



MICROBIOLOGY

Antarctic fungi applied to textile dye bioremediation

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Abstract: Antarctica has one of the most hostile conditions on the planet. The environmental characteristics found in this region favor the development of extremophile microorganisms, which are poorly explored biotechnologically. In this context, this study aimed at selectively isolating fungi with potential for the bioremediation of a textile dye. A total of 11 filamentous fungi were isolated from Antarctic samples after incubation in Minimal Mineral medium with the addition of Sulphur Indigo Blue dye. The Antarctic-derived fungi were submitted to textile dye decolorization analysis and biomass production. Isolates LAMAI 2400 and LAMAI 2402 showed more than 90% decolorization at 15 °C, whereas at 28 °C these isolates showed 81.86 and 98.89%, respectively. In general, the toxicity of the bioassays, evaluated using *Cucumis sativus*, was higher than in the control. Both isolates, LAMAI 2400 and LAMAI 2402, were identified as *Penicillium cf. oxalicum* and classified as mesophilic-psychrotolerant. This fungal species has rarely been reported in the Antarctic environments. The results presented herein indicate the potential of the fungi recovered from Antarctic marine sediments for bioremediation of textile dyes at low and moderate temperatures, broadening the perspectives in the field of Antarctic mycology.

Key words: Antarctica, environmental biotechnology, extremophiles, mycology, pollutants.

INTRODUCTION

The Antarctic environment presents hostile conditions, being characterized by low temperatures, high incidence of radiation (mainly UV), high salinity, frequent freeze-thaw cycles, dryness, low nutrient content and long periods of darkness (Onofri et al. 2007, D'elia et al. 2009, Yergeau & Kowalchuk 2008). All these factors hinder the development of many forms of life (Wilkins et al. 2013), and the survival in such extreme conditions requires adaptations (Margesin et al. 2002, Duarte et al. 2018a). Nevertheless, there is evidence that Antarctica is a reservoir of microbial diversity, where new species, genes, and biochemical pathways with

biotechnological applications can be found (Varrella et al. 2021).

Microbial life in Antarctica has a key role in nutrient recycling, providing the basis for the function of marine and terrestrial environments (Onofri et al. 2007, Ruisi et al. 2007). The fungi that inhabit marine sediments and soil are dominant components of the biomass in Antarctic ecosystems (Wynn-Williams 1996) and can be classified as psychrophilic, psychrotolerant (psychrotrophic), and mesophilic-psychrotolerant. The first includes microorganisms able of growing at 0 °C, with optimum growth at temperatures ≤ 15 °C and maximum growth at temperatures ≤ 20 °C. The second includes microorganisms that can grow

at 0 °C, but with maximum growth temperature between 15 and 25 °C. The third includes microorganisms that have the ability to grow at low temperatures, with optimum growth at temperatures > 25 °C and ≤ 40 °C (Ruisi et al. 2007, Pesciaroli et al. 2012). The production of cold-adapted enzymes, exopolysaccharides, antifreeze proteins, photoprotective pigments and cryoprotective carbohydrates are some of the important adaptations for fungal survival in harsh ecosystems like Antarctica (Krishnan et al. 2011, Duarte et al. 2018a).

According to Selbmann (2019), extremophile fungi can exhibit exceptional resistance to stress, with resistance mechanisms not only related to their natural environment. Fungal strains isolated from Antarctic environments are able to produce hydrolytic and oxidative (oxidoreductases) enzymes at low/moderate temperatures (Duarte et al. 2018a). Among oxidoreductases, the ligninolytic enzymes MnP, LiP and laccase stand out, which can be used to decolorize a wide range of industrial dyes due to the structural similarity of these compounds to lignin (Chauhan & Choudhury 2021).

The significant quantities of textile dye that are discharged into effluents modify the whole ecosystem, impeding light penetration into water and, consequently, reducing photosynthesis rates and inhibiting the growth of aquatic biota (Enayatzamir et al. 2009, Hossain et al. 2018). Furthermore, these pollutants can cause damage to the human body because of their toxic properties, even increasing the risks of developing some diseases, like cancer (Chung et al. 1992, Chequer et al. 2011). Considering this, currently, there has been interest in the exploration of fungi for dye decolorization and degradation (Mostafa et al. 2019). Pramanik & Chaudhuri (2018) studied the decolorization of Rose Bengal dye by the fungus *Podoscypha elegans*, obtaining 70.4% of decolorization. A

study performed by Ortiz-Monsalve et al. (2019) using dye-containing wastewaters resulted in 90% of color removal using the fungus *Trametes villosa* SCS-10. Additionally, Ameen et al. (2016) reported the ability of four *Aspergillus* species (*A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus*) to degrade the azo dyes Acid Blue 29, Disperse Red 1 and Congo Red, in a synthetic wastewater. Although textile dye decolorization and degradation are of utmost relevance, toxic intermediates can be formed during the biodegradation process (Sen et al. 2016). Since the biodegradation of dyes depends on the fungal species, strain, dye molecule, culture medium and environmental conditions, the degradation products (metabolites) may differ (Mostafa et al. 2019). In this sense, the assessment of growth medium toxicity resulting from dye degradation is relevant for the formulation of bioremediation strategies (Giovannella et al. 2020, Vieira et al. 2021). Despite the relevance of the fungal metabolic arsenal for dye decolorization and detoxification, studies that use Antarctic-derived fungi for the bioremediation of textile dyes are still scarce.

