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Fungi from Antarctic marine sediment: characterization and assessment for textile dye decolorization and detoxification

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Abstract
Cold-adapted microorganisms can produce enzymes with activity at low and mild temperatures, which can be applied to environmental biotechnology. This study aimed to characterize 20 Antarctic fungi to identify their genus (ITS rDNA marker) and growth temperatures and evaluate their ability to decolorize and detoxify the textile dye indigo carmine (IC). An individual screening was performed to assess the decolorization and detoxification of IC by the isolates, as well as in consortia with other fungi. The isolates were affiliated with seven ascomycete genera: *Aspergillus* ($n=4$), *Cosmospora* ($n=2$), *Leuconeurospora* ($n=2$), *Penicillium* ($n=3$), *Pseudogymnoascus* ($n=6$), *Thelebolus* ($n=2$), and *Trichoderma* ($n=1$). The two isolates from the genus *Leuconeurospora* were characterized as psychrophilic, while the others were psychrotolerant. The *Penicillium* isolates were able to decolorize between 60 and 82% of IC. The isolates identified as *Pseudogymnoascus* showed the best detoxification capacity, with results varying from 49 to 74%. The consortium using only Antarctic ascomycetes (C1) showed 45% of decolorization, while the consortia with the addition of basidiomycetes (C1 + *Peniophora* and C1 + *Pholiota*) showed 40% and 50%, respectively. The consortia C1 with the addition of the basidiomycetes presented a lower toxicity after the treatments. In addition, a higher fungal biomass was produced in the presence of dye when compared with the experiment without the dye, which can be indicative of dye metabolization. The results highlight the potential of marine-derived Antarctic fungi in the process of textile dye degradation. The findings encourage further studies to elucidate the degradation and detoxification pathways of the dye IC by these fungal isolates.

Keywords Antarctic mycology · Marine fungi · Bioremediation · Extremophiles

Introduction

The harsh conditions of the Antarctic continent and its subregions can challenge macro and microscopic forms of life. In the marine environment, in addition to the low temperatures, conditions like salinity and pressure demand morphologic and metabolic adaptations to survival [1–3]. Microorganisms that grow in such harsh conditions are known as extremophiles. Concerning the temperature of growth, they are classified as psychrophilic and psychrotolerant. Psychrophilic microorganisms grow at maximum temperatures below 20 °C with optimum growth temperatures lower than, or equal to, 15 °C. Psychrotolerant microorganisms can grow at low temperatures and have optimal growth in the temperature range of mesophilic organisms (20–40 ºC) [1].

Some genera commonly found in the Antarctic marine environment are *Aspergillus*, *Cadophora*, *Cladosporium*, *Cosmospora*, *Mortierella*, *Penicillium*, *Pseuderotium*, *Pseudogymnoascus*, *Geomyces*, *Trichoderma*, *Phaeoacremonium*, *Phenoliferia*, and *Pseudocercosporella*, among others [4–7]. These extremophile fungi have also been gaining attention due to their biotechnological potential. Targeted

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biomolecules are antibiotics, antitumoral compounds, and a range of enzymes like hydrolases and oxidases that can have important uses in industrial and environmental areas, including bioremediation of environmental pollutants [2, 8–9]. An industrial sector that can benefit from these enzymes is the textile sector, considered one of the major industrial waste generators, with a complex and varied composition [10–11]. The complexity of the effluent comes from the presence of synthetic dyes, softeners, metals, and different salts, which, even at lower concentrations, can impact the water body, the fauna, and the ecosystem [12].

Synthetic dyes are one of the most concerning components of textile effluents. Their different physicochemical properties are a major cause of the recalcitrancy as they can be further oxidized into more toxic or mutagenic forms [13–15]. Indigo dyes are known to be resistant to light and heat exposure, insoluble, and generally need an adjuvant for the proper adhesion to the textile fiber [16]. They are traditional dyes, whose natural form has been used for centuries, and their synthetic form was introduced in the 1800s [17, 18]. One of the most popular forms is the indigo carmine dye, produced by reducing indigo with $Na_2S_2O_4$. Currently, indigo dyes are used in the textile industry and also in the food and cosmetic sectors, resulting in a worldly consumption per year of 50.000 tons of indigo dyes and their derivatives [11, 17, 19].

The treatment of such effluents is required for their disposal back into the environment, given the toxic and disruptive potential. Different approaches can be used, including physical and biological methods, either isolated or in combination, to attend to the final parameters demanded by the local legislation [20]. In the textile sector, the chemical methods comprise oxidative processes that can disrupt the chromophore part of the dye molecule, removing the color of the effluent. Nonetheless, these methods do not deal with the toxicity aspect and can cause an increase in dye toxicity in some cases [14, 20]. The physical methods comprise the transference of dye from a liquid to a solid phase, generating sludges that need to be disposed of in landfills and do not tackle the toxicity and recalcitrance of the waste [20, 21]. The biological methods (bioremediation) include the action of microorganisms (e.g. fungi, bacteria, and algae), either isolated or in consortia, and/or their enzymes to achieve the degradation of waste or the treatment of an effluent, aiming at the complete mineralization into carbon and hydrogen [21].

Among the benefits of bioremediation is the eco-friendly nature, and the chance of completing dye degradation, thus eliminating or at least lowering the toxicity factor. This approach can be cheaper than chemical and physical treatments and can be performed with organisms that can tolerate the salt and other recalcitrant characteristics of the waste or effluent [15, 22].

