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Bioremediation Potential of Basidiomycetes in a Medium with Apple-Based Detergent

Potencial de Bioremediação de Basidiomicetos em Meio com Detergente de Maçã

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David Patrick Almeida Correia: Universidade Federal de Sergipe, Departamento de Engenharia Agronômica, Laboratório de Microbiologia. Campus São Cristóvão, SE, Brasil. E-mail: davidpatrickcorreia@gmail.com

Michele Santos de Jesus: Universidade Federal de Sergipe, Departamento de Engenharia Agronômica, Laboratório de Microbiologia. Campus São Cristóvão, SE, Brasil.

Leonel Bismarck Belo Pereira: Universidade Federal de Sergipe, Departamento de Engenharia Agronômica, Laboratório de Microbiologia. Campus São Cristóvão, SE, Brasil.

Igor Daniel Alves Ribeiro: Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Programa de Pós-Graduação em Genética e Biologia Molecular. RS, Brasil.

Luciane Maria Pereira Passaglia: Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Programa de Pós-Graduação em Genética e Biologia Molecular. RS, Brasil.

Eliana Midori Sussuchi: Universidade Federal de Sergipe, Departamento de Química, Laboratório de Corrosão e Nanotecnologia/NUPEG. Campus São Cristóvão. SE, Brasil.

Pedro Roberto Almeida Viégas: Universidade Federal de Sergipe, Departamento de Engenharia Agronômica. Campus São Cristóvão. SE, Brasil.

Regina Helena Marino: Universidade Federal de Sergipe, Departamento de Engenharia Agronômica, Laboratório de Microbiologia. Campus São Cristóvão. SE, Brasil.

Abstract

The extensive use of detergents and inadequate waste disposal practices have caused significant environmental pollution. To mitigate these effects, this study aimed to evaluate the bioremediation potential of the basidiomycetes *Lentinula edodes* (LED 96/18) and *Pycnoporus sanguineus* (PS) in a medium containing apple-based detergent, with or without methylene blue, as well as the phytotoxicity of these mediums following fungal colonization. The following bioassays were carried out: (1) Influence of apple-based detergent on mycelial growth in potato-dextrose-agar (PDA) and

sawdust; 2) *In vitro* bioremediation potential of fungal isolates in PDA, detergent, and methylene blue; 3) Degradation of the detergent and methylene blue by fungal isolates; and 4) Phytotoxicity of the detergent and methylene blue after fungal cultivation. The apple-based detergent inhibited the mycelial growth of LED96/18 and PS at 200 µL.L^{-1}. The detergent-dye medium inhibited the growth of PS, but stimulated the growth of LED 96/18. PS and LED 96/18 promoted the decolorization of 32.7% of the detergent-dye medium. The detergent-dye medium, with and without fungal colonization, did not inhibited the germination of 'Rasteiro Rio Grande' tomato seeds, but negatively influenced the growth of the radicle with the increase from 1% to 10% of the medium. The isolates LED 96/18 and PS show promise for *in vitro* bioremediation of a medium containing apple detergent and methylene blue.

Keywords: Phytotoxicity. Mushroom. Environmental Microbiology.

Resumo

O uso extensivo de detergentes e práticas inadequadas de descarte de resíduos tem causado significativa poluição ambiental. Para mitigar esses efeitos, este estudo teve como objetivo avaliar o potencial de biorremediação dos basidiomicetos *Lentinula edodes (*LED 96/18) e *Pycnoporus sanguineus* (PS) em meio de cultura contendo detergente à base de maçã, com ou sem azul de metileno, bem como a fitotoxicidade destes meios após a colonização fúngica. Foram realizados os seguintes bioensaios: 1) Influência do detergente à base de maçã no crescimento micelial em meio batata-dextrose-ágar (BDA) suplementado com serragem; 2) Potencial de biorremediação *in vitro* de isolados fúngicos em BDA, detergente e azul de metileno; 3) Degradação do detergente e do azul de metileno pelos isolados fúngicos; e 4) Fitotoxicidade do detergente e do azul de metileno após cultivo fúngico. O detergente à base de maçã inibiu o crescimento micelial do LED96/18 e do PS a 200 µL.L^{-1}. O meio detergente-azul de metileno inibiu o crescimento do PS, mas estimulou o crescimento do LED 96/18. PS e LED 96/18 promoveram a descoloração de 32,7% do meio detergente-corante. O meio detergente-corante, com e sem colonização fúngica, não inibiu a germinação de sementes de tomate 'Rasteiro Rio Grande', mas influenciou negativamente no crescimento da radícula com o aumento de 1% para 10% do meio. Os isolados LED 96/18 e PS têm potencial de biorremediação *in vitro* de meio com detergente de maçã e azul de metileno.

Palavras-chave: Fitotoxicidade. Cogumelos. Microbiologia Ambiental.

1 Introduction

The increasing global population has led to a growing demand for surfactants in detergents used for dishwashing, laundry, and personal hygiene products. This rise in usage has raised significant concerns regarding environmental contamination. In Brazil, only 42.6% of sewage is collected and treated, with effluent treatment stations achieving less than 60% efficiency in removing biochemical oxygen demand (Brasil, 2017; Silva; Ledo, 2023).

According to the Globally Harmonized System (GHS) classification, the toxicity of surfactants ranges from harmful to highly toxic, depending on factors such as the target species, exposure time, concentration, and ambient temperature (Cetesb, 2022; Effendi *et al.*, 2017; Sobrino-Figueroa, 2018). This makes the release of these products into water bodies particularly concerning in the context of global warming.