The importance of filamentous fungi for textile dye decolorization and the lack of information on the potential of Antarctic-derived fungi for the bioremediation of this kind of environmental pollutant motivated the performance of this study. In this context, the enrichment strategy for the selective isolation of fungi from marine and terrestrial Antarctic samples was applied. The isolated strains were analyzed for the potential of Sulphur Indigo Blue textile dye decolorization and biomass production. The selected strains were evaluated regarding their dye detoxification potential and were taxonomically characterized. An effort was made in this study to broaden the innovation of Antarctic microbial research.

MATERIALS AND METHODS

Sampling sites

Soil and marine sediment samples were collected from two different Islands in the Antarctic Peninsula (Figure 1) during the XXXVII Brazilian Antarctic Operation (OPERANTAR) in December 2018 (Process number 02000.005323/2018-52 authorized by the Brazilian Environmental Assessment Group - GAAM/PROANTAR on July 2nd, 2018). At Botany and Hennequin Points, superficial soil was collected, while at Comandante Ferraz Brazilian Station EACF Helipad (King George Island) and Whalers Bay (Deception Island), marine sediments were collected using a box-corer. Three simple samples were collected from each point using sterilized tools, stored in sterile “Whirl-Pak”

bags (Nasco, United States), and maintained at 7 °C during transportation, until the arrival at the laboratory in Brazil, where they were also maintained at 7 °C. Only one sample from each point was used for the enrichment procedure.

Isolation of filamentous fungi from soil and marine sediment

For fungal isolation, the enrichment technique was conducted as described by Spini et al. (2018), with modifications. In this process, 5 g of samples were added into an Erlenmeyer flask containing 50 mL of Minimum Mineral medium (In g L⁻¹: 0.7 KCl, 2.5 NaH₂PO₄, 3.0 Na₂HPO₄, 1.0 (NH₄)₂SO₄), 1 mL of micronutrient solution (In g L⁻¹: 4 MgSO₄, 0.2 FeSO₄, 0.2 MnCl₂, 0.2 CaCl₂), supplemented with 150 mg L⁻¹ of chloramphenicol and 400 mg

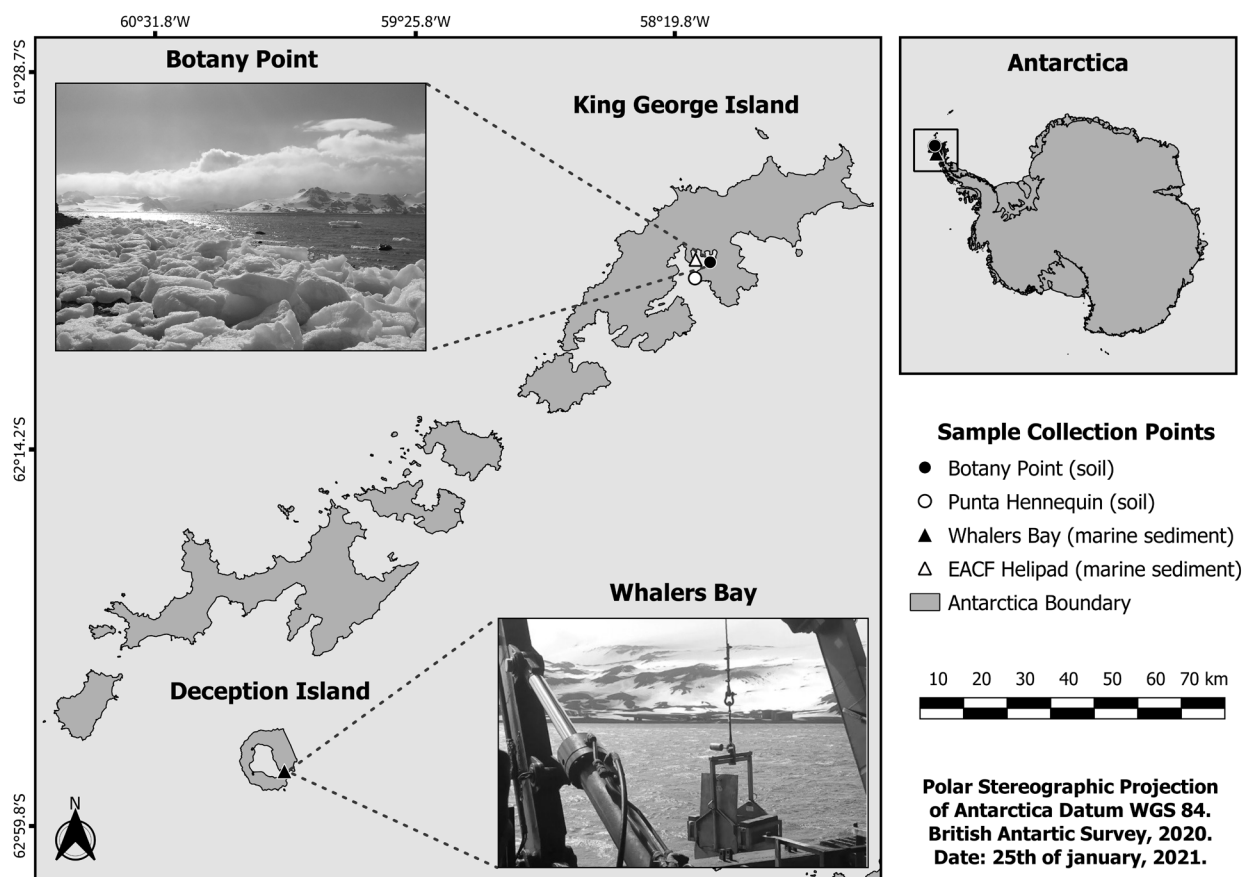


Figure 1. Sampling sites in King George Island (Admiralty Bay) and Deception Island (Whalers Bay), South Shetlands Archipelago, Maritime Antarctica.

