Degradation of the herbicide paraquat by macromycetes isolated from southeastern Mexico

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Abstract Fifty-four macromycetes, isolated from southeastern Mexico, were used in order to evaluate their capacity for degradation and tolerance to the herbicide paraquat. Ten of these strains were capable of growing in a solid culture medium in the presence of 200 ppm paraquat. Subsequently, assays to evaluate the degradation of the xenobiotic in a liquid medium were carried out. Of the ten strains evaluated, three presented the highest levels of degradation of the compound, which were *Trametes pavonia* (54.2%), *Trametes versicolor* (54.1%) and *Hypholoma dispersum*. They presented the highest overall degradation percentage (70.7%) after 12 days culture. The presence of ligninolytic enzymes in these strains was evaluated. *H. dispersum* only presented aryl alcohol oxidase activity; however, with the data obtained, it was not possible to conclude whether this specific enzyme is responsible for paraquat degradation. The level of degradation obtained is above the one reported for *Pseudomonas putida*, one of the few reports on paraquat degradation. This is the first report on the contaminant degradation capacity of *H. dispersum*.

Keywords Pesticide · Ligninolytic enzymes · Mycoremediation · Paraquat

Introduction

Paraquat (*N*, *N'*-dimethyl-4, 4'-bipyridinium dichloride) is one of the most frequently used herbicides in agriculture. It is a cationic non-systematic, non-selective contact compound that instantaneously interferes with the photosynthesis processes of plants. Once the compound comes into contact with the plants' leaves, where the reaction occurs, it has an immediate effect (Kumar et al. 2016; PMEP 2017). The world health organization (WHO) estimates that the minimum lethal dose in humans is 35 mg/kg of concentrated paraquat (Tsai 2013). This compound results in neurological damage, renal and hepatic insufficiency, lacerations in mouth, nose and throat and severe damage to the lungs (Blanco-Ayala et al. 2014). In the soil, paraquat is absorbed by organic matter and depending on soil composition, can remain there for up to 5 years (Pateiro-Moure et al. 2009; Muhamad et al. 2011; Conde-Cid et al. 2017).

The transformation of paraquat in soil can take place through a variety of mechanisms, including photodegradation, chemical degradation, and microbial metabolism. Photo degradation reactions occur on the surface, a few centimetres within the soil (Rongchapo et al. 2016; Jaiswal et al. 2017). Soil surface photodegradation depends on the intensity of ultraviolet light within the wavelength range of 285–310 nm (Jafarinejad 2015; Zahedi et al. 2015).

There are several groups of microorganisms capable of using paraquat as a nitrogen source (Gondar et al. 2012; Devashree et al. 2014), including bacteria and actinomycetes. *Pseudomonas putida* was used to evaluate paraquat degradation using activated charcoal as an intermediary. In the absence of activated charcoal, paraquat degradation levels have reached 47.29%, while 95% degradation was attained when activated charcoal was

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present; this is due to the strong absorption of paraquat by this component (Kopytko et al. 2002).

The capacity of fungi to degrade paraquat has not yet been evaluated; however, there are many reports that indicate its potential in the biodegradation processes of other similar compounds. For example, ligninolytic or white rot fungi (WRF) have developed a unique non-specific enzymatic system that functions in an extracellular environment. This mechanism is based on the production of free radicals and allows participant enzymes to be catalytically active on a wide diversity of organic substrates, including those that are recalcitrant for the environment (Rhodes 2014). *Phanerochaete chrysosporium* and *Trametes versicolor* are among the most studied fungi and have been evaluated for the degradation of aromatic hydrocarbons, colourants and agrochemicals, among others (Fulekar et al. 2013; Ding et al. 2013; Bautista et al. 2015). WRF possess unique characteristics that allow the degradation of such compounds and the most studied enzymes from these organisms include laccases, manganese peroxide and arylalcohol oxidase (Rhodes 2014).