Resolution Nº 694/2022 by the Agência Nacional de Vigilância Sanitária orders that anionic surfactants in detergents must be biodegradable (Brasil, 2022). However, linear alkylbenzene sulfonate (LAS), a key component of biodegradable detergents, remains recalcitrant under anaerobic conditions commonly found in sewage treatment systems (Kaida *et al.*, 2021). Thus, the biodegradable nature of detergents does not eliminate their potential ecological impact (Kogawa *et al.*, 2017; Toledo *et al.*, 2021).

Microorganisms have been widely reported as a cost-effective solution for the treatment of effluents, including the removal or detoxification of xenobiotics such as organic pollutants, hydrocarbons, plastics, textile effluents, and pesticides (Aniceto; Irazusta, 2023; Beltrane; Oliveira; Limeira, 2023). Among these, white-rot basidiomycetes such as *Agaricus bisporus*, *Lentinula edodes*, *Pycnoporus sanguineus*, and *Pleurotus* spp. have been extensively studied for their bioremediation potential in vitro, particularly with synthetic dyes that act as bioindicators (Eichlerová; Baldrian, 2020; Doi; Otaguiri; Souza, 2021). These fungi can decolorize dyes either through absorption by the mycelium (Singh, 2017; Bouras *et al.*, 2021) or by utilizing xenobiotic-dye compounds as a carbon source, coupled with the release of oxidative enzymes that break down aromatic rings and double bonds (Eichlerová; Baldrian, 2020; Zhao *et al.*, 2024).

Although *L. edodes* and *P. sanguineus* have not been previously studied for their ability to decolorize detergent-dye mixtures, the white-rot fungus *Phanerochaete chrysosporium* has demonstrated the capacity to decolorize LAS-containing agar media via oxidative enzymes, which are also produced by *L. edodes* and *P. sanguineus* (Backes *et al.*, 2023; Yadav *et al.*, 2001; Zhao *et al.*, 2024).

It is crucial to recognize that during bioremediation, microorganisms may degrade or transform xenobiotics into compounds with varying toxicity levels (Kogawa *et al.*, 2017; Liu; Deng; Yang, 2021; Santos *et al.*, 2023). Phytotoxicity evaluations of commercial detergents on seeds have been reported (Hernández-Branda *et al.*, 2023), but no studies have assessed the phytotoxicity of detergent-dye solutions after fungal colonization by white-rot fungi.

The objective of this study was to evaluate the in vitro bioremediation potential of the white-rot basidiomycetes *Lentinula edodes* and *Pycnoporus sanguineus* in a medium containing apple-based detergent, as well as the phytotoxicity of this medium after fungal colonization, to propose a biotechnological alternative for wastewater treatment.

2 Material and Methods

2.1 Fungal inoculum

The fungal isolates used in this study were *Lentinula edodes* (LED 96/18) and *Pycnoporus*

sanguineus (PS), both classified as white-rot basidiomycetes. The isolates were propagated in a commercial potato dextrose agar (PDA) medium (39 $g \cdot L^{-1}$) under controlled conditions of 28 ± 1 °C with an 8-hour light photoperiod for 7 days. The PDA medium colonized by each fungal isolate served as the inoculum for subsequent experiments.

2.2 Detergent

The dishwashing detergent used in this study was an apple-scented commercial product, Ypê®, which, according to the manufacturer, consists of anionic surfactants, a sequestrant, preservatives, a thickener, adjuvants, coloring agents, fragrance, water, and linear sodium alkylbenzene sulfonate (LAS) as the active ingredient.

2.3 Influence of an apple-based detergent on mycelial growth

The experiment followed a completely randomized 2 × 6 factorial design, involving two fungal isolates (LED 96/18 and PS) cultivated on potato-dextrose-agar (PDA) medium supplemented with sawdust (1g L^{-1}) and six detergent concentrations (0, 50, 100, 200, 400, and 800µL L^{-1}), with four replicates per treatment. The control treatment contained no detergent (0µL L^{-1}).

The sawdust used in the cultivation medium was sourced from medium-density fiberboard (MDF) with particle sizes smaller than 20 mesh, autoclaved at 120°C for 20 minutes, to stimulate the synthesis of oxidative enzymes responsible for compound degradation (Coelho *et al.*, 2021; Silva; Moreira, 2024). Detergent was added to the autoclaved and cooled medium, which was then transferred to Petri dishes. After solidification, a 7-mm diameter mycelial disc of fungal inoculum was deposited and incubated at 28 ± 1 °C under an 8-hour photoperiod.

The variables analyzed included mycelial diameter (MD), growth rate (GR), percentage inhibition of mycelial diameter (PIC-MD), and percentage inhibition of growth rate (PIC-GR), evaluated after six days of cultivation.

Mycelial diameter (MD, cm) was measured as the means of two perpendicular measurements using a millimeter ruler. Growth rate (GR, $cm \cdot day^{-1}$) was calculated using the formula $GR = (MDf - MDi)/CP$, where MDf is the final mycelial diameter, MDi is the initial mycelial diameter, and CP is the cultivation period in days.

Percentage inhibition of mycelial diameter (PIC-MD, %) and growth rate (PIC-GR, %) were determined using the formula $PIC = [(A - B)/A] \times 100$, where A represents the variable's value (mycelial diameter or growth rate) in the control treatment without detergent, and B represents the variable value in the detergent-treated fungal cultures.

2.4 *In vitro* bioremediation potential of fungal isolates

The experiment followed a completely randomized 2 × 5 factorial design, corresponding to the cultivation of two fungal isolates (LED 96/18 and PS) on commercial PDA medium supplemented with detergent (200µL L^{-1}) and five concentrations of methylene blue (MB) dye (0, 20, 40, 80, and 160 mg L^{-1}), with five repetitions per treatment.