Considering the above mentioned, this study was designed to characterize 20 filamentous fungi from marine sediments collected from two different Antarctic islands and assess their ability to degrade and detoxify the dye indigo carmine in consortia at moderate temperatures under the perspective of a future application in the bioremediation of textile industrial effluents.

Materials and methods

Microorganisms

The fungi used in this study were selectively isolated using two enrichment treatments (sulfur indigo dye and sawdust) from two marine sediments collected at Deception Island Whalers Bay (SMWB1–95.4 m depth) and King George Island - EACF Helipad SMEACF1–60 m depth) (OPERANTAR XXXVII, December 2018) [23]. The fungi were cultivated in malt extract 2% agar (MEA: malt extract 20 g; bacteriological agar 15 g; deionized water 1,000 mL) at 15 °C. All fungal isolates are deposited in the UNESP's Central of Microbial Resource (CRM-UNESP) (Biosciences Institute, São Paulo State University– UNESP, Rio Claro, SP, Brazil) and have been maintained in the biobank by freezing at -80 °C and employing the method of Castellani using glycerol 10% and water, respectively.

Characterization of the fungi

Molecular identification

The ITS barcode was used for the molecular characterization of the fungi. The DNA was extracted from the colonies using the CTAB method [24, 25]. The ITS region was amplified according to dos Santos et al. [6] using the primers ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and 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 sequences generated in this study were deposited in Genbank under the accession numbers PP003958-PP003975 (Table 1). The reference sequences retrieved from the NCBI database and the fungal sequences from this study were aligned using MAFFT v7.520 [26]. Manual refinements were performed using Aliview v. 1.28 [27].

Table 1 Data related to the sites, fungi codes, and taxonomic identification

Enrichment treatment	Site of origin	LAMAI code	Identification	GenBank acession number
Sawdust	Deception Island Whalers Bay	2758	*Aspergillus* sp.	PP003962
		2759	*Aspergillus* sp.	PP003963
		2755	*Leuconeurospora* sp.	PP003959
		2756	*Leuconeurospora* sp.	PP003960
		2754	*Penicillium* sp.	PP003958
		2757	*Pseudogymnoascus* sp.	PP003961
		2760	*Pseudogymnoascus* sp.	PP003964
	King George Island - EACF Helipad	2777	*Cosmospora* sp.	PP003966
		2778	*Cosmospora* sp.	PP003967
		2776	*Pseudogymnoascus* sp.	PP003965
		2780	*Trichoderma* sp.	PP003968
Sulfur indigo dye	Deception Island Whalers Bay.	2400	*Penicillium* cf. *oxalicum**	MZ376947
		2402	*Penicillium* cf. *oxalicum**	MZ376948
		2398	*Pseudogymnoascus* sp.	PP003969
		2401	*Pseudogymnoascus* sp.	PP003971
		2399	*Thelebolus* sp.	PP003970
	King George Island - EACF Helipad	2424	*Aspergillus* sp.	PP003974
		2425	*Aspergillus* sp.	PP003975
		2422	*Pseudogymnoascus* sp.	PP003972
		2423	*Thelebolus* sp.	PP003973

*Identified as described by Kita et al. [23]

The best evolutionary models were determined using jModelTest 2 v. 2.1.10 [28] with the Akaike Information Criterion (AIC) and 95% confidence intervals. A maximum likelihood phylogenetic tree was inferred using RAxML-NG 1.2.0 [29] with 20 distinct starting trees using the best-fit model (Table S1) and 1000 bootstrap replicates. All trees were visualized in FigTree 1.4.4 [30] and edited in Inkscape v. 1.2.2.

Growth temperatures

For the determination of the optimum growth temperatures, the fungi were cultivated on Petri plates (90 × 15 mm) containing MEA medium for 7 days at 4 different temperatures: 15, 20, 25, and 30 °C. A suspension was prepared at 2×10^6 spores/mL and 10 µL of each suspension was inoculated in the center of a Petri dish, each fungus was inoculated in triplicate. After incubation, the growth area was scanned and measured with the software ImageJ using the free hand mode. The growth area (cm^2) was determined by the software using a relation between the image pixels and the measured area [31].

Decolorization of the textile dye indigo carmine

All isolates were cultivated in 50 mL of Malt Extract Broth (MEB - malt extract 20 g; deionized water 1,000 mL) with 100 mg/L of the dye, the Indigo Carmine dye used ($C_{16}H_8N_2Na_2O_8S_2$,) was purchased from Sigma-Aldrich (85% of purity). The fungi were inoculated as a calibrated spore suspension at 1×10^6 spores/mL and the flasks were incubated at 20 °C for seven days and 140 rpm in the dark. After incubation, the mycelium was separated by vacuum filtration (Unifil– 80 g/m^2), and the supernatant was used to measure decolorization and detoxification. As a control experiment, flasks with MEB without the fungi were incubated under the same conditions. All assays were conducted in triplicate.