Another group of fungi that has been evaluated for degrading contaminants is the brown rot fungi (BRF). These fungi degrade cellulose and hemicellulose present in trees and plants and the production of free radicals by the Fenton reaction promotes the degradation of contaminant compounds, the most important including chlorofenol and 2,4,6, trinitrotoluene (Newcombe et al. 2002; Zhu et al. 2017). Other studies include the degradation of 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) by brown rot fungi such as *Fomitopsis pinicola* and *Daedalea dickinsii*. These fungi can transform DDT to DDE and DDD via the Fenton reaction (Purnomo et al. 2010a, b, 2011).

The present study aims to identify both the degradation and tolerance capacity of macromycetes isolated from the southern part of Chiapas Mexican state, to the paraquat herbicide. Furthermore, the study evaluates the influence of the ligninolytic enzymes system in the removal of this harmful compound from the culture medium.

Materials and methods

Biological material

Fifty-four strains of fungi, collected in the southeastern region of the state of Chiapas, Mexico, were used in this study. The collected fungi were isolated in a selective medium and taxonomically identified by using their particular macro and microscopic characteristics. All of them were subsequently deposited in the mycology collection of ECOSUR. The fungi were grown in Petri dishes on malt extract agar (MEA) and were maintained at 28 °C, until

complete colonization. Among them, ten strains were selected for the degradation in liquid medium experiments and were identified by using specific initiators for the ITS-4 and ITS-5 regions (Ma et al. 2009). The PCR products were analyzed in a capillary sequencer (MACROGEN, inc., Seoul, Korea) while the sequences acquired were analyzed by means of BLAST type algorithms provided by the National Centre of Biotechnology Information (NCBI BLAST). The strains tested were identified as: strain ECS-49 *Fomitopsis meliae*; ECS-50 *Trametes maxima*; ECS-56, ECS-58 and ECS-59 *Polyporus tricholoma*; ECS-66 *T. Villosa*; ECS-67 *T. Pavonia*; ECS-68 *T. Ellipsospora*; ECS-79 *T. versicolor* and ECS-705 *Hypholoma dispersum*.

Mycelial growth in the presence of paraquat

The tolerance to paraquat, by the 54 fungal strains that were previously isolated, was analyzed. In order to determine the radial extension rate (RER) of each strain in presence of the herbicide, an agar disc (5 mm diameter) with mycelium was placed in the centre of a Petri plate (9 cm diameter) with the minimum salt and peptone medium (MSPM, in g/l: potassium monobasic phosphate 5, magnesium sulphate 0.4, ammonium sulphate 10, sodium chloride 10, casein peptone 2, dextrose 5 and bacteriological agar 16). 200 mg/l paraquat were added (sterilized with Acrodisc® filters; 0.2 µm pore size). Three repetitions per treatment were carried out and the petri dishes were maintained at 28 °C. The controls were monitored using MSPM, without paraquat. Growth was measured daily until total colonization of the Petri dish (4–30 days, depending on the strain).

Liquid culture with paraquat

With regard to the degradation of paraquat in liquid medium, pre-inocula of the tolerant strains were prepared by adding eight agar with mycelium discs (5 mm diameter) to each 125 ml flask, containing 30 ml ME liquid medium. Flasks were maintained at 28 °C and were shaken constantly at 125 rpm for 10 days. Afterwards, mycelium produced in the flasks was filtered (under aseptic conditions) and crushed in 30 ml sterile distilled water until attaining a homogenous suspension. Subsequently, 1 ml of this suspension was used to inoculate 125 ml Erlenmeyer flasks with 30 ml MSPM. The material was agitated at 125 rpm and 28 °C. After 3 days incubation, paraquat (100 ppm final concentration) was added and flasks were incubated for 15 days total. All experiments were repeated three times.

Samples were taken at 3, 6, 9 and 12 days after seeding. Two controls were used: (1) heat-inactivated mycelium (by autoclaving the flasks at 121 °C, 30 min) was used as an

adsorption control and (2) a non-inoculated sample was included as an abiotic control for other types of losses. Both controls with same paraquat concentration were prepared according to each strain. All samples were filtered (Whatman filters, grade 6) and centrifuged (5000 rpm, 10 min), at 4 °C. Filtered mycelium was used to determine biomass through dry weight while supernatant was used to determine the remaining concentration of paraquat and the enzymatic activity (laccase, manganese peroxidase, lignin peroxidase, aryl alcohol oxidase and versatile peroxidase).