The dye was added to evaluate detergent removal from the medium, as detergents with anionic surfactants can bind to cationic synthetic dyes, enabling detergent degradation assessment through medium discoloration (Cesar-Ribeiro; Prado; Rosa, 2022; Mastroti *et al.*, 1998). The detergent concentration was based on results from a prior bioassay, where the selection criterion was a reduction in fungal growth rate (PIC-GR) of less than 50%. The dye used, manufactured by PROQUÍMIOS, had the chemical formula $C_{16}H_{18}ClN_3S \cdot 3H_2O$ and a molar mass of 373.90g mol^{-1}.

Detergent was added to the autoclaved PDA-dye culture medium, and the mixture was transferred to Petri dishes. A 7-mm diameter mycelial disc of fungal inoculum was placed at the center of the solidified culture medium and incubated under the same conditions described in the prior bioassay for four days.

The variables analyzed included mycelial diameter (MD), growth rate (GR), percentage inhibition of mycelial diameter (PIC-MD), percentage inhibition of growth rate (PIC-GR), identification of contaminating fungi, genetic characterization of contaminating bacteria, medium discoloration, and medium absorption by the mycelium.

Mycelial diameter, growth rate, PIC-MD, and PIC-GR were measured according to the methodology of the previous bioassay. Contaminating fungi were identified following the classification by Barnett and Hunter (1998). Bacterial identification involved genomic DNA extraction using the phenol-chloroform method (Sambrook; Russel, 2001; Alippi; Aguilar, 1998). Amplification of complete 16S rRNA gene sequences was performed using primers 27F (GAGTTTGATCCTGGCTCAG) and 1525R (AGAAAGGAGGTGATCCAGCC) (Rainey *et al.*, 1996).

PCR amplification used a 25 µL reaction mixture containing 1 x Taq polymerase buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, 1 U of Taq DNA polymerase, and 100 ng of template DNA. Conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 sec, and extension at 72°C for 1.5 min, with a final extension at 72°C for 10 min.

Amplicons were sequenced using the Sanger method by Macrogen. Low-quality sequences were trimmed using Chromas Software (v2.6.6), and contigs were assembled with CAP3 (Huang; Madan, 1999) implemented in UGENE (Okonechnikov; Golosova; Fursov, 2012). The 16S rRNA

sequences were submitted to BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and EzBioCloud (www.ezbiocloud.net/) databases. Nucleotide sequences were deposited in the GenBank database.

Phylogenetic analysis used type strain sequences of the closest related species. Sequence alignment was performed with the MUSCLE algorithm (Edgar, 2004), and phylogenetic trees were constructed using IQ-TREE software (Minh *et al.*, 2020) with the maximum-likelihood method and 1,000 bootstrap replications. Sequences of Alteribacillus bidgolensis CCM7963 were used as the outgroup. The resulting phylogenetic trees were edited using iTOL (Letunic; Bork, 2019).

Medium discoloration and/or absorption by the mycelium were evaluated after ten days of fungal cultivation.

2.5 Detergent-dye degradation rate and phytotoxicity of the medium after fungal cultivation

The experimental design was a completely randomized 2 × 3 factorial method, corresponding to the cultivation of two isolates (LED 96/18 and PS) in a liquid yeast extract culture medium (25g L^{-1}) with detergent (200µL L^{-1}) and three concentrations of methylene blue (MB) dye (0, 40, and 80mg L^{-1}), with four repetitions per treatment. The concentrations of the dye and detergent were determined based on results from previous bioassays.

The dye and detergent were incorporated into the autoclaved yeast extract medium according to the respective treatments. After the medium cooled, two 7-mm diameter mycelial discs were transferred as inoculum. Control treatments did not include inoculum discs. The incubation conditions were the same as described in previous bioassays and lasted nine days.

The discoloration rate of the medium, representing detergent-dye degradation (Cesar-Ribeiro; Prado; Rosa, 2022; Mastroti *et al.*, 1998), was quantified as the percentage of discoloration of the medium with detergent and dye (D, %) using the equation $D = [(ABSc - ABSf) / ABSc] \times 100$, where ABSc is the absorbance of the culture medium in the control treatment (without fungus), and ABSf is the absorbance of the culture medium with fungus. The absorbance was measured using a Varian CARY-100 UV-VIS spectrophotometer at a wavelength of 612 nm. The adsorption of the culture medium containing detergent and dye by the mycelium was visually evaluated.

Phytotoxicity of the culture medium was evaluated through two experiments using tomato seeds (*Solanum lycopersicum* L.) cultivar ‘Rasteiro Rio Grande’ following a modified methodology by Guevarra *et al.* (2019). Two bioassays were conducted with solutions containing 1% and 10% yeast extract (25g L^{-1}) with detergent (200µL L^{-1}) and dye concentrations of 40mg L^{-1} and 80mg L^{-1}, respectively, which were not contaminated by fungi or bacteria.

In the first experiment, a completely randomized design with four treatments was applied:

Control 1: autoclaved distilled water only; Control 2: 1% yeast extract-detergent solution with 40mg L^{-1} MB dye (without fungal inoculation); Treatment LED 96/18: 1% yeast extract-detergent solution with 40mg L^{-1} MB dye, colonized for nine days by isolate LED 96/18; Treatment PS: same as Treatment LED 96/18 but colonized by isolate PS. Each treatment had four repetitions.

In the second experiment, a completely randomized experimental design was used with four treatments: Control 1: autoclaved distilled water only; Control 2: 10% yeast extract-detergent solution with 80mg L^{-1} MB dye (without fungal inoculation); and 10% solution of yeast extract-detergent and 80mg L^{-1} of methylene blue, and colonized for nine days by the isolates LED 96/18 and PS, with four repetitions.