To determine decolorization, 1 mL of the supernatants was evaluated on a UV-Vis spectrophotometer (Shimadzu UV-1240, Kyoto, Japan) [32]. The decolorizing activity was calculated from the maximum absorption peak (610 nm) of the dye, according to Eq. 1.

$$\text{Decolorization } (\%) = \frac{(\text{medium absorbance of control} - \text{medium absorbance of treatment})}{(\text{medium absorbance of control}) \times 100} \quad (1)$$

Detoxification of the textile dye indigo carmine

The phytotoxicity assessment was conducted using seeds of cucumber (*Cucumis sativus*) to evaluate the impact of degradation metabolites on the germination process and overview the toxicity [33]. Each Petri dish contained 15 seeds (without pesticides) and 3 mL of the supernatant (adjusted to pH 7.0) was placed on filter paper. The seeds were incubated at 24 °C in the dark for 5 days. The positive control consisted of a solution of 0.05 M $ZnSO_4$, and the negative control was

distilled water, as well as, a solution of the dye (100 mg/L). After incubation, the root length (RL) and hypocotyl length (HL) were measured (cm), and they were used to calculate the toxicity and detoxification according to Eqs. 2 and 3.

$$\text{Toxicity } (\%) = \frac{(\text{RL} + \text{HL of negative control} - \text{RL} + \text{HL of treatments})}{(\text{RL} + \text{HL of the negative control}) \times 100} \quad (2)$$

$$\text{Detoxification } (\%) = \text{control toxicity} - \text{treatment toxicity} \quad (3)$$

Consortia structuration

A major fungal consortium was structured based on the taxonomic characterization and considering the results of individual performance in the decolorization and detoxification experiments. Additional experiments were performed adding to the consortium the basidiomycete *Peniophora* sp. CBMAI 1063 (=111), which was isolated from a marine sponge [34], and *Pholiota* sp. LAMAI 2484 (=B9.320), which was isolated from Antarctic soil [6]. Both fungi can produce ligninolytic enzymes and degrade/adsorb textile dyes [6, 32, 35] and were introduced to diversify the consortia metabolisms.

Decolorization and detoxification of the textile dye indigo carmine by the consortia

The analysis of decolorization and detoxification was conducted using 50 mL of Minimum Mineral broth (MM1–1,000 mL of distilled water; 3 g of Na_2HPO_4; 2.5 g of NaH_2PO_4; 1 g of $(NH_4)_2SO_4$; 0.7 g of KCl; 1 mL of a micronutrient solution (1,000 mL of distilled water; 4 g of $MgSO_4$; 0.2 g of Fe_2SO_4; 0.2 g of $MNCl_2$; 2 g of $CaCl_2$) with 100 mg/L of the dye and 0.1% of glucose. The fungi of the major consortium were inoculated as a calibrated spore suspension at 1×10^6 spores/mL. After 2 days of incubation, the fungi *Peniophora* sp. CBMAI 1063 and *Pholiota* sp. LAMAI 2484 were inoculated (in separate experiments) by the addition of three plugs of 5 mm (in diameter) to the medium MM1 containing the inoculants (spore suspension) of the major consortium. The flasks were incubated at 20 °C for seven days and 140 rpm in the dark. After incubation, the mycelia were separated by centrifugation at 10,000 rpm (13,416 g) and 4 °C for 15 min according to Vieira et al. [36]. The supernatant was used to measure decolorization and detoxification, as mentioned above. As a control experiment, flasks with MM1 broth without the fungi were incubated under the same conditions. All assays were conducted in triplicate.

Dry biomass

The dry biomass was evaluated according to Vieira et al. [36]. Falcon tubes were dried at 105 °C until constant mass, and the weight (g) was measured with the empty tubes. In these same flasks, the mycelia were centrifuged at 10,000 rpm (13,416 g) at 4 °C for 15 min. After supernatant removal, the mycelia were taken to dry at 105 °C until constant mass. Then, the flasks with the mycelium were weighted and the dry biomass was calculated using Eq. 4.

$$\text{Dry Biomass } (g) = \text{weight of flasks with mycelium} - \text{weight of the empty flask} \quad (4)$$

Statistical analysis

The statistical analysis was executed in GraphPad Prism 9 GraphPad Software, United States, using One-Way ANOVA. For the growth temperature, decolorization and detoxification, decolorization by the consortia, and dry biomass, $p < 0.05$ were considered. For the phytotoxicity in the consortia experiments, $p < 0.10$ was considered.

Results

Taxonomic characterization

Using the ITS rDNA marker (fungal molecular barcode), the isolates were associated with seven different ascomycete genera (Table 1): *Aspergillus* ($n=4$), *Cosmospora* ($n=2$), *Leuconeurospora* ($n=2$), *Penicillium* ($n=3$), *Pseudogymnoascus* ($n=6$), *Thelebolus* ($n=2$), and *Trichoderma* ($n=1$). The molecular taxonomic data (phylogeny trees) are available in the Supplementary material (Figures S1-S6).

The phylogenetic analysis allowed the inference that the representatives of the genus *Aspergillus* belong to different species, with the closest relatives being the species *A. sydowii* and *A. creber.* The results for *Pseudogymnoascus* also indicate the presence of more than one species, with the closest relatives being *P. griseus*, *P. lanuginosus*, *P. lindineri*, *and P. turneri*. On the other hand, representatives of *Cosmospora*, *Leuconeurospora*, and *Thelebolus* appear to be a single species, with the closest relatives being *C. viridescens*, *L. capsici*, *T. microspores*, and *T. balaustiformis*, respectively, while the representative of *Thichoderma* clustered with the species *T. obovatum*. Concerning the three isolates of the genus *Penicillium*, two of them, LAMAI 2400 and 2402, have been previously identified as *Penicillium* cf. *oxalicum* based on molecular taxonomy (ITS rDNA) and morphology [23]. In the present study, the sequences of LAMAI 2754

are grouped in the same cluster of 2400 and 2402, suggesting they belong to the same *Penicillium* species.

Regarding distribution (Table 1), it was observed that the fungi from the genera *Aspergillus*, *Thelebolus*, and *Pseudogymnoascus* were recovered from both marine sediment samples, collected from different islands in the South Shetlands Archipelago, Antarctica: Deception Island and King George Island. Fungi from the genera *Penicillium* and *Leuconeurospora* were recovered only from the sample from Deception Island and representatives of the genera *Cosmospora* and *Trichoderma* were isolated only from the samples from King George Island.