Measured variables

Radial Extension Rate (RER): this index measured daily mycelial growth along two perpendicular directions in each Petri dish, using a Vernier scale (Mitutoyo®, ±0.05) until the complete colonization of the dish. The RER was calculated by applying the linear growth function $Y = krX + C$ (where Y is distance and X is time); the results were expressed in mm/day.

Rate of growth (rg) was calculated for each strain through the following formula:

$$rg = \frac{[(\text{final biomass} - \text{initial biomass}) / (\text{final time} - \text{initial time})]}{}$$

Inhibition of mycelial growth (%) due to paraquat was determined by means of the following formula:

$$\text{Inhibition (\%)} = \left[\frac{(\text{RER}_{\text{control}}) - (\text{RER}_{\text{sample}})}{\text{RER}_{\text{control}}} \right] \times 100.$$

Strains presenting less than 50% inhibition were considered as tolerant to the herbicide and were used for later assays.

Determination of paraquat

In order to determine the concentration of paraquat, an enzymatic immunoassay was carried out, using the Paraquat Elisa Kit KA1424 (Abnova: Taipei, Taiwan). The test is based on the competition between paraquat contained in the sample and the horseradish peroxidase to combine the antibody directly against the paraquat, immobilized in the micropores of the Elisa dish.

Enzymatic activity

Laccase (Lac): A reaction blend with 2,6 dimethoxyphenol (DMP) 50 mM in a buffer solution of sodium 200 mM pH 5, H₂O and enzymatic extract was used. The reaction was measured over two minutes and DMP oxidation was monitored by an increase in absorbance at 469 nm ($\epsilon_{469} = 49,600/\text{M}/\text{cm}$) (Tinoco et al. 2001).

Aryl alcohol oxidase (AAO): This was determined through the oxidation of 25 Mm veratryl alcohol in a buffer solution of 500 Mm sodium phosphate pH 6, H₂O and enzymatic extract. The reaction was measured for two minutes at 310 nm ($\epsilon_{310} = 9300/\text{M}/\text{cm}$) (Guillén et al. 2000).

Manganese peroxidase (MnP): A reaction blend consisting of 20 mM manganese sulphate tetrahydrate (MnSO₄ 4H₂O), 100 mM sodium malonate pH 4.5, H₂O₂ 10 mM, H₂O and enzymatic extract. Oxidation was spectrophotometrically monitored for one minute at 270 nm ($\epsilon_{270} = 1159 \text{ M}/\text{M}/\text{cm}$) (Wariishi et al. 1992).

Lignin peroxidase (LiP): This was estimated using a reaction solution consisting of 50 mM veratrilic alcohol in a buffer solution of 200 Mm sodium tartrate pH 3, H₂O₂ 100 mM, H₂O and enzymatic extract. Changes in absorbance at 310 nm ($\epsilon_{310} = 9300/\text{M}/\text{cm}$) were monitored during 2 min (Tien and Kirk 1984).

Versatile peroxidase (VP): Determined by the oxidation of 1 Mm black five reactive in a buffer solution of 200 mM sodium malonate pH 3, H₂O₂ 10 mM, H₂O and enzymatic extract. Changes in absorbance at 598 nm ($\epsilon_{598} = 30,000/\text{M}/\text{cm}$) were monitored over a period of 5 min (Rodríguez et al. 2004).

In all cases, enzymatic unity (U) was defined as the quantity of enzyme required for the transformation of 1 µmol of substrate per minute.

Experimental design

A completely random design was used. All the experiments were carried out three times, under identical conditions. The IBM SPSS Statistics software was used for the statistical analysis; in all cases a multivariate or unifactorial ANOVA was applied and the data was analyzed through a comparison between the Tukey and Bonferroni tests, both with a significance level of 0.05.

Results and discussion

Collection and isolation of macromycetes

Seven collections were carried out in the south of the state of Chiapas in southeast Mexico (municipalities of Tapachula, Unión Juárez, Cacahoatán and Motozintla). A total of 126 specimens of macromycetes were collected and subsequently taken to the laboratory for identification, morphological analysis and isolation. A total of 54 strains were isolated (Table 1).