L⁻¹ of Sulphur Indigo Blue dye. Subsequently, the Erlenmeyer flasks were refrigerated at 15 °C with agitation at 140 rpm for 15 days. After this period, 5 mL of the microbial culture were transferred to another Erlenmeyer flask containing the same medium described above and incubated under the same conditions for additional 15 days. At the end of three transfers, 100 µL of the culture was removed to make dilutions up to 10⁻⁴, which were used for the inoculation on Malt Agar medium (20 g L⁻¹ malt extract and 20 g L⁻¹ agar) supplemented with 150 mg L⁻¹ of chloramphenicol. The plates were incubated at 15 °C until the appearance of colonies, which were isolated according to the morphological differences and purified for later experiments.

Fungal isolates were preserved by two distinct methods: cryopreservation (-80 °C) and Castellani (4 °C), using cryotubes with 10% glycerol and water, respectively. All fungal isolates were deposited in the Microbial Resource Center Culture Collection (CRM-UNESP) of the São Paulo State University, Rio Claro, Brazil.

Decolorization and detoxification of Sulphur Indigo Blue textile dye

The cultivation of the filamentous fungi was performed as described by Vieira et al. (2021), with modifications. All isolates were cultivated on Petri dishes containing Malt Agar medium and 150 mg L⁻¹ of chloramphenicol for 7 days at 15 °C or 28 °C. Subsequently, three culture discs (5 mm in diameter) from the edge of the colony were aseptically transferred to an Erlenmeyer flask containing 50 mL of Malt Broth medium (20 g L⁻¹ of malt extract) supplemented with Sulphur Indigo Blue textile dye (400 mg L⁻¹) and incubated for 7 days at 15 °C or 28 °C and 140 rpm. All assays were conducted in triplicate.

Dry biomass

For the measurement of the dry biomass, the procedures reported by (Vieira et al. 2021) were applied. Cultures of filamentous fungi were filtered in qualitative filter paper (previously dried) to separate the fungal biomass from the supernatant. The fungal biomass was dried at 105 °C and measured after 24 h. The difference between the final mass (dry mycelium + filter paper) and initial mass (filter paper) was the fungal biomass value. The same procedure was performed in the control group containing the culture medium and the Sulphur Indigo Blue textile dye.

Decolorization assay

To evaluate decolorization, the supernatants were diluted in water (5:5) and evaluated on a UV-Vis spectrophotometer (Shimadzu UV-1240, Kyoto, Japan), according to Bonugli-Santos et al. (2012). The decolorizing activity was calculated from the maximum absorption peak (600 nm) of the dye, according to the following formula:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Detoxification assay

Phytotoxicity assessment using *Cucumis sativus* was conducted in Petri dishes based on the study performed by Wang et al. (2001), with modifications. Each Petri dish containing 15 seeds without pesticides and 5 mL of the supernatant (adjusted to pH 7.0) placed on a filter paper was individually wrapped with plastic film to prevent water evaporation, and incubated at 24 °C in the dark, for 5 days. The negative control consisted in a solution of 0.05 M ZnSO₄ and the positive control was distilled water. At the end of the experiment, the hypocotyl and root of each seed were measured. Only the seeds with

growth of 5 mm or more were considered. The calculation of hypocotyl + root growth inhibition was performed using the following equation:

$$\text{Inhibition(\%)} = \frac{\text{Mean of the positive control} - \text{Mean of the bioassay}}{\text{Mean of the positive control}} \times 100$$

Fungal identification and classification

The best-performing fungi in the decolorization experiment (80% or more of dye decolorization) were selected for taxonomic identification and classification (based on their growth temperature). For molecular taxonomy, the DNA from the Antarctic fungi was extracted following the method described by Lacerda et al. (2018). The internal transcribed spacer (ITS1-5.8S-ITS2) was amplified according to Santos et al. (2020) using the universal primers ITS4 (5'TCCTCCGCTTATTGATATGC3') and ITS5 (5'GGAAGTAAAAGTCGTAACAAGG3'). The amplicons were purified using the enzymes Exonuclease I and alkaline phosphatase (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The samples were quantified in NanoDrop (Thermo Scientific, MA, USA) and sequenced by a specialized company using the Sanger sequencing method. The generated sequences were processed (trimmed tails and quality Phred checking) using BioEdit v.7.2.5 (Hall 1999) and assembled into contigs using CAP3 (Huang & Madan 1999). All sequences were compared with GenBank and CBS Fungal Biodiversity Centre sequences using BLAST (Altschul et al. 1990), and alignments were performed using MUSCLE (Edgar 2004) in the MEGA X version 10.2.6 package (Kumar et al. 2018). Evolutionary distances were calculated using the Maximum Likelihood algorithm and Kimura-2p nucleotide substitution model (Kimura 1980), which takes into account differences in transitions and transversions. The robustness of the trees was

calculated using the bootstrap method, with 1000 generations. The tree was produced using the iTOL web application (Letunic & Bork 2021). The ITS sequences of the selected fungal strains were deposited in GenBank under the following accession numbers: MZ376947 and MZ376948. For the morphological characterization, the fungi were cultivated in Malt Agar blocks under a cover glass to obtain the reproductive structures for further investigation. The system was incubated at 15 °C until sporulation. After sporulation, the cover glass was transferred to a slant with a drop of cotton blue and then observed under light microscopy.