Growth temperature

Based on the area of growth for each isolate in the four different temperatures, two isolates can be considered psychrophilic and the eighteen others as psychrotolerant. Figures 1 and 2 show the growth temperatures for the marine-derived fungi isolated using sawdust and sulfur indigo dye, respectively. The isolate LAMAI 2780, identified as *Trichoderma* sp., grew in all available areas in the Petri dishes by the end of the incubation period for all temperatures tested. For this fungus, it was not possible to infer an optimum growth temperature.

The psychrophilic isolates were LAMAI 2755 and 2756, identified as *Leuconeurospora* sp., which showed the best growth at 15 °C, and did not grow at temperatures higher than 20 °C. The fungi considered psychrotolerant showed different temperature ranges for their growth. Among them, the isolates of the genus *Pseudogymnoascus* (LAMAI 2760, 2422, 2757, 2776, 2401, and 2398) and *Thelebolus* (LAMAI 2399 and 2423) presented the best growth temperature at 20 °C. A total of four isolates showed the optimum temperature at 25 °C, two of the genus *Cosmospora* (LAMAI 2777 and 2778) and the other two of the genus *Aspergillus* (LAMAI 2758 and 2759). A total of five isolates presented the best growth at 30 °C, two of the genus *Aspergillus* (LAMAI 2424 and 2425), and three of the genus *Penicillium* (LAMAI 2754, 2400, and 2402).

Decolorization and detoxification of the textile dye indigo carmine by the Antarctic fungi

Among the 20 fungal isolates tested, three presented a high capacity to decolorize the dye indigo carmine (from 60 to 82%): LAMAI 2754 (82%), 2400 (76%), and 2402 (60%), all of them of the genus *Penicillium*. The fungi LAMAI 2756, 2758, and 2759 presented a moderate ability for dye decolorization (from 30 to 42%) and were identified as *Leuconeurospora* sp. and *Aspergillus* sp., respectively (Table 2).

A total of six isolates (LAMAI 2754, 2758, 2759, 2780, 2400, and 2402) showed higher toxicity after the treatment when compared with the control (Table 2). On the other hand, the isolates LAMAI 2760 and 2776, both identified as *Pseudogymnoascus* sp., presented low toxicity after the treatment. The isolate LAMAI 2425 presented moderate toxicity. For the three isolates that presented some detoxification (LAMAI 2760, 2776, and 2425), the decolorization could not be quantified, since the absorbance measured was higher than the control, which can be a result of a modification in the chemical structure of the dye, or the production of secondary metabolites that had an overall influence on toxicity.

Decolorization and detoxification of the textile dye indigo carmine by the fungal consortia

To improve the decolorization and detoxification rates of the textile dye Indigo Carmine, the fungal consortia were structured considering the results of individual performance in the decolorization and detoxification experiments and based on the taxonomic diversity (Fig. 3). The consortium (C1) composed of marine Antarctic isolates of different genera, LAMAI 2756 (*Leuconeurospora* sp.), 2760 (*Pseudogymnoascus* sp.), 2425 (*Aspergillus* sp.), and 2400 (*Penicillium* cf. *oxalicum*), showed 45% of dye decolorization. When the white-rot basidiomycete fungi *Peniophora* sp. CBMAI 1063 (marine origin) and *Pholiota* sp. LAMAI 2484 (Antarctic soil origin) were added to the consortia C1 (C1 PEN and C1 PHO, respectively), and the decolorization rates were 40 and 50%, respectively. Nevertheless, there was no statistical difference for any of the treatments.

Considering the toxicity analyses (Fig. 4), the experiment with the consortium C1, composed of only the Antarctic ascomycetes, presented the highest toxicity (61%), while the consortia with the addition of basidiomycetes (C1 PHO and C1 PEN) showed 43% and 32% of toxicity, respectively, without statistical difference in comparison to the control.

Dry biomass of the fungal consortia

Data on the dry biomass (Fig. 5) revealed that the consortium C1 without the dye (C1 WD) generated less biomass (0.069 g) than the other treatments. When the dye was present, the biomass of the consortia was higher (0.122 g for C1 PHO, 0.111 g for C1 PEN, and 0.108 g for C1). Although there was no control (without dye) for the treatments with the basidiomycetes (C1 PEN and C1 PHO), there was no statistical difference between the accumulation of biomass in the presence of the dye in the three consortia, even with an extra fungus in the consortia C1 PEN and C1 PHO.