Table 1 Radial Extension Rate (mm/day) of the strains grown in MMSP with 200 mg/l paraquat at 28 °C

Strain	Control (mm/day)	Paraquat (mm/day)	Inhibition (%)	Group ^a	Strain	Control (mm/day)	Paraquat (mm/day)	Inhibition (%)	Group ^a
ECS-46	9.74d	4.82cde	50.5	NT	ECS-74	3.1efg	0	100	NG
ECS-47	7.1de	2.2fg	61.3	NT	ECS-75	5.1ef	0	100	NG
ECS-48	2.3 fg	0	100	NG	ECS-76	9.3cde	2.6efg	72.1	NT
ECS-49	3.1efg	1.7fg	45.1	T	ECS-77	2.8efg	0	100	NG
ECS-50	9.4cde	5.7cd	39.3	T	ECS-78	8.1de	3ef	63	NT
ECS-51	5.2ef	2.1fg	59.6	NT	ECS-79	5.9def	3.1ef	47.4	T
ECS-52	1.7 g	0.2h	88.2	NT	ECS-80	7.9de	1.9 fg	76	NT
ECS-53	9.2cde	3.2ef	65.2	NT	ECS-81	21.8b	4.1de	81.1	NT
ECS-54	4.4ef	2.3fg	47.7	NT	ECS-82	19.1bc	3.1ef	83.7	NT
ECS-55	5.5ef	1.9fg	65.4	NT	ECS-85	1.6 g	0	100	NG
ECS-56	3.5f	1.9fg	45.7	T	ECS-86	5.3ef	0	100	NG
ECS-57	4.4ef	1.3fg	70.4	NT	ECS-87	4.4ef	0	100	NG
ECS-58	12.5cd	6.4c	48.8	T	ECS-88	13.1bcd	4.4de	72	NT
ECS-59	22.9b	12.4b	45.8	T	ECS-89	14.2c	4.2de	70.4	NT
ECS-60	8.2de	4.1de	50	NT	ECS-90	18.4bc	0	100	NG
ECS-61	4.8ef	0	100	NG	ECS-91	16.1bc	1.1g	93.1	NT
ECS-62	6.3e	1.5fg	76.1	NT	ECS-92	2.1 fg	0	100	NG
ECS-63	7.6de	2.3fg	69.7	NT	ECS-93	22.9b	4.6cde	80	NT
ECS-64	8.2de	3.6e	56.1	NT	ECS-94	4.2ef	0	100	NG
ECS-65	12.1 cd	0	100	NG	ECS-95	10d	0	100	NG
ECS-66	35.2a	18.3a	48.1	T	ECS-96	2.6efg	1.4fg	80	NT
ECS-67	19.6bc	10.7bc	45.4	T	ECS-97	14.9c	2.5efg	83.2	NT
ECS-68	8.1de	5.5cd	32.1	T	ECS-100	4ef	0	100	NG
ECS-70	8.3de	2.6f	68.7	NT	ECS-703	1.5g	0	100	NG
ECS-71	11.3cd	0	100	NG	ECS-705	1.7g	1.3fg	23.5	T
ECS-72	2.2fg	1.1g	50	NT	ECS-706	22.1b	4.9d	77.8	NT
ECS-73	4.1ef	1.6fg	60.9	NT	ECS-707	2.3fg	1.1g	52.1	NT

Incubation 15 days

Mean of three repetitions

MSPM minimum salt and peptone mediums

^a T tolerant strain (<50% inhibition), NT non-tolerant strain (≥50% inhibition) and NG strain that did not experience growth in the presence of paraquat (100% inhibition). The control corresponds to the grown sample without paraquat. Different small letters in the same column indicate that there are significant differences between treatments, according to the Tukey test ($\alpha = 0.05$)

Evaluation of paraquat tolerant strains

Growth rate in the presence of the herbicide and the resulting inhibition in each of the strains evaluated is presented in Table 1. Growth fluctuated between 0 (16 strains) and 18.3 mm/day (strain ECS-66). Inhibition by paraquat varied between 23.5% (strain ECS-705) and 100% (16s-trains). The statistical analysis determined significant differences between strains ($P = 0.05$); strain ECS-66 obtained the highest RER, for both with or without paraquat (18.3 and 35.2 mm/day, respectively).