Ten seeds of the tomato 'Rasteiro Rio Grande' were used for each treatment and repetition. The seeds were immersed for 3 min. into the solution according to the treatment and transferred to a Petri dish with two autoclaved filter papers and 10 mL of autoclaved distilled water. Incubation occurred at 28 ± 1 ºC with an 8 h photoperiod per day for five days.

The variables analyzed included pH, germination rate (G), radicle length (RL), percentage inhibition of radicle length (PIC-RL), and phytotoxicity indicators, namely percentage of normalized residual germination (ING) and percentage of normalized residual radical elongation (IRE).

The pH of autoclaved distilled water and media containing detergent and dye was measured following Silva (2009). The germination rate (G, %) of seeds was calculated using the formula G = (NSG / NTS) × 100, where NSG is the number of germinated seeds and NTS is the total number of seeds. The radicle length of seedlings was measured using a digital caliper. The normalized residual germination index (ING) was calculated as ING = (GT – GTC) / GTC, where GT is the average germination percentage in the treated medium, and GTC is the average germination percentage in the control. The normalized residual radicle elongation index (IRE) was calculated as IRE = (RLT – RLC) / RLC, where RLT is the average radicle length in the treated medium, and RLC is the average radicle length in the control. The ING and IRE values were classified as toxicity according to Bagur-Gonzalez *et al.* (2011).

2.6 Statistical analysis

The results were submitted to ANOVA and Tukey's test, while regression analysis was evaluated using the F-test and t-test at 1% and 5% probability utilizing the SISVAR version 5.8 software.

3 Results and Discussion

3.1 Influence of the apple-based detergent on mycelial growth

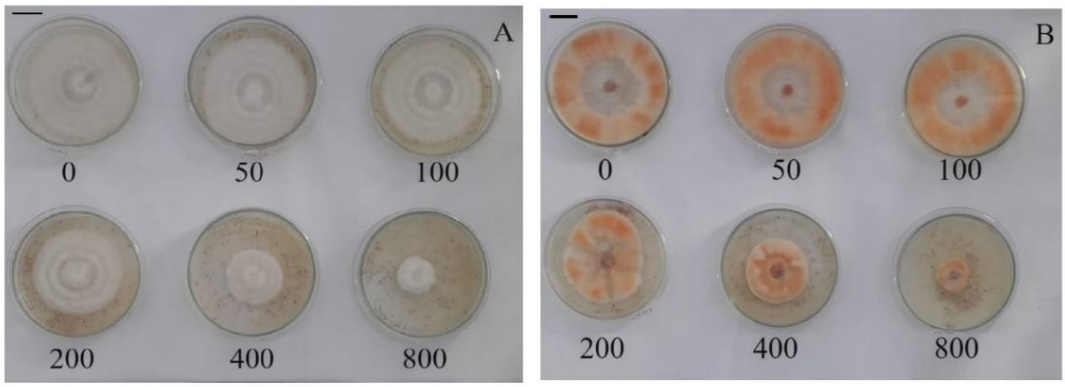
Increasing the concentration of detergent from 200µL L^{-1} significantly reduced the mycelial growth in diameter and growth rate of the fungal isolates tested, with the percentage of inhibition (PIC-MD, PIC-GR) exceeding 50% in the medium containing 800µL L^{-1} of detergent (Table 1; Figure 1).

Table 1 - Mycelial diameter, percentage of inhibition of mycelial diameter (PIC-MD), growth rate (GR), and percentage of inhibition of growth rate (PIC-GR) of fungal isolates in PDA medium with apple-based detergent, after six days of cultivation

Detergent	**Mycelial diameter (cm)**		**PIC-MD (%)**	
(µL L^{-1})	**LED 96/18**	**PS**	**LED 96/18**	**PS**
0	7.6aB	9.0aA	-	-
50	7.3aB	8.7aA	4.0dA	3.8dA
100	7.2aB	8.7aA	4.9dA	3.6dA
200	6.5bB	7.3bA	14.5cA	19.2cA
400	4.4cA	4.8cA	42.3bA	46.6bA
800	2.8dA	2.9dA	63.4aA	69.9aA
VC[b]	5.0%		14.6%	
Regression; R^2	**Linear 0.97**[c]**	**Linear 0.96****	**Linear 0.96****	**Linear 0.95****
Detergent	Growth rate (cm.day^{-1})		PIC-GR (%)	
(µL L^{-1})	LED 96/18	PS	LED 96/18	PS
0	1.2aB	1.4aA	-	-
50	1.1aB	1.3aA	4.4dA	4.1dA
100	1.1aB	1.3aA	5.4dA	3.9dA
200	0.9bB	1.1bA	16.0cA	20.8cA
400	0.6cA	0.7cA	46.6bA	50.5bA
800	0.3dA	0.4dA	69.9aA	73.2aA
VC	5.6%		14.6%	
Regression; R^2	**Linear 0.97****	**Linear 0.96****	**Linear 0.96****	**Linear 0.95****

[a]Means followed by the same letter (lowercase in the column; uppercase in the row) do not differ from each other using the *Tukey* Test at 5% probability;
[b]VC = Variation coefficient; and
[c](**) = significant at 1% ($p < 0.01$).
Source: research data.

Figure 1 - Mycelial growth of isolates LED 96/18 (A) and PS (B) in PDA culture medium with 0, 50, 100, 200, 400, and 800µL L^{-1} of the apple-based detergent (bar = 2 cm)



Source: the authors.