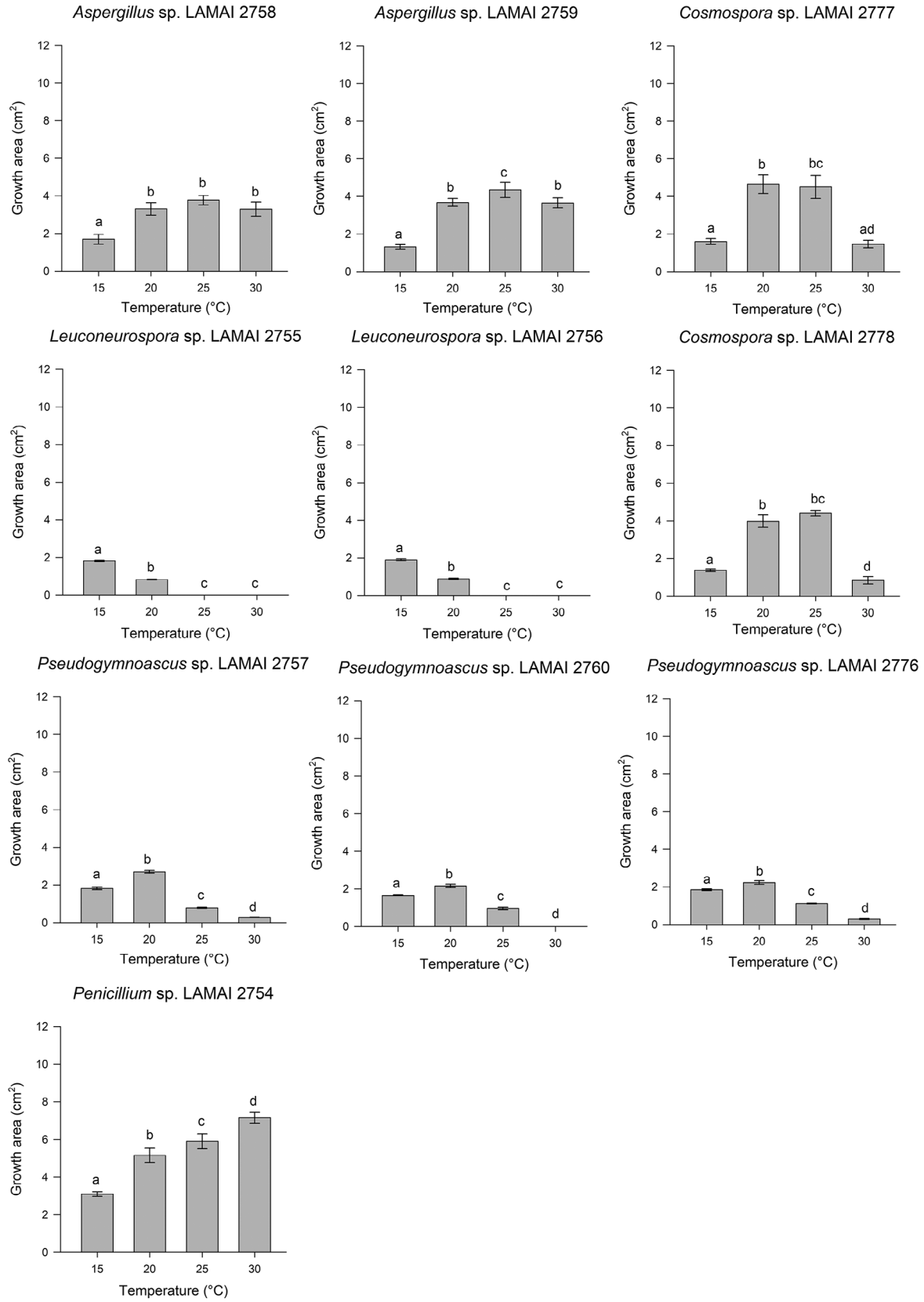

Fig. 1 Growth (cm²) of the fungi isolated from sawdust treatment at four different temperatures (15, 20, 25, and 30 °C) after 7 days of incubation in MEA medium. LAMAI 2780 is not shown, since it grew in all areas at all temperatures. One-way analysis of variance (ANOVA), with Tukey posttest ($p < 0.05$)