Consistent with the level of herbicide tolerance established for this study (<50% inhibition) three main groups were evident: (a) 10 strains tolerant to paraquat (T), (b) 29

strains not tolerant to paraquat (NT), presenting an RER on paraquat containing medium that was less than half the one observed on the control without paraquat, and (c) strains presenting no growth in presence of paraquat (NG). The ten strains that were tolerant to paraquat were selected for subsequent degradation assays.

Paraquat degradation assays

Table 2 displays the increase in biomass in each of the strains cultivated in MSMP with 100 ppm paraquat, at 28 °C and 120 rpm during 12 days. At the start of the experiments, all the samples contained an initial biomass concentration of 1 g/l. *F. meliae* ECS- 49, *T. maxima* ECS-

Table 2 Growth rate and biomass production of the selected strains, grown in MSPM with 100 ppm of paraquat, at 28 °C, 120 rpm during 12 days

Strain	Growth rate (g/day)	Biomass (g/l)
<i>Fomitopsis meliae</i> (ECS-49)	0.22a	4.10b
<i>Trametes maxima</i> (ECS-50)	0.23a	3.50c
<i>Polyporus tricholoma</i> (ECS-56)	0.22a	4.20b
<i>Polyporus tricholoma</i> (ECS-58)	0.33c	4.23b
<i>Polyporus tricholoma</i> (ECS-59)	0.29bc	5.02b
<i>Trametes villosa</i> (ECS-66)	0.41d	5.30b
<i>Trametes pavonia</i> (ECS-67)	0.42d	4.82b
<i>Trametes ellipsospora</i> (ECS-68)	0.34c	4.79b
<i>Trametes versicolor</i> (ECS-79)	0.34c	5.89a
<i>Hypholoma dispersum</i> (ECS-705)	0.57e	6.20a

Identical small letters in the same column indicate that there are no significant differences between strains, according to the Tukey's test ($P < 0.05$)

Fig. 1 Degradation of 100 ppm paraquat by different fungal strains in a liquid culture at 28 °C, 120 rpm, and 3, 6, 9 and 12 days culture. C₁ MMSP without inoculation; C₂ heat-inactivated mycelium 3; both controls were treated as with the samples (100 mg/l de paraquat). Same small letter in two bars indicate that there are no significant differences between strains on time 12, according to the Tukey's test ($P < 0.05$). The experiments were triplicated

50 and the three strains of *P. tricholoma* (ECS-56, 58 and 59) did not demonstrate an increase in biomass after day 6. Taking into consideration that the addition of paraquat took place on day 3, the lack of growth of these strains could be due to inhibition caused by the presence of paraquat (results not shown). In contrast, the strains *T. villosa* ECS-66, *T. pavonia* ECS-67, *T. ellipsospora* ECS-68, *T. versicolor* ECS-79 and *H. dispersum* ECS-705, experienced an increase in biomass. *T. versicolor* ECS-79 and *H. dispersum* ECS-705 presented the largest amount of biomass at 12 days growth (5.89 and 6.2 g/l respectively). *T. maxima* ECS-50 demonstrated the lower growth with 3.5 g/l biomass produced over 12 days. *H. dispersum* ECS-705 demonstrated the highest rate of growth, (0.57 g/day, $\alpha < 0.05$) while the strains *P. tricholoma* ECS-56, *F. meliae* ECS-49 and *T. maxima* displayed the lowest ones (0.22, 0.22 and 0.23 g/day respectively, statistical group “a”, $\alpha < 0.05$).

