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Degradation of 4-nitrophenol by the white-rot polypore *Trametes versicolor*

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ABSTRACT

The ability of *Trametes versicolor* strain BAFC 2234 to degrade 4-nitrophenol *in vivo* and *in vitro* was evaluated. *T. versicolor* grew in the presence of 0.5 mM 4-nitrophenol and degraded 98.4% of this toxic compound in less than 96 h. The strain secreted different ligninolytic oxidoreductases such as laccase, Mn-peroxidase and versatile peroxidase. Substantial conversion of nitrophenol, a typical high-redox potential phenolic substrate, is reported for versatile peroxidase for the first time; 2,4-dinitrophenol and a dimer were identified as products. *T. versicolor* immobilized on natural sponge-like material from *Luffa aegyptiaca* removed 97% of 4-nitrophenol (1 mM) over a period of 72 h. 4-Nitrophenol phytotoxicity decreased noticeably after fungal treatment.

1. Introduction

Nitro-aromatic compounds are broadly distributed contaminants, by-products of the industrial manufacturing of insecticides, herbicides, fungicides, dyes and explosives. These compounds tend to accumulate in the environment due to their persistence, high toxicity and water solubility (Spain, 1995). 4-Nitrophenol is a common pollutant that enters the environment through industrial release and degradation of parathion-based pesticides (Zhang et al., 2012). Due to the nitro group these compounds are more resilient to biodegradation than their non-substituted analogs. As a consequence, wastewater containing these toxic compounds needs careful treatment before discharge into the receiving water bodies (Santos and Linardi, 2004). Conventional methods for dephenolization are often high-priced, do not assure complete purification, can render dangerous by-products and are frequently applicable only to a restricted concentration range.

The aim of biotechnology is to develop efficient, cost effective and environmentally safe bioremediation methods to replace or suitably complement existing technologies as well as to provide unique solutions for the remediation of contaminated waste streams (Ryan et al., 2007). Various degrading microorganisms have been extensively studied in order to develop and improve the technological processes of biodegradation and biotransformation. Most of the information on metabolism of nitrophenols has been reported for bacteria (Venkateswarlu and Spain, 1998; Zhang et al