To define growth temperature range, a spore suspension (1×10^6) was prepared and 10 μ L were inoculated in Malt Agar medium for 7 days at four different temperatures: 15, 20, 25 and 30 °C. After the incubation period, the plates were photo-documented, and the growth area (cm^2) was measured using the software ImageJ (Abramoff et al. 2004).

Statistical analysis

The data obtained were analyzed by XLSTAT 2020. The normality of the data was checked for each treatment (Ryan-Joyner test with p-value >0.05). For parametric distributions, the data were compared using ANOVA followed by the Tukey's test (p-value of 0.05). For non-parametric distributions, the data were compared using the Kruskal Wallis test followed by the Conover-Iman's post-hoc test.

RESULTS

Isolation of filamentous fungi from soils and marine sediments

A total of 29 microorganisms were isolated and purified from Antarctic soil and marine sediment samples after 45 days of enrichment with Sulphur Indigo Blue dye. Among them, 11

were characterized as filamentous fungi based on morphological features. A higher number of filamentous fungi were recovered from marine sediment samples ($n = 9$) compared to soil samples ($n = 2$) (Table I). From the marine sediment samples, 5 isolates were recovered from the sample collected in Whalers Bay (Deception Island) and 4 from the sample collected in EACF Helipad (King George Island). From the soil samples, 1 isolate was recovered from the soil collected in Punta Hennequin and the other, from the soil collected in Botany Point, both placed at King George Island.

Decolorization and detoxification of Sulphur Indigo Blue textile dye

Selectively isolated filamentous fungi were able to decolorize the textile dye Sulphur Indigo Blue at 15 °C and the decolorization rates varied from 0.64% to 94.88% (Figure 2a). In the experiments performed at 28 °C, the percentage of decolorization varied from 0.35% to 98.89% (Figure 2b). At this temperature, two isolates (LAMAI 2399 and LAMAI 2423) did not show dye decolorization.

In general, the filamentous fungi presented similar decolorization rates at 15 and 28 °C. Isolates LAMAI 2400 and LAMAI 2402 stood out as the best decolorizers at both temperatures (Figure 2a, b) with more than 93% of dye decolorization at 15 °C, and 81.86% (LAMAI 2400) and 98.89% (LAMAI 2402) at 28 °C. Both of them were recovered from the marine sediment collected in Deception Island. Isolates LAMAI 2398, LAMAI 2399, LAMAI 2401, LAMAI 2410, LAMAI 2422 and LAMAI 2423 were able to decolorize the dye at rates above 20% at both temperatures. Isolates LAMAI 2424 and LAMAI 2425 showed a lower capacity to decolorize the dye at 15°C (less than 10%) than at 28 °C (more than 40%).

There was less variation in the biomass produced by the Antarctic filamentous fungi in

the bioassays conducted at 15 °C (Figure 3a) in comparison with the bioassays performed at 28 °C (Figure 3b). The biomass at 15 °C varied between 0.506 g L⁻¹ and 0.859 g L⁻¹, while at 28 °C, biomass variation was between 0.162 g L⁻¹ and 0.959 g L⁻¹ (Figure 3a). The fungal isolate LAMAI 2400 produced the highest amount of biomass at 15 °C, whereas the isolate LAMAI 2425 showed the best biomass production at 28 °C (Figure 3b).

Isolates LAMAI 2399 and LAMAI 2423, which did not show dye decolorization at 28 °C (Figure 2b), were not able to produce biomass at this temperature (Figure 3b). Nonetheless, at 15 °C, the biomass produced was 0.663 g L⁻¹ (LAMAI 2399) and 0.816 g L⁻¹ (LAMAI 2423) (Figure 3a). It was not possible to establish a direct relationship between the amount of biomass produced and the Sulphur Indigo Blue decolorization rates. Nevertheless, for the best decolorizers (LAMAI 2400 and 2402), the amount of biomass produced was among the highest at both temperatures.

Based on the results of decolorization and biomass production, isolates LAMAI 2400 and LAMAI 2402 were selected and the bioassays conducted with these isolates were submitted to the toxicity analyses using *C. sativus* as bioindicator.

The results at 15 °C showed that the toxicity of the bioassay performed with the selected isolates was higher than the toxicity of the control, composed of Malt Broth medium with Sulphur Indigo Blue textile dye (Figure 4a). LAMAI 2400 and LAMAI 2402 showed 83.44 and 83.07% of *C. sativus* hypocotyl + root inhibition, respectively, while in the control the inhibition was 27.64%. However, at 28 °C (Figure 4b), the bioassay performed with the isolate LAMAI 2400 showed toxicity (41.16%) at the same level as the control (46.16%). At this temperature, the toxicity in the bioassay with the isolate LAMAI 2402 (73.41%) was also higher than the toxicity of the control.

Table I. Data on the soils and marine sediments collected at Maritime Antarctica during OPERANTAR XXXVII (December 2018) and fungal strains isolated from these samples.

Site	Geographic coordinates	Depth (m)	Average T (°C)	Sample	Filamentous fungi	LAMAI code
Deception Island Whalers bay (SMWB1)	62°59.071` S 60°33.720` W	95.4	ND*	Marine sediment	D2A	LAMAI 2398
					D2B	LAMAI 2399
					D2C	LAMAI 2400
					D2D	LAMAI 2401
					D2P	LAMAI 2402
King George Island EACF Helipad (SMEACF1)	62°05.168` S 58°22.829` W	60.0	ND*	Marine sediment	D8A	LAMAI 2422
					D8B	LAMAI 2423
					D8C1	LAMAI 2424
					D8C2	LAMAI 2425
King George Island Punta Hennequin (SOH2)	62°07.429` S 58°23.697` W	Superficial (0.5 cm)	6.9	Soil	D3D	LAMAI 2406
King George Island Botany point (SOBP1)	62°05.744` S 58°19.145` W	Superficial (0.5 cm)	7.3	Soil	D4C	LAMAI 2410

*ND = Not Determined.