Figure 1 shows the concentration of residual paraquat for each fungi strain tested at this point. Degradation percentages vary between 15 and 22% for *F. meliae* ECS-49, *T. villosa* ECS-66, *T. maxima* ECS-50 and the three strains of *P. tricholoma* ECS-56, ECS-58 and ECS-59, coinciding with low biomass production for at least five of them. Growth inhibition is reflected in a low percentage of paraquat degradation. The degradation percentages for *T. ellipsospora* ECS-68 and *P. tricholoma* ECS-56, ECS-58 and ECS-59 were also around 28–29%, while they were higher for the strains *T. pavonia* ECS-67, *T. versicolor* ECS-79 and *H. dispersum* ECS-705 (54%–70% after 12 days growth). *H. dispersum* ECS-705 achieved the greatest degradation of paraquat with 70.7% (29.23 ppm) after 12 days growth.

The data obtained does not reveal if the compound was totally degraded and incorporated into the energetic metabolism of the fungi; however, two controls were

Fig. 2 Laccase activity of the extracellular culture medium (substrate DMP, pH 5 at 26 °C) during paraquat degradation. Activity was measured on day 6, 9 and 12 of the culture. Same small letters in each bar indicate that there are no significant differences between strains after the culture was 12 days old, according to the Tukey test ($P < 0.05$). The experiments were triplicated and the mean comparisons for each strain were shown

Fig. 3 Activity of the manganese peroxidase enzyme in the extracellular culture medium (H_2O_2 substrate with $MnSO_4$), pH 4.5 at 26 °C during paraquat degradation. Activity was measured at day 6, 9 and 12 of the culture. Identical small letters in each bar indicate that there are no significant differences between strains after the culture was 12 days old, according to the Tukey test ($P < 0.05$). The experiments were triplicated

Fig. 4 Activity of the aryl alcohol oxidase (AAO) enzyme from the extracellular culture medium (veratrylic alcohol substrate, pH 6, 26 °C) during paraquat degradation. Activity was measured at day 6, 9 and 12 of the culture. Identical small letters in each bar indicate that there are no significant differences between strains after the culture was 12 days old, according to the Tukey test ($P < 0.05$). The experiments were triplicated

included in the experiments: C1 corresponding to the culture medium with 100 mg/l paraquat without mycelium, and C2 presenting sterile mycelium with paraquat, in order to identify the possible adhesion of paraquat to the fungal structures as well as eliminating the possibility of a decrease in the concentration of the compound due to physical (shaking, evaporation, light, absorption) or chemical processes, through interaction with the culture medium. In both controls, the concentration of paraquat remained constant over time, demonstrating that its decrease in the treatments corresponded to degradation by the strains tested. Thus, it is possible to observe the capacity of the strains in transforming the compound, although the degradation pathway remains to be clarified. Several reports refer to the presence of intermediaries that have been analyzed during the photocatalytic degradation of paraquat, such as pyridine paraquat, protonated dipyridine paraquat and 4-carboxy-1-methyl pyridine (Cantavenera et al. 2007). The metabolites associated with the degradation of paraquat by microorganisms or macromycetes have yet to be identified. In the case of the oxidation pathway, there are no reports on the intermediaries produced during the degradation of the compound (Florêncio et al. 2004; Cantavenera et al. 2007; Zahedi et al. 2015).

The above-mentioned samples were also used for the measurement of enzymatic activity to ascertain any possible association with paraquat degradation.

Five ligninolytic activities were determined: Lac, MnP, AAO, LiP and VP. In the majority of the strains, only the first three enzyme activities were observed.

Both LiP and VP did not present activity in any of the extracts analyzed. *T. versicolor* ECS-79 presented the highest Lac activity (Fig. 2) (65.78 U/ml) on day 12. The strain *H. dispersum* ECS-705, which presented the highest percentage of paraquat degradation, did not demonstrate any Lac activity. Manganese peroxidase activity (Fig. 3), reported as responsible for several processes of xenobiotic degradation, also demonstrated degradation percentages between 28 and 50% for *T. pavonia* ECS-67, *T. ellipsospora* ECS-68 and *T. versicolor* ECS-79. *H. dispersum* ECS-705 did not present Lac and MnP activity. Finally, AAO activity was evident in all isolates (Fig. 4); AAO presented the highest activity in *P. tricholoma* ECS-56 and *T. villosa* ECS-66. These strains did not demonstrate a considerable degradation of paraquat; however, *T. pavonia* ECS-67 and *T. ellipsospora* ECS-68 presented acceptable levels of this enzyme with values of 16 and 24 U/ml respectively, coinciding with paraquat degradation. AAO was the only enzymatic activity identified for *H. dispersum* ECS-705, the strain that presented the highest percentage of paraquat degradation, although the level of activity was intermediate when compared with the other isolated strains.