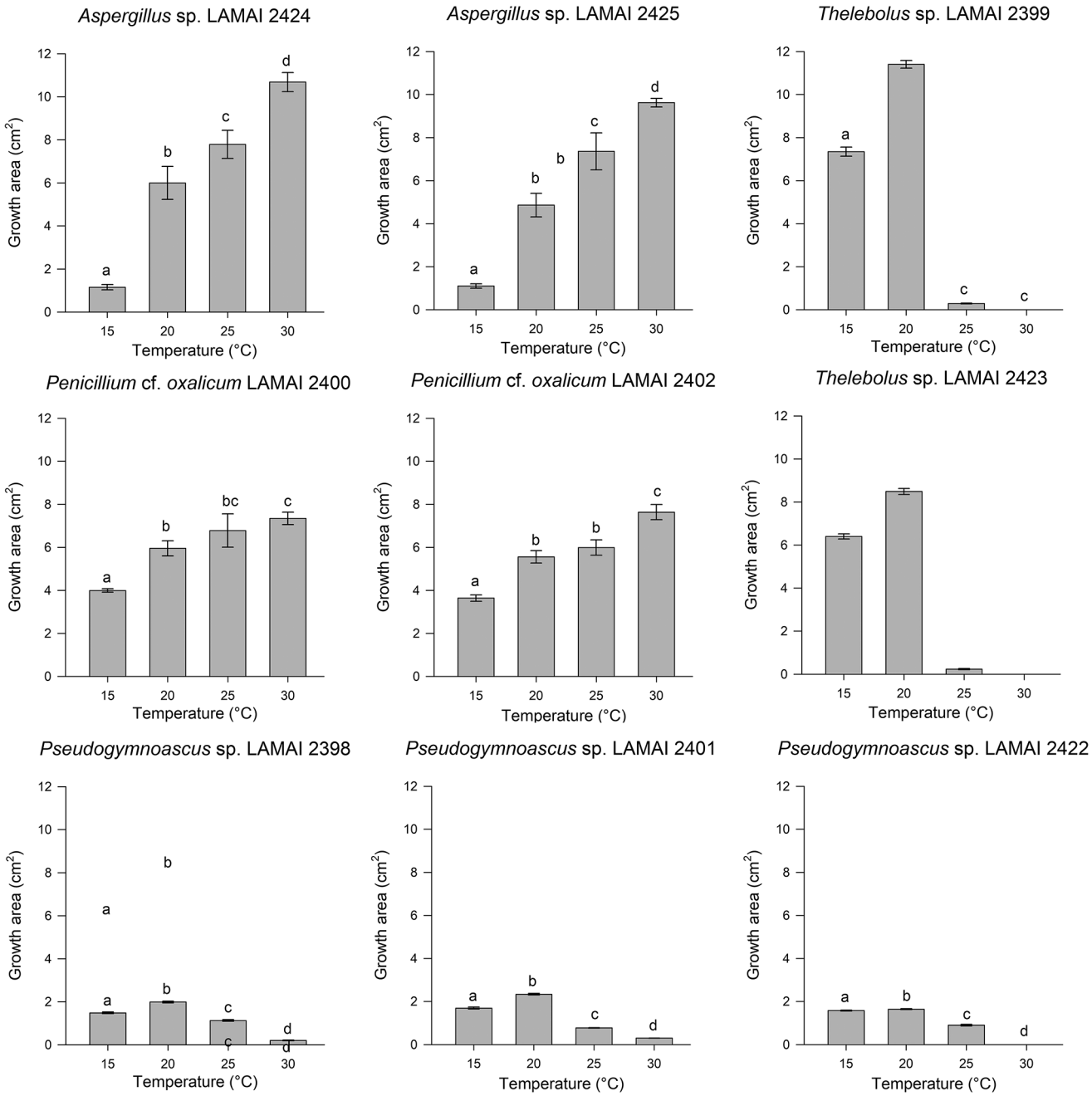

Fig. 2 Growth (cm²) of the fungi isolated from the sulfur indigo dye treatment at four different temperatures (15, 20, 25, and 30 °C) after 7 days of incubation in MEA medium. One-way Analysis of variance (ANOVA), with Tukey posttest ($p < 0.05$)

Discussion

The fungi isolated from marine sediments, after selective pressure (enrichment), were affiliated with seven different genera in the phylum Ascomycota: *Aspergillus*, *Cosmospora*, *Pseudogymnoascus*, *Leuconeurospora*, *Penicillium*, *Thelebolus*, and *Trichoderma*. The data from the phylogenetic analysis allowed the inference of the closest relatives. For some of these genera, the fungal barcoding marker (ITS rDNA) is not sufficient for a conclusive evolutionary position, being necessary for a secondary marker or more (multilocus phylogenetic analyses), as in the case of the genus *Pseudogymnoascus*, for which the molecular taxonomy includes the markers ITS, LSU, TEF1, RPB2, and MCM7 [37]. It is worth mentioning that the species *P. griseus* and *P. lanuginosus*, which clustered with the marine Antarctic *Pseudogymnoascus* isolates, have recently been described as associated with Antarctic environments [37].

Table 2 Data on pH, decolorization, toxicity and detoxification of the dye indigo carmine by fungi after 7 days of incubation in MEB with the addition of 100 mg/L of indigo carmine, at 20 °C and 140 rpm. CTR1– batch 1 control; CTR2– batch 2 control; CRT 3– batch 3 control

Fungal isolate	pH	Decolorization (%)	Toxicity (%)	Detoxification (%)
CTR1	5.20	---	77.3 ± 4.71	---
LAMAI 2398	5.67	0.0	62.5 ± 7.66	14.8 ± 6.26
LAMAI 2401	6.33	0.0	65.5 ± 4.68	11.8 ± 3.83
LAMAI 2754	3.23	82.5 ± 2.75	90.0 ± 2.69	*12.6 ± 2.20
LAMAI 2755	5.63	0.0	70.4 ± 17.35	6.9 ± 14.17
LAMAI 2756	4.83	37.7 ± 2.05	71.0 ± 9.78	6.2 ± 7.98
LAMAI 2757	4.63	0.0	60.7 ± 4.33	16.6 ± 3.53
LAMAI 2760	6.70	0.0	2.5 ± 7.99	74.7 ± 8.07
LAMAI 2776	5.77	0.0	27.9 ± 22.73	49.3 ± 18.57
CTR2	5.20	----	87.3 ± 2.30	----
LAMAI 2400	2.30	76.9 ± 3.59	100.0 ± 0.00	*12.7 ± 0.00
LAMAI 2402	2.17	60.0 ± 6.47	100.0 ± 0.00	*12.7 ± 0.00
LAMAI 2422	6.10	0.0	68.4 ± 7.31	18.8 ± 5.98
LAMAI 2424	5.90	0.0	78.3 ± 7.17	9.0 ± 5.86
LAMAI 2425	6.20	0.0	54.0 ± 6.94	33.2 ± 5.67
LAMAI 2758	5.77	30.1 ± 12.53	90.6 ± 6.40	*3.3 ± 5.23
LAMAI 2759	5.50	42.3 ± 5.79	94.2 ± 1.84	*6.8 ± 1.50
LAMAI 2780	7.10	0.0	92.0 ± 3.42	*4.7 ± 2.79
CTR3	5.13	----	88.9 ± 3.23	----
LAMAI 2399	5.03	0.0	72.1 ± 16.49	16.7 ± 13.46
LAMAI 2423	6.17	0.0	62.5 ± 22.71	26.4 ± 18.55
LAMAI 2777	5.13	0.0	73,9 ± 16,16	15.0 ± 13.20
LAMAI 2778	5.37	0.0	69.0 ± 11.77	19.8 ± 9.62