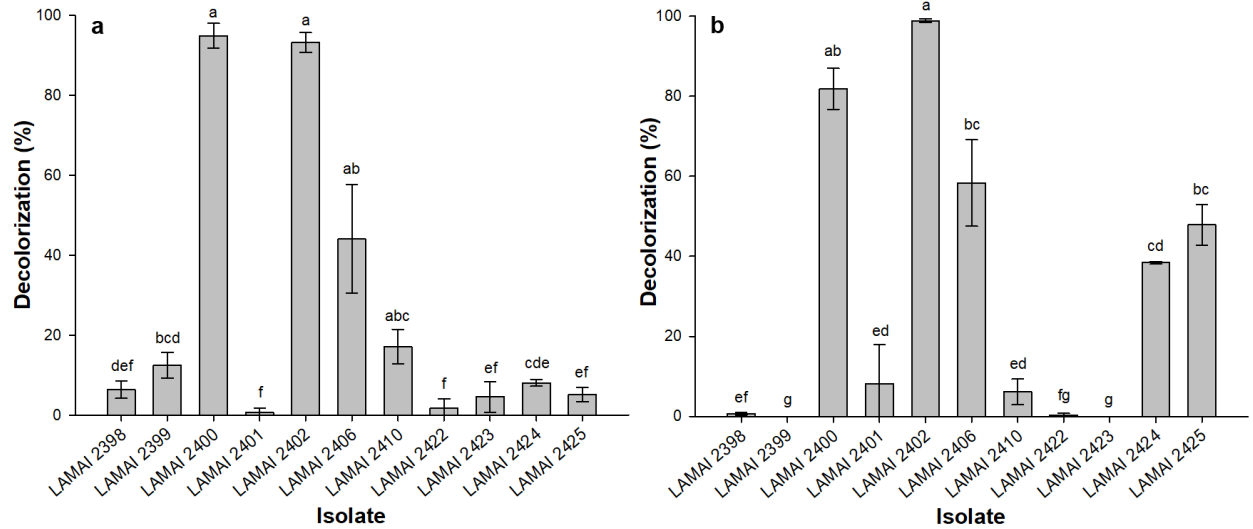


Figure 2. Percentage of Sulphur Indigo Blue decolorization by Antarctic filamentous fungi after 7 days of cultivation in Malt Broth medium containing 400 g L⁻¹ of the textile dye. a) Incubation at 15 °C and 140 rpm and b) Incubation at 28 °C and 140 rpm. Values indicated by different letters are significantly different according to the Conover-Iman’s test (p < 0.05). The vertical lines represent the standard error.

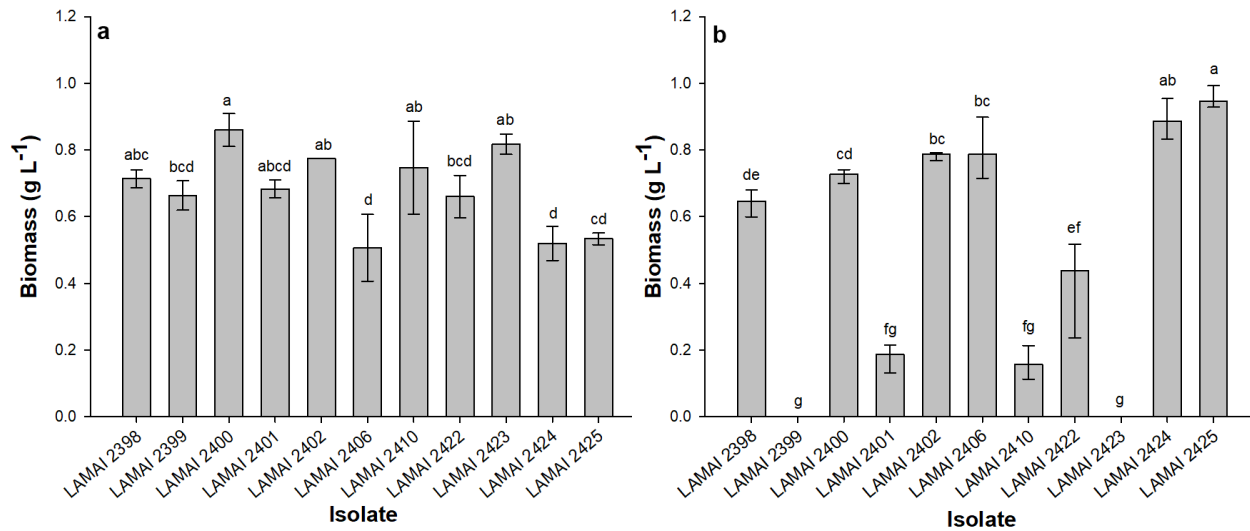


Figure 3. Dry biomass of the Antarctic filamentous fungi after 7 days of cultivation in Malt Broth medium containing 400 g L⁻¹ of the textile dye. a) Incubation at 15 °C and 140 rpm - Values indicated by different letters are significantly different according to the Tukey's test (p < 0.05) and b) Incubation at 28 °C and 140 rpm. Values indicated by different letters are significantly different according to the Conover-Iman's test (p < 0.05). The vertical lines represent the standard error.