It remains to be established whether this enzyme is responsible for paraquat degradation.

Discussion

Fifty-four strains of macromycetes were analyzed for paraquat degradation. Ten of those strains were considered as tolerant to paraquat. It is worth to point out the high concentration of paraquat (200 mg/l) used for the solid medium experiments. Anasonye et al. (2015) found that the growth of the fungi *Phanerochaete chrysosporium* was inhibited at concentrations of 24 ppm TNT when malt extract agar was used as a base medium. In 2005, Mukherjee and Mittal (Mukherjee and Mittal 2005), clearly identified inhibition of *Aspergillus terreus* and *Cladosporium oxysporum*, when exposed to the insecticide endosulfan at concentration of 100 µg/l.

However, the quantities used in degradation experiments vary considerably, depending on the compound used, fluctuating between 1 and 500 mg/l (Kästner et al. 1999; Sasaki et al. 2005; Lee et al. 2014). *Aspergillus flavus* was capable of degrading 800 ppm (99.6%) of glyphosate in a liquid medium after 16 days growth (Eman et al. 2013). The above implies that the concentrations of contaminants that fungi can tolerate, varies significantly. In nature, when a herbicide is added to the soil, microorganisms demonstrate different response times; a part of the soil microbiota is poisoned, resulting in cellular lysis (Bending et al. 2002). However, several microorganisms are resistant and tolerant to the contaminant, increasing their biomass in order to reduce competition (Gondar et al. 2012; Rongchapo et al. 2016). Only a few of them can grow and degrade the compound efficiently. Many of the strains used in this study present similar patterns of intoxication, tolerance and degradation when in contact with the herbicide paraquat.

Three strains demonstrated the highest level of degradation in the experiments carried out in liquid mediums: *T. versicolor* ECS-79 and *T. pavonia* ECS-67 presented 55% degradation, while *H. dispersum* ECS-705 presented 71% degradation after 15 days of growth. We have no knowledge of any reported study on paraquat degradation by macromycetes; there are only two reports concerning microorganisms, one is with *P. putida* and the other with *Lipomyces* sp. *P. putida* attained 47.29% degradation in a culture medium that was free of an absorbent agent used to increase percentage degradation to a maximum of 95% (Kopytko et al. 2002). In contrast, the yeast *Lipomyces* sp. attained 100% degradation of paraquat in a liquid culture after 3 days incubation; however, the contaminant concentration was only 27 ppm. When the paraquat concentration was increased two fold (54 ppm), biomass and paraquat degradation decreased notably to less than

10% (Hata et al. 1986). The degradation obtained by *H. dispersum* ECS-705 (71%) is less than previously reported for *P. putida*. In our study, the concentration of paraquat was five times greater. A similar effect was reported by Hua Fang (Fang et al. 2008), for *Verticillium* sp on chlorpyrifos, which experienced a degradation percentage of 88.5%, although the paraquat concentrations were much lower (50 mg/l). This also coincides with a study by Da Silva Coelho (da Silva Coelho et al. 2010), where levels of diuron and bentazon degradation were 74 and 61%, respectively, after 10 days culture, under conditions similar to those evaluated in this study.

The strain ECS-79 was identified as *Trametes versicolor*. There are several reports on the degradation potential of this species and together with *P. chrysosporium*, is one of the most frequently studied macromycete species regarding the degradation of xenobiotic compounds such as aromatic polycyclic hydrocarbons (anthracene and naphthalene) (Marco-Urrea et al. 2010; Jelic et al. 2012; Wolfand et al. 2016; Kamei 2017). This paper represents the first study that analyses the breakdown of the herbicide paraquat by both of these strains. There is only one study of the strain ECS-67, *T. pavonia*; examining the degradation of ferulic acid (Tanruean et al. 2013). Other studies have analyzed the productive capacity of ligninolytic enzymes such as laccase and manganese peroxidase (Kenkebashvili et al. 2012; Singh et al. 2013; Sari et al. 2016). This is associated with the degradation of recalcitrant compounds and coincides with the observations made in this study where this strain demonstrated a high percentage of degradation.