*higher toxicity in comparison to the control of the batch

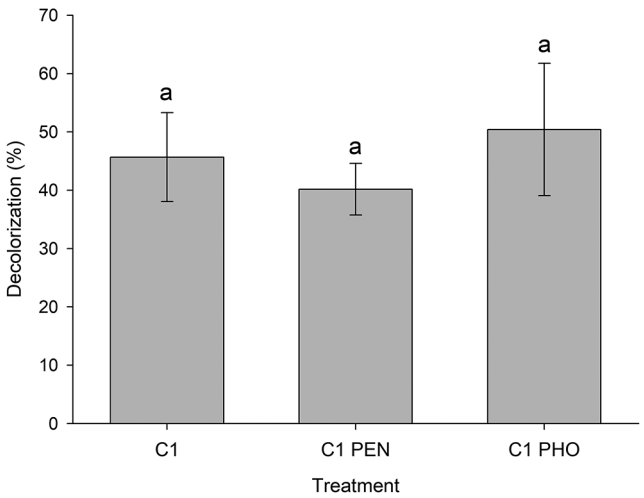

Fig. 3 Decolorization analysis for the three consortia in MM1 medium, with 0.1% glucose and 100 mg/L of indigo carmine dye after incubation for 7 days at 20 °C and 140 rpm. (C1) *Leuconeurospora* sp. LAMAI 2756, *Pseudogymnoascus* sp. LAMAI 2760, *Aspergillus* sp. LAMAI 2425, and *Penicillium* cf. *oxalicum* LAMAI 2400; (C1 PEN) C1 + *Peniophora* sp. CBMAI 1063; (C1 PHO) C1 + *Pholiota* sp. LAMAI 2484. One-way Analysis of variance (ANOVA) with Tukey test ($p < 0.05$)

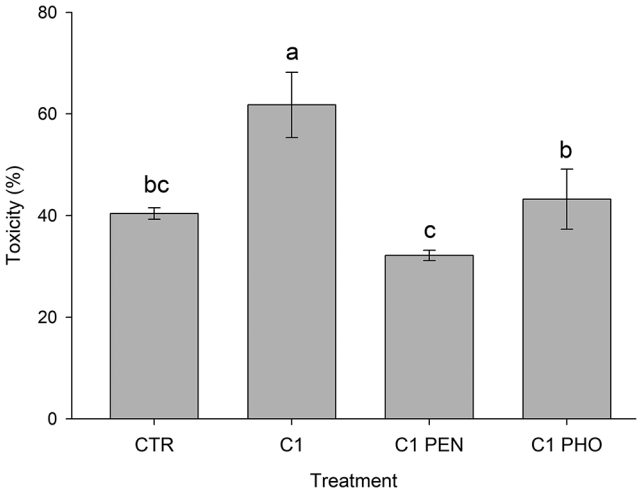

Fig. 4 Phytotoxicity analysis using *Cucumis sativus* as bioindicator of the three consortia in MM1 medium with 0.1% glucose and 100 mg/L of indigo carmine dye after incubation for 7 days at 20 °C and 140 rpm. (CTR) Control– dye solution (C1) *Leuconeurospora* sp. LAMAI 2756, *Pseudogymnoascus* sp. LAMAI 2760, *Aspergillus* sp. LAMAI 2425, and *Penicillium* cf. *oxalicum* LAMAI 2400; (C1 PEN) C1 + *Peniophora* sp. CBMAI 1063; (C1 PHO) C1 + *Pholiota* sp. LAMAI 2484. One-way Analysis of variance (ANOVA) with Tukey test ($p < 0.10$)

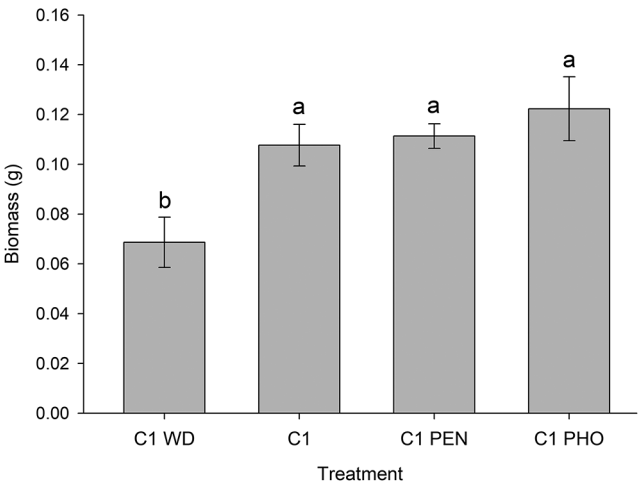

Fig. 5 Dry biomass of the mycelium in MM1 with 0.1% glucose with and without dye (100 mg/L) after incubation at 20 °C for 7 days at 140 rpm. (C1 WD) Control without dye; (C1) *Leuconeurospora* sp. LAMAI 2756, *Pseudogymnoascus* sp. LAMAI 2760, *Aspergillus* sp. LAMAI 2425, and *Penicillium* cf. *oxalicum* LAMAI 2400; (C1 PEN) C1 + *Peniophora* sp. CBMAI 1063; (C1 PHO) C1 + *Pholiota* sp. LAMAI 2484. Analysis of variance (one-way ANOVA) with Tukey posttest ($p < 0.05$)

The representatives of the genera identified in the present study have been reported in different samples from the Antarctic environments [3, 6, 7, 38–41]. Filamentous fungi from Antarctic marine sediments were previously reported as a promising alternative to bioremediate cold

environments contaminated with polluting compounds, including synthetic dyes [23, 42].