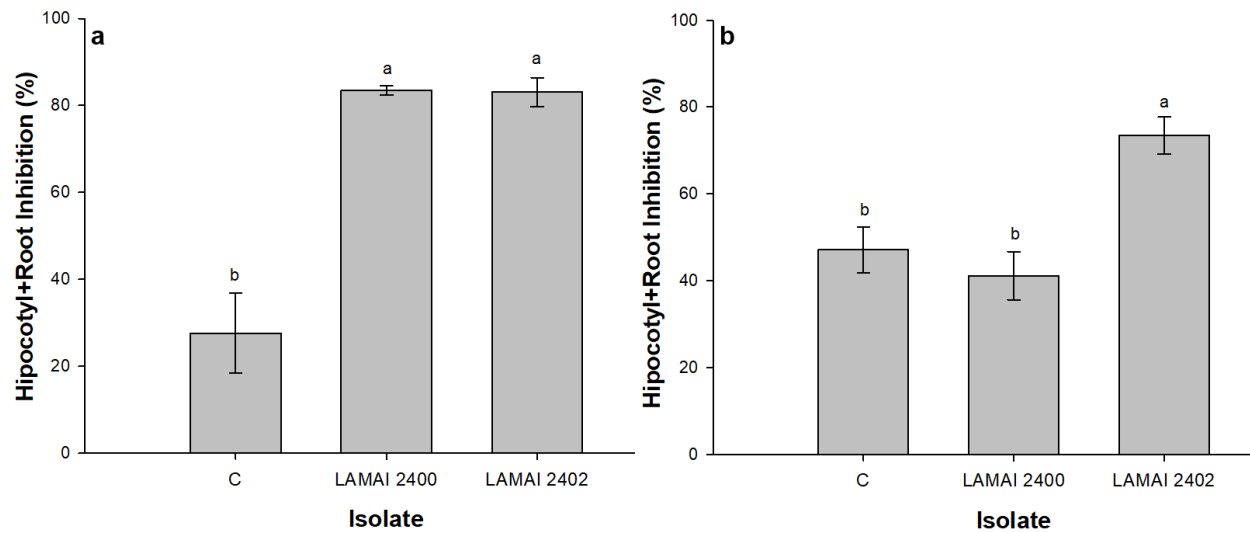


Figure 4. Toxicity of the bioassays performed with the Antarctic filamentous fungi that showed more than 80% of decolorization using *C. sativus* as bioindicator. a) Incubated at 15 °C and b) Incubated at 28 °C. Values indicated by different letters are significantly different according to the Tukey's test (p < 0.05). The vertical lines represent the standard error.

Identification and classification of the selected fungi

The ITS sequence (DNA barcode for fungi) of the selected Antarctic-derived fungi LAMAI 2400 and LAMAI 2402 showed 99% of similarity

with the type (CBS 219.30^T) and neotype (NRRL 787^{NT}) strains of *Penicillium oxalicum*, and with the type strain of *Penicillium asturianum* CBS 173.81^T (current *P. oxalicum*). Additionally, the sequences of the Antarctic fungi were 98% similar to the type strains of *Penicillium soosanum*.

Sequence similarity with type strains of other *Penicillium* species was less than 96.80%. In the phylogenetic tree, the sequences of both fungi (LAMAI 2400 and LAMAI 20402) grouped in a cluster composed only of sequences of *P. oxalicum* isolates, with a bootstrap value of 95% (Figure 5), indicating that these isolates belong to the group of *P. oxalicum*. Macro and micromorphological features after the growth of both fungi on malt extract agar were similar to the characteristics of *P. oxalicum* reported by Kubátová et al. (2019). Culture characteristics include submerged marginal hyphae; velutinous texture; light green to dark yellowish green conidia *en masse*; absent exudate and soluble pigment; pale reverse (data not shown). The asexual state (micromorphology) includes terminally branched conidiophores, typically bi-verticillate or monoverticillate, cylindrical phialides and ellipsoidal conidia (data not shown). These results allow the identification of the Antarctic-derived fungi as *Penicillium* cf. *oxalicum*.

The Antarctic fungi *Penicillium* cf. *oxalicum* LAMAI 2400 and LAMAI 2402 were able to grow at all temperatures tested (from 15 to 30 °C) as shown in Table II. The growth area increased with the rise in temperature and the best growth was achieved at 25-30 °C for strain LAMAI 2400 and 30 °C for strain LAMAI 2402. This behavior is characteristic of mesophilic-psychrotolerant microorganisms.

DISCUSSION

Isolation of filamentous fungi from soils and marine sediments

The enrichment strategy applied in this study allowed the isolation of 11 potentially textile dye-degrading filamentous fungi from marine and terrestrial samples collected during OPERANTAR XXXVII (December 2018). All of them showed some Sulphur Indigo Blue dye decolorization at 15 and 28 °C, with the exception of two isolates that were not able to grow at 28 °C. According to Pham & Kim (2012), this methodology