In the selection of the fungi with bioremediation potential, the highest growth rate has been used as an indicator (Su; Rodrigues, 2022). In this study, the mycelial diameter and growth rate of the PS were significantly higher than those of the LED 96/18 with 0 to 200µL L^{-1} of the detergent. There was no significant difference between LED 96/18 and PS with 400 and 800µL L^{-1} of detergent (Table 1).

No reports were found on the influence of detergents and/or surfactants on the mycelial growth in diameter and growth rate of *L.edodes* and *P. sanguineus*. However, the growth of the basidiomycete *Armilaria sp.* F022 in a medium containing the detergent Tween 80 was favored by the presence of the lignocellulolytic enzyme laccases (Hadibarata; Kristanti, 2014). Likewise, the basidiomycete *Coriolus brevis* synthesized laccase capable of oxidizing aromatic compounds with industrial and environmental application (Kim *et al.*, 2024).

The production of laccase enzymes has also been described for the white rot fungi *Pycnoporus spp.* and *Lentinula edodes* (Liu *et al.*, 2020; Doi; Otaguiri; Souza, 2021), mainly in lignocellulosic waste (Freitas *et al.*, 2021), as the sawdust added in this study. However, the laccase synthesis can be influenced by substrate, surfactants, temperature, pH, organic and inorganic carbon, and nitrogen sources (Aiswarya; Nayana; Nambisan, 2018; Mathur; Sanyal; Dey, 2021; Khatami *et al.*, 2022; Backes *et al.*, 2023), which may have influenced the growth of the LED 96/18 and PS. Furthermore, residual compounds with different degrees of toxicity can be formed (Kogawa *et al.*, 2017; Liu; Deng; Yang, 2021; Santos *et al.*, 2023) and influence microbial growth.

3.2 Bioremediation potential of fungal isolates *in vitro*

The bioremediation potential of microorganisms can be assessed through the growth, the decolorization and/or absorption of the medium with dyes and/or detergent-dyes by the fungus due to its hydrophobic-hydrophilic interaction with the mycelium (Mastroti *et al.*, 1998; Yadav *et al.*, 2001; Singh, 2017; Cesar-Ribeiro; Prado; Rosa, 2022; Zhao *et al.*, 2024).

The addition of the dye to the medium did not negatively influence the mycelial diameter and growth rate of LED96/18, but there was an increase in growth of 1.2 to 18.5% in PIC-MD and 2.8 to 32.6% in PIC-GR with 20 to 160mg L^{-1} of dye, respectively, compared to the control (detergent only) (Table 2), probably because it used the detergent-dye as a carbon source for its metabolism, as occurs with hydrocarbons (Tiso *et al.*, 2022).

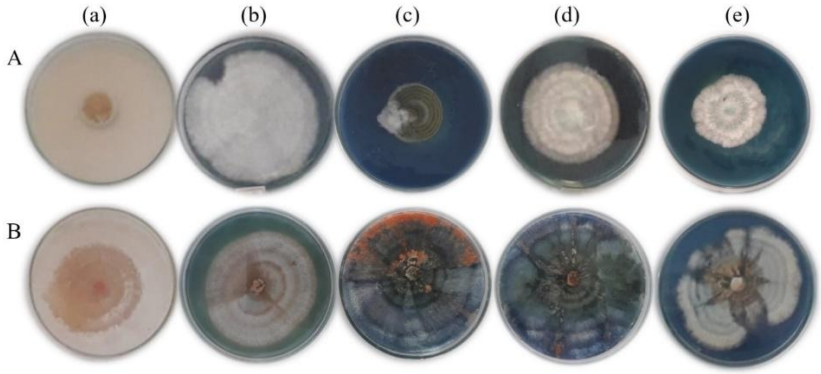
Table 2 - Mycelial diameter, percentage of inhibition of mycelial diameter (PIC-MD), growth rate (GR), and percentage of inhibition of growth rate (PIC-GR) of fungal isolates in PDA medium with apple-based detergent and the methylene blue dye (MB dye), after six days of cultivation

MB dye concentration (mg L^{-1})	Mycelial diameter (cm)		PIC-MD (%)	
	LED 96/18	PS	LED 96/18	PS
0	1.6aB	6.5aA	-	-
20	1.6aB	5.9aA	-1.2	9.5
40	1.9aB	4.7bA	-18.5	27.8
80	1.9aB	4.3bA	-18.5	34.6
160	1.6aB	2.3cA	-1.2	64.5
VC[c]	15.15%			
Regression; R^2	Linear R^2 = 0.09 ns[d]	Linear R^2 = 0.96**		
MB dye concentration (mg.L^{-1})	Growth rate (cm.day^{-1})		PIC-GR (%)	
	LED 96/18	PS	LED 96/18	PS
0	0.2aB	1.5aA	-	-
20	0.2aB	1.3aA	-2.7	10.6
40	0.3aB	1.0bA	-32.6	31.2
80	0.3aB	0.9bA	-32.6	38.7
160	0.2aB	0.4cA	-2.8	72.3
VC	19.25%			
Regression; R^2	Linear R^2 = 0.09 ns	Linear R2 = 0.96**		

[a]Means followed by the same letter (lowercase in the column; uppercase in the row) do not differ from each other using the *Tukey* Test at 5% probability; [b]Positive values = inhibit mycelial growth and negative values = stimulate mycelial growth; [c]VC = Variation coefficient; and [d](ns) not significant (p>0/.05); and (**) = significant at 1% (p < 0.01).
Source: research data.