Most of these fungi tolerated or grew best at temperatures lower than 20 °C, indicating an adaptation to the Antarctic environments. The isolates that had better growth at higher temperatures LAMAI 2777 and 2778 (*Cosmospora*), 2758, 2759, 2424 and 2425 (*Aspergillus*), 2754, 2400, and 2402 (*Penicillium*), and 2780 (*Trichoderma*) are known as cosmopolite and psychrotolerant. Although they could grow at moderate temperatures, they showed more vigorous growth at 25 °C and 30 °C. Similar results were reported by Martorell et al. [43] in the evaluation of the optimum growth temperature of 51 Antarctic fungi, when 12 isolates were considered psychrophilic and 29 psychrotolerant, with the optimum temperature in the range of 15 °C and 25 °C for most of the isolates. In another study reported by Azmi and Seppelt [44], the maximum growth of the fungi isolated from Antarctica was observed between 15 and 25 °C, being 20 °C the best growth temperature for most of the isolates.

The use of extremophilic fungi in studies of textile dye degradation can bring new insights and reveal biological advantages [6, 45]. The results of indigo carmine decolorization by the isolates LAMAI 2400 and 2402, both identified as *Penicillium* cf. *oxalicum* [23], were already expected since they presented a potential for the decolorization of Sulphur Indigo Blue dye in a previous study [23]. Studies have reported the decolorization and detoxification of textile dyes by *Penicillium oxalicum* enzymes [46] and by mycelial adsorption [47].

A higher toxicity in comparison to the control was observed in some of the treatments, it can be related to the fact that the degradation of the dye molecule during the 7 days may have generated byproducts with higher toxicity than the dye itself. These results have also been observed in previously reported studies [48, 49]. New strategies may be necessary to tackle the toxicity aspect, and maybe increasing incubation time, or adding some complementary nutrients, may help boost the consortia metabolism. It is important to highlight that in the present study, just one culture condition was used to evaluate the decolorization and detoxification of the textile dye. Considering that culture conditions have different effects on the behavior and metabolism of microorganisms, further optimization experiments and analyses of different variables in these processes can amplify the ability of these Antarctic fungi to degrade and detoxify the pollutant.

Most of the studies related to the degradation of indigo carmine use basidiomycete fungi [48–53] since they are considered the best degraders of aromatic and complex molecules due to their ability to produce ligninolytic enzymes. However, some studies have shown that ascomycete fungi can also be applied in textile dye degradation, and the production of ligninolytic enzymes has already been reported for the genera *Penicillium*, *Aspergillus*, and *Pseudogymnoascus* [7, 45, 50, 56].

Considering that a synergistic metabolism composed of different groups could advance pollutant degradation in the environment, the strategy of introducing a "new metabolism" was applied, since all fungi selectively isolated from Antarctic marine sediment samples belong to the phylum Ascomycota. The fungus *Pholiota* sp. LAMAI 2484, used in consortium C1 PHO, isolated from Antarctic glacier retreated soil [6], presents optimum growth at 20 °C and showed the ability to decolorize and detoxify the textile dye Congo red, as well as to produce ligninolytic enzymes (data not published). Species from the genus *Pholiota* are producers of ligninolytic enzymes and can degrade textile dyes and other pollutants [57, 58]. *Peniophora* sp. CBMAI 1063, used in consortium C1 PEN, isolated from marine sponge [34], has already been reported as a great degrader of reactive black 5 dye [35], reaching 94% of decolorization in 7 days of incubation in an optimized condition. This fungus is a laccase hyper-producer [59–61] and can also produce other ligninolytic enzymes [32]. Although there were differences in the composition of the three consortia, there were no significant differences among them in terms of decolorization. Other conditions can favor the metabolism of these fungi and may boost the enzymatic production for the degradation of the dye and its byproducts.

On the other hand, in the toxicity assay, the treatments C1 PEN and C1 PHO showed lower toxicity in comparison with the consortium C1. Although both consortia with the basidiomycetes showed lower toxicity among the treatments, the results were not statistically different from the control (CTR), indicating that toxicity does not have a particular correlation with decolorization. De Jesus Fontes et al. [48] observed an increase in toxicity in treatments of Reactive Blue dye with the enzymatic extract of *Peniophora* sp. CBMAI 1063. This result suggests the other fungi belonging to the consortium could act on the intermediate metabolites, reducing their toxicity.

Concerning biomass production, other studies have also reported better biomass production in the presence of the dye indigo carmine than without the dye, suggesting the assimilation of the dye as a carbon source [62]. The significant difference found in the present study for the three consortia (C1, C1 PEN, and C1 PHO), in comparison with the control without dye (C1 WD) might be an indication that there is an assimilation of the dye as a carbon source, although more investigation needs to be conducted to confirm this hypothesis.

The results from the present study can be considered a good start for understanding the applicability of extremophile Antarctic fungi in the biodegradation of textile dye. Further analyses can be useful and help in finding a better dynamic for the consortia in the decolorization and detoxification processes, including experimental design (for optimization) and the use of different trophic levels as bioindicators of toxicity. Additional analyses, such as metabolite identification using chromatography associated with mass spectroscopy and metatranscriptomics (expressed genes) may help elucidate the pathways involved in the degradation and detoxification of the dye and overall process by the consortia.

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Data availability Data supporting this study are openly available from NCBI (GenBank) at accession numbers listed in Table 1.

Declarations

Conflict of interest The authors declare no competing interests.

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