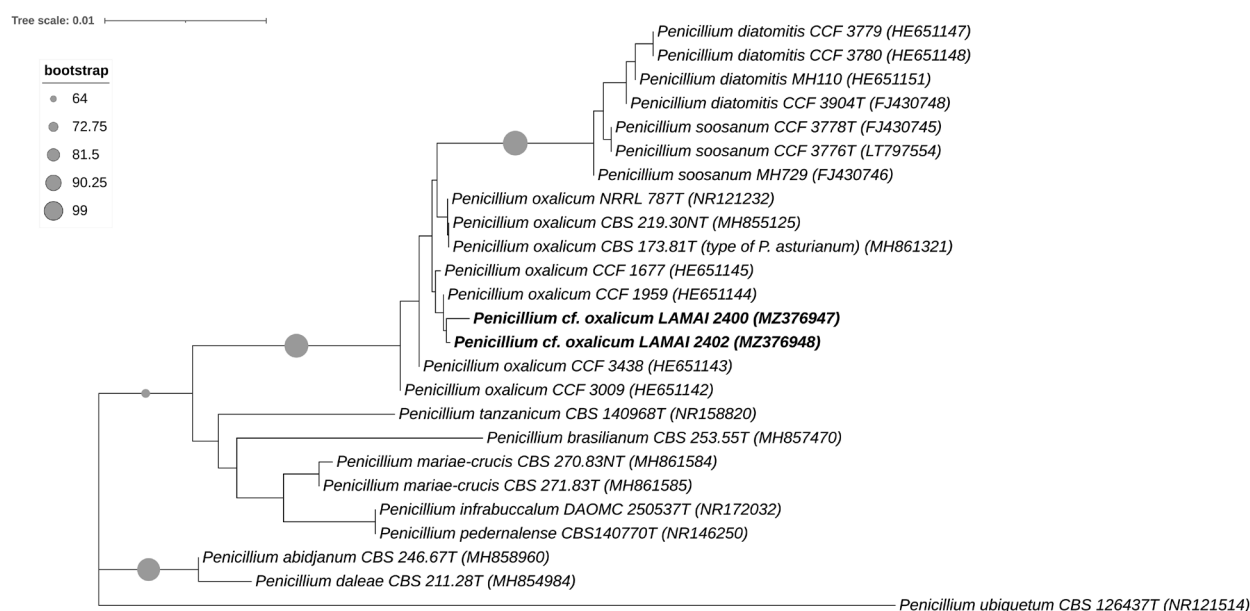


Figure 5. Phylogenetic tree based on ITS sequences showing the relatedness of the Antarctic-derived fungi LAMAI 2400 and LAMAI 2402 and related taxa. Evolutionary distances were calculated using the Maximum Likelihood algorithm and Kimura-2p nucleotide substitution model. Bootstrap values (1000 replicate runs) >50% are listed.

is applicable to select desired organisms by providing them a low-nutrient growth environment to guarantee the specificity for the isolation of effective strains. Enrichment is a valuable tool for isolating microbial consortium by serial inoculations in mineral media with the addition of the pollutant as the sole carbon source (Omrani et al. 2018).

The use of the enrichment strategy for the selective isolation of cold-adapted microorganisms capable of degrading hydrocarbons has been reported in the literature (Pham & Kim 2012, Wang et al. 2016, Chaudhary & Kim 2019, Omrani et al. 2018). This process is based on the assumption that hydrocarbons are the only carbon and energy source for the microbial community that can grow under the established conditions. In the study performed by Wang et al. (2016), cold-adapted bacterial strains with great biodegrading potential were isolated. According to Chaudhary & Kim (2019), this technique could allow a wider evaluation of the yet unexplored psychrophilic population that can be applied in bioremediation processes. Omrani et al. (2018) reported that the fungal consortium submitted to an enrichment process showed a significant potential for degradation, with more pathways during hydrocarbon degradation than bacteria. Additionally, although the use of culture media is a limiting factor to fully know the diversity of microorganisms in the world (Santos et al. 2020), the enrichment strategy can be a useful

methodology to reduce this gap, simulating a harsh environment and also discovering new microorganisms with specific properties.

In the present study, a higher number of filamentous fungi was selectively isolated from marine samples in comparison to soil samples, indicating that the fungal diversity from the marine sediment adapted better to the enrichment conditions. Usually, soil samples provide a higher number of isolates in studies conducted without selective pressure. Wentzel et al. (2019) isolated 492 Antarctic filamentous fungi from soil and 24 from marine sediment samples after 7 days of incubation in different culture media at 5 and 15 °C. The low number of isolates recovered in the present study probably occurs because of the restriction of nutrient and carbon sources and to the presence of the dye (pollutant).

Decolorization and detoxification of Sulphur Indigo Blue textile dye

Among the 11 filamentous fungi selectively isolated from marine and terrestrial Antarctic samples, two (LAMAI 2400 and LAMAI 2402) showed more than 80% of textile dye decolorization and relatively great biomass production at 15 and 28 °C. Data from molecular and morphological analyses allowed the identification of both fungi as *Penicillium* cf. *oxalicum*. The morphological characteristics of both fungi were very similar. Nevertheless, the

Table II. Growth area (cm²) of the isolates LAMAI 2400 and LAMAI 2402 in Malt Agar medium at different temperatures. Values indicated by different letters are significantly different according to the ANOVA's test.

Fungi	Area of growth (cm ²)			
	30 °C	25 °C	20 °C	15 °C
LAMAI 2400	7.341 ± 0.248 a	6.778 ± 0.673 a	5.952 ± 0.303 b	3.992 ± 0.074 c
LAMAI 2402	7.632 ± 0.353 a	5.988 ± 0.356 b	5.559 ± 0.290 b	3.641 ± 0,45 c

ITS sequences of LAMAI 2400 and LAMAI 2402 were a little different. In the study performed by Kubátová et al. (2019), *P. oxalicum* isolates exhibited a difference of 1.7% in the ITS region. For accurate taxonomic identification of species from the group of *P. oxalicum*, additional studies should be performed including analyses of multigene sequencing (e.g. ITS-LSU, *TUB2*, *RPB2*, and *CAM*), secondary metabolites (extrolites), and other morphological characteristics as reported by Kubátová et al. (2019). *Penicillium oxalicum* is considered a biotechnologically important species with strains patented as biofertilizers, producers of commercial enzymes, and degraders of pollutants (Kubátová et al. 2019).