PS exhibited greater mycelial growth in terms of both diameter and growth rate compared to LED 96/18 in the detergent-dye medium across all dye concentrations. However, at dye concentrations of 40mg L^{-1} and above, growth was reduced when compared to the control (detergent only). In contrast, the dye added to the detergent medium also inhibited mycelial growth starting at a concentration of 20mg L^{-1} in both the PIC-MD (9.5-64.5%) and PIC-GR (10.6-72.3%) (Table 2; Figure 2), as reported by Menezes *et al.* (2017) with PS in a medium containing 50mg L^{-1} of MB dye, but without detergent.

Figure 2 - Mycelial growth of isolates LED 96/18 (A) and PS (B) in PDA medium supplemented with an apple-based detergent (200µL L^{-1}) and 0 (a), 20 (b), 40 (c), 80 (d), and 160 (e) mg L^{-1} of methylene blue dye, after six days of cultivation



Source: the authors.

LED 96/18 absorbed the detergent-dye medium containing 20, 80, and 160mg L^{-1} of dye, while PS absorbed the medium starting at 80mg L^{-1}. Both LED 96/18 and PS decolorized the medium from 20mg L^{-1} of the dye. The differences observed between the fungal isolates during growth may have been due to contamination, probably because of the addition of the dye after autoclaving the medium.

In the case of LED 96/18, bacterial contamination occurred with 20mg L^{-1} of the dye, and with *Penicillium* sp. at 0, 20, and 160 mg.L^{-1} of dye, which likely influenced the medium decolorization. Costa *et al.* (2020) observed that *Penicillium chrysoxylum* degraded 99.5% of the linear sodium alkylbenzene sulfonate (LAS), an anionic surfactant used in biodegradable detergents. Thus, the presence of *Penicillium* may have contributed to the detergent-dye medium discoloration in the LED 96/18 treatment.

LED 96/18 in the detergent-dye medium containing 20mg L^{-1} of dye, bacterial contamination was detected with the isolates BAC2, BAC3, and BAC5. BAC2 showed 99.87% similarity with

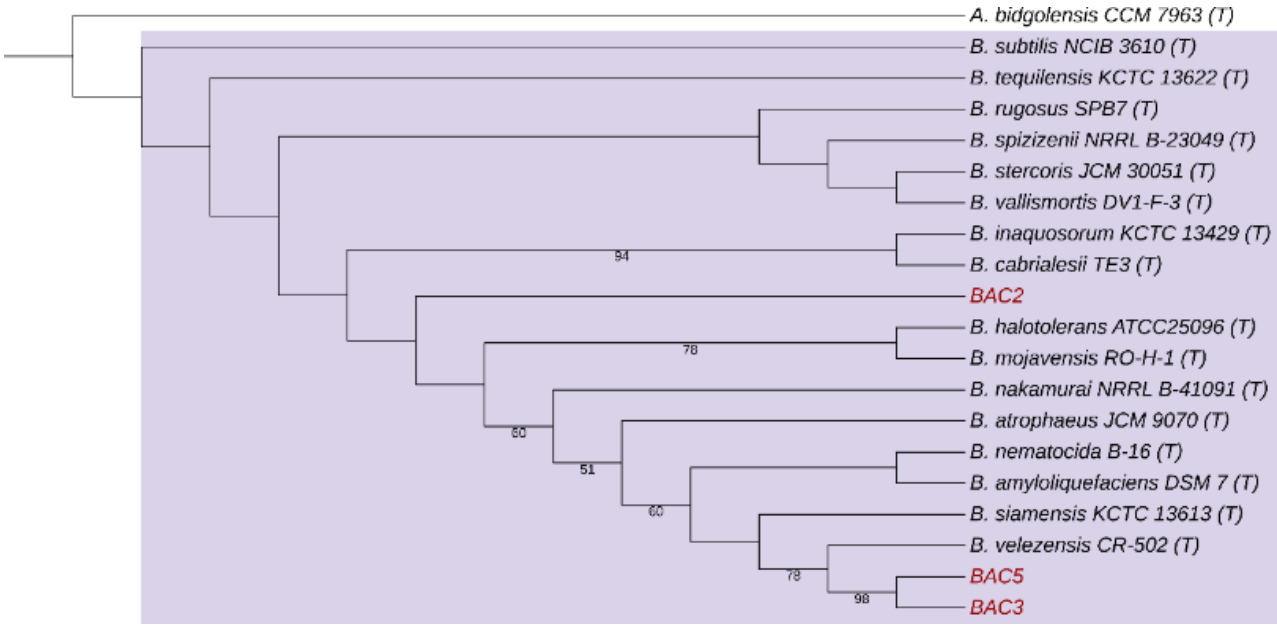
Bacillus subtilis NCIB 3610, while BAC3 and BAC5 exhibited 100% similarity with *Bacillus velezensis* CR-502 (Table 3; Figure 3), which may also have influenced the medium discoloration,since *B. velezensis* isolate AB has also been shown to promote the discoloration of methyl red, methyl orange, and Congo red dyes (Bafana, 2022).

Table 3 - Bacterial isolates obtained in the medium with the apple-based detergent colonized by LED 96/18

Isolates	Closest type strain	Similarity (%)	GenBank accession number
BAC2	*Bacillus subtilis* NCIB 3610	99.87	PP177411
BAC3	*Bacillus velezensis* CR-502	100.00	PP177412
BAC5	*Bacillus velezensis* CR-502	100.00	PP177413

Source: research data.

Figure 3 - Phylogenetic tree of species *Bacillus* based on 16S rRNA gene sequences. The tree was reconstructed using the Maximum Likelihood (ML) method with IQ-TREE software. Bootstrap values below 70% are not displayed. Isolate labels are shown in red. *Alterbacillus bidgolensis* CCM 7963^{T} was designated as the outgroup



Source: research data.

