use of a secondary marker (partial *act* gene) together with the ITS marker revealed a better resolution of the phylogenetic relationships between the studied isolates and the closest *Cladosporium* species in comparison with the ITS-based phylogenetic tree (data not shown). Nevertheless, to have a more accurate taxonomic position and to suggest possible new species, additional taxonomic analyses are necessary using other concatenated markers (e.g. secondary marker *tef*1), as well as conventional taxonomy. The results shown herein highlight the potential of the Antarctic *Cladosporium* for biotechnological applications, especially isolate LAMAI 595, which presented relevant results of resistance, opening new perspectives in the field of extremophiles and their biomolecules, including cold-adapted enzymes, protective solar radiation substances, and application in saline processes/environments.

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SUPPLEMENTARY MATERIAL

Table SI-SII.

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