This is the first study on the potential for xenobiotic degradation by the species *H. dispersum*. Fungi of the genus *Hypholoma* play an important role in decomposition of woody materials, in particular wood polymers such as lignin, cellulose and hemicellulose. Species in this genus are easily recognizable because the dark spores create a distinctive greenish effect on the yellow cap underside. *Hypholoma* means mushrooms with threads, because of the threads-like veil that connects the cap to the stem when young (Parker 1933; Cortez and Silveira 2007).

H. fasciculare is the only species belonging to the *Hypholoma* genus that has been studied for the degradation of chlorpyrifos and the pesticides atrazine and diuron. It also has shown ability to degrade over 88% of terbutylazine, a triazine herbicide in liquid culture after 42 days (Gramss et al. 1999; Bending et al. 2002).

Only one of the three strains that presented the highest degradation percentages presented an increase in laccase enzyme activity (*T. versicolor* ECS-79). This is consistent with a study by Mougin (Mougin et al. 2002), who also reported an increase in laccase activity for *T. versicolor* ECS-79, in the presence of different environmental contaminants. However, the absence of this enzyme in the

strain *T. pavonia* ECS-67, contrasts with the results presented by Arana-Cuenca (Arana-Cuenca et al. 2004), who reported the presence of laccase in this species. The absence of Lac and other ligninolytic enzymes during the assays does not provide conclusive proof that these strains are incapable of producing them. The lack of these enzymes could be due to diverse factors, primarily culture medium composition, in addition to time and incubation conditions (Saparrat et al. 2002; Zucca et al. 2011).

The ligninolytic enzymes that constitute the fungi degradation systems vary for each species. Some rot fungi selectively degrade lignin; consequently, these species lack one or two ligninolytic enzymes (Yang et al. 2013; Rouches et al. 2016). It should be emphasised that the conditions evaluated at the laboratory level are different from those found in nature. The synthesis and secretion of ligninolytic enzymes to the extracellular medium occur under conditions of limited levels of carbon or nitrogen; however, this is suppressed by agitation in a submerged liquid medium (Elisashvili et al. 2009; Zhuo et al. 2017). Therefore, the precise conditions for each species and strain must be assessed in order to make the most of their potential for degradation.

Activity could only be detected in three of the five ligninolytic enzymes monitored and only in the case of the strain *T. versicolor* ECS-79, did there appear to be a relationship between paraquat degradation and the production of these enzymes. However, the strain that presented the highest percentage of degradation, demonstrated only a low level of aryl alcohol oxidase activity. Jauregui, Kües and Shin (Jauregui et al. 2003; Kües 2015; Shin et al. 2017), found the presence of an intracellular system of enzymes that corresponds to the cytochrome P-450 system, which could be linked to contaminant degradation. This system may explain paraquat degradation in the absence of several evaluated ligninolytic enzymes; however, results obtained so far do not provide sufficient evidence to conclude if this is attributable to this type of intracellular system.

In summary, the strain *H. dispersum* ECS-705 presented the highest degradation of the herbicide. This coincides with the greatest production of biomass (6.2 g/l) and high speed growth when paraquat is present (0.57 g/day); however, when ligninolytic activity was analyzed, only AAO activity was observed. The strain *T. versicolor* ECS-79 attained 54.9% paraquat degradation and presented three of the five evaluated activities, with a note worthy laccase activity of 65.8 U/ml. This strain also demonstrated the highest MnP activity, with 55.7 U/ml.

The strains that demonstrated high levels of degradation capability have potential as bioremediators of the herbicide paraquat. In future studies, the degradation and culture conditions for the strain *H. dispersum* ECS-705 could be optimized in order to increase the degradation rate and

favour the production of enzymes that may be involved in the degradation process. Furthermore, it is essential that other types of emerging contaminants be evaluated.

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Compliance with ethical standards

Conflict of interest Authors report no conflict of interest.

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