Consequently, LED 96/18 and PS also demonstrate bioremediation potential for this xenobiotic, as evidenced by the detergent-dye medium discoloration containing 40 and 80mg L^{-1} of methylene blue, in the absence of contaminants.

However, it must be considered that sewage is not a sterile environment, and the presence of

other microorganisms may interfere with the bioremediation potential of these isolates. It is important to evaluate in future studies the interaction between LED 96/18 and PS fungi with bacteria (BAC2, BAC3, and BAC5), as well as with the contaminating fungus and/or the native sewage microbiota. This consortium may enhance efficiency and reduce the bioremediation time of detergents in the environment (Talukdar *et al*., 2020; Torres-Farradá *et al.*, 2024).

3.3 Detergent-dye degradation rate and phytotoxicity of the medium after fungal cultivation

The average decolorization rate of the detergent-dye medium was 32.7%, with no significant difference observed between the fungal isolates and the dye concentrations (Table 4).

Table 4 - Discoloration (%) of the yeast extract medium apple-based detergent and methylene blue dye by the fungal isolates, after nine days of cultivation

Fungal isolates	Discoloration (%) of detergent-dye medium	
	40mg L^{-1}	**80mg L^{-1}**
LED 96/18	27.6%aA	36.7%aA
PS	37.5%aA	28.6%aA
VC[b]	25.4%	

[a]Means followed by the same letter (lowercase in the column; uppercase in the row) do not differ from each other using the *Tukey* Test at 5% probability; [b] VC = Variation coefficient.
Source: research data.

The decolorization of the surfactant medium LAS-agar by the basidiomycete *P. chrysosporium* occurred through oxidative enzymes (Yadav *et al.*, 2001), such as laccases synthesized by *L. edodes* and *P. sanguineus* (Eichlerová; Baldrian, 2020; Zhao *et al.*, 2024), which may have contributed to the medium discoloration by LED 96/18 and PS, as well as the degradation of the detergent dye.

Araújo *et al.* (2019) observed that extending the cultivation period of the white rot fungus *Panus lecomtei* led to an increase in laccase activity, promoting the industrial dyes discoloration after 20 days of cultivation. Therefore, it is important to extend the cultivation period of white rot fungi to evaluate their bioremediation efficiency. Furthermore, the microbial degradation products of xenobiotics can be non-toxic, less toxic, or more toxic, depending on the microbial interaction with the contaminant (Liu; Deng; Yang, 2021; Santos *et al.*, 2023).

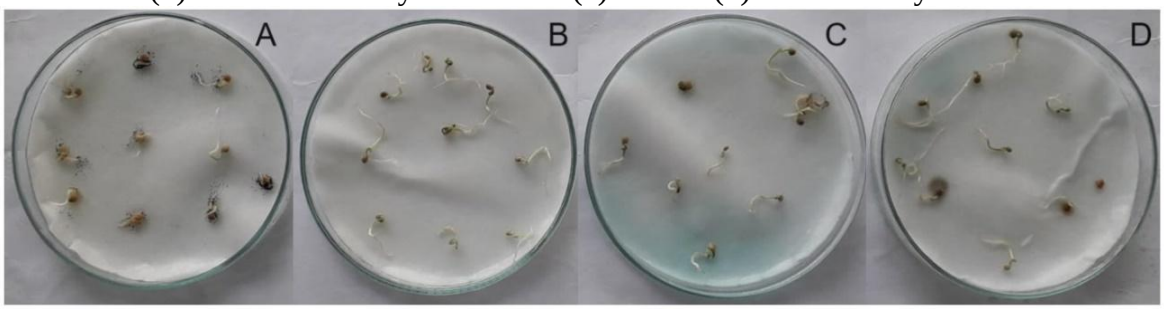
In the phytotoxicity bioassay, the use of solutions containing 1% and 10% of the detergent-dye medium after the cultivation of LED 96/18 and PS did not inhibit the germination of tomato seeds due to their low toxicity (ING < 0.25) (Table 5; Figures 4 and 5). Similarly, the solution with commercial detergent did not inhibit cucumber seed germination; however, increasing the concentration inhibited the radicle growth of cucumber (Hernández-Branda *et al.*, 2023).

Table 5 - Germination (G) and normalized residual seed germination index (ING), radicle length (RL), percentage of radicle length inhibition (PIC-RL), and normalized residual radical root elongation index (IRE) of seedlings of the tomato 'Rasteiro Rio Grande', after five days of sowing; and pH of the solution of 1% and 10% of the culture medium of the fungal isolates

Treatments[a]		G (%)	ING[b]	RL (mm)	PIC-RL[c] (%)	IRE[b]	pH
1% detergent-dye Medium	Control 1	95.0a	-	16.3b	-	-	7.6a
	Control 2	95.0a	-0.01	21.0a	-29.4	0.29	6.5b
	LED 96/18	100.0a	-0.14	21.5a	-53.7	0.54	6.2b
	PS	85.0a	- 0.05	25.0a	-32.3	0.32	5.8c
	VC[d]	9.2%		25.8%			2.8%
10% detergent-dye medium	Control 1	98.0a	-	36.6a	-	-	8.6a
	Control 2	93.0a	-0.06	16.0b	57.3	-0.57	6.1b
	LED 96/18	93.0a	-0.03	19.9b	50.7	-0.51	6.0b
	PS	95.0a	-0.06	17.5b	46.0	-0.46	4.2c
	VC	9.3%		25.0%			3.7%