The genus *Penicillium* is spread worldwide, and several species can develop under different and extreme conditions. Although representatives of the genus *Penicillium* have been frequently isolated from a variety of samples collected in Antarctica (Loque et al. 2010, Gonçalves et al. 2013, Duarte et al. 2018b, Wentzel et al. 2019, Tosi et al. 2010, Godinho et al. 2019, Ogaki et al. 2020, Menezes et al. 2020, Santos et al. 2020), only a few studies have reported the presence of *P. oxalicum* in the Antarctic environments. Ding et al. (2016) reported the recovery of four *P. oxalicum* isolates from soil collected at Fields Peninsula. In the study conducted by Connell & Staudigel (2013), one clone of *P. oxalicum* was obtained from a dark oligotrophic volcanic ecosystem on Mont Erebus using a culture-independent approach.

Despite their origin, both *Penicillium* cf. *oxalicum* strains (LAMAI 2400 and 2402) were classified as mesophilic-psychrotolerant. They grew at all temperatures tested, showing smaller growth areas at 15 °C, with an increase in the growth area when the temperatures were raised. Similar results were reported by Gocheva et al. (2006) in the study performed

with two *Penicillium* specimens, one from the polar region (Antarctica) and the other from a temperate climate. Both fungi were able to grow at temperatures ranging from 12 °C to 35 °C, and the best growth temperature was between 25 °C and 30 °C. In the study reported by Marín et al. (1998), spores from the mesophilic *P. hordei* and *P. aurantiogriseum* were able to germinate and grow at temperatures ranging from 5 °C to 30 °C (being 25 °C the fastest), as well as 37 °C and 42 °C (more slowly). The majority of the filamentous fungi recovered from samples collected in the Maritime Antarctica are psychrotolerant, and few are psychrophilic (Godinho et al. 2013).

Penicillium cf. *oxalicum* LAMAI 2400 and LAMAI 2402 were recovered from the same marine sediment sample. In the study performed by Wentzel et al. (2019), fungi from the genus *Penicillium* were the most abundant in marine sediments. Marine-derived fungi are considered an important microbial resource in the field of textile dye decolorization/degradation, since they are adapted to the high salt and pH found in marine environments, which are features of textile effluents (Bonugli-Santos et al. 2015).

Bioremediation of colored effluents by fungal cells includes a degradative process and/or biosorption. Enzymatic decolorization/degradation can be achieved using ligninolytic peroxidase enzymes (e.g., manganese peroxidase, lignin peroxidase and horseradish peroxidase), laccases, azoreductase, among others (Giovanella et al. 2020, Vieira et al. 2021). In the case of biosorption, the dye is bound to cellular functional groups and can also accumulate inside the fungal cells (Dzionek et al. 2016, Giovanella et al. 2020). As in degradative processes, biosorption is also considered an efficient, economic and eco-friendly process for industrial wastewater treatment (Singh et al. 2020).

Fungi from the genus *Penicillium* have been reported as capable to degrade different classes of synthetic dyes. *P. pinophilum* decolorized 87% of malachite green (10 mg L⁻¹) in four days at 28 °C (Jasińska et al. 2012). In another study, 80-99% of different azo dyes (100-300 mg L⁻¹) were decolorized by *P. oxalicum* in a process mediated by manganese peroxidase at 30 °C (Saroj et al. 2014). *P. oxalicum* has also been reported as able to absorb dyes. In the study performed by Zhang et al. (2003), the mycelial pellet of the fungus *P. oxalicum* absorbed up to 60% of the Reactive Blue 19 dye in 10 min, and 91% in 80 min. In the present study, this phenomenon might have occurred, since the colonies in Malt Broth medium with the addition of Sulphur Indigo Blue textile dye acquired a dark blue color. On the other hand, the higher toxicity found in the bioassays performed with *Penicillium* cf. *oxalicum* LAMAI 2400 and LAMAI 2402 in comparison to the control (medium with the textile dye) suggests these fungi transform enzymatically the dye molecule. Microbial degradation can result in the mineralization of the compound or its transformation into less toxic and/or more water-soluble molecules, which can be used as nutrients and consequently, be degraded by other microorganisms (Cerniglia 1997, Cerniglia & Sutherland 2001). Nevertheless, intermediates of the degradation process of an environmental pollutant can be more toxic than the original compound (Bergsten-Torralba et al. 2009, Giovanella et al. 2020, Vasconcelos et al. 2019). To certify if the color removal of the Sulphur Indigo Blue textile dye by the Antarctic filamentous fungi happened by metabolic pathways, biosorption, or both processes, additional experiments have to be undertaken.

The approach used for fungal isolation from the Antarctic samples allowed the acquisition of microbial resources with the ability to decolorize high rates of Sulphur Indigo Blue dye at 15 and

28 °C, highlighting the potential of the Antarctic fungi *Penicillium* cf. *oxalicum* LAMAI 2400 and LAMAI 2402 for textile dye bioremediation in processes conducted at low and moderate temperatures. Considering the biotechnological relevance of the fungus *P. oxilacum*, as well as the fact that representatives of this species have rarely been reported in Antarctic environments, the two selected filamentous fungi recovered from Antarctic marine sediments added value to the UNESP Microbial Culture Collection (CRM-UNESP) and will be available for further studies related to Antarctic mycology and biotechnological application in industrial and environmental microbiology. Additionally, the lack of information about representatives of this fungal species in the field of textile dye bioremediation opens new perspectives for studies related to pathways and genes involved in the degradative process.

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