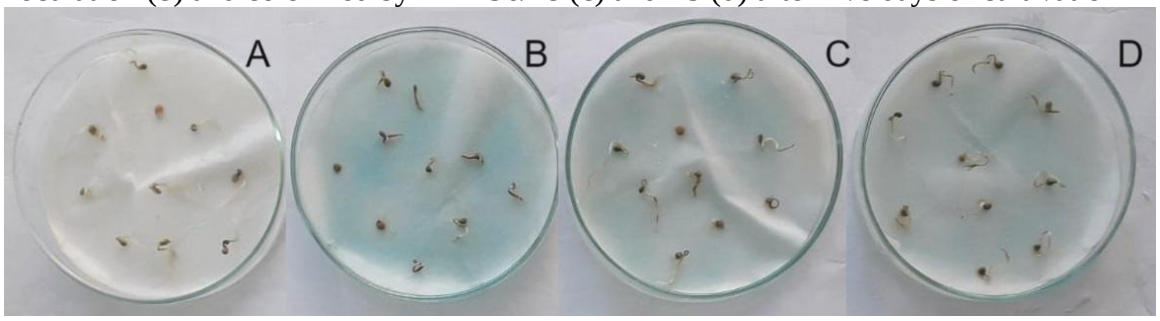
[a]Means followed by the same letter (lowercase in the column; uppercase in the row) do not differ from each other using the *Tukey* test at 5% probability; [b]IGN and IER toxicity classification as: low (0.00 to -0.25), moderate (-0.25 to -0.50), high (-0.50 to -0.75), very high (-0.75 to -1.00) and without toxicity (>0.00); [c]Positive values = inhibit mycelial growth and negative values = stimulate mycelial growth; and [d]VC = Variation coefficient. See Material and Methods for experimental details.
Source: research data.

Figure 4 - Germination of 'Rasteiro Rio Grande' tomato seeds in treatments: control 1 - autoclaved distilled water (a) and 1% solution of yeast extract culture medium with detergent (200µL L^{-1}) and methylene blue (40mg L^{-1}) in control 2 - without fungal inoculation (b) and colonized by LED 96/18 (c) and PS (d) after five days of cultivation



Source: the authors.

Figure 5 - Germination of 'Rasteiro Rio Grande' tomato seeds in treatments control 1 - autoclaved distilled water (a) and 10% solution of yeast extract culture medium with detergent (200µL L^{-1}) and methylene blue (80mg L^{-1}) in control 2 - without fungal inoculation (b) and colonized by LED 96/18 (c) and PS (d) after five days of cultivation



Source: the authors.

In this study, the use of a 1% detergent-dye medium solution (control 2, LED96/18, and PS) stimulated seedling radicle growth (PIC-RL = 29.4-53.7%), due to the absence of toxicity (IRE 0.29-0.54) in the medium. Increasing the solution concentration from 1% to 10% resulted in a decrease in radicle length of the seedlings compared to control 1, due to the high toxicity of the 10% detergent-dye solution in control 2 (IRE = -0.57) and LED96/18 (IRE = -0.51). However, in the 10% solution, PS reduced the toxicity to moderate levels (IRE = -0.46) compared to control 2 (IRE = -0.57) and exhibited lower inhibition of radicle length (PIC-RL = 46%) compared to the other treatments (Table 5).

The addition of detergent can increase the electrical conductivity of the medium due to the presence of salts, promoting the accumulation of toxic ions and reducing the absorption of water and nutrients by the seeds (Ehilen *et al.*, 2017). This may also have occurred in this study with the 10% detergent-dye solution, despite the average pH of 6.0 being within the recommended range for tomato plants (Miranda *et al.*, 2023).

In the solutions containing 1% and 10% detergent-dye medium in control 2 and LED96/18, a reduction in pH was observed compared to control 1 (autoclaved distilled water). PS also reduced the pH of the medium in the 1% and 10% solutions compared to the other treatments (Table 5).

Menezes *et al.* (2017) also observed a reduction in the pH of the culture medium with MB dye after the growth of *P. sanguineus* and *Pleurotus ostreatus*. The decrease in pH of the medium colonized by basidiomycetes is linked to the production of ammonium during the degradation of dyes by oxidative enzymes (Kanagaraj; Senthilvelan; Panda, 2015), whose optimal laccase activity occurs at an acidic pH for white rot fungi (Backes *et al.*, 2023; Mathur; Sanyal; Dey, 2021;).

In general, the basidiomycetes PS and LED 96/18 have bioremediation potential for apple-based detergent. However, the by-products of the fungal isolates' cultivation medium should be evaluated in future studies. Seed germination is not a reliable indicator of the toxicity of the detergent-dye culture medium after colonization by bioremediating microorganisms. Radicle length, on the other hand, may serve as a more accurate measure in this context. The seeds of the tomato variety ‘Rasteiro Rio Grande’ were sensitive enough to evaluate the phytotoxicity of the apple-based detergent medium with MB dye and may serve as an alternative to the lettuce and cucumber seeds commonly used in phytotoxicity tests.

4 Conclusion

The apple-based detergent inhibits the mycelial growth of the isolates LED96/18 and PS at concentrations greater than 200µL L^{-1}. The detergent-methylene blue medium inhibits the growth of PS but stimulated the growth of LED 96/18. Both PS and LED 96/18 promote the discoloration of

32.7% of the detergent-dye medium. The detergent-dye medium, with and without fungal colonization, did not inhibit the germination of 'Rasteiro Rio Grande' tomato seeds, but negatively affect root growth with an increase in concentration from 1% to 10% of the medium.

Isolates LED 96/18 and PS demonstrate *in vitro* bioremediation potential for the medium containing apple-based detergent and methylene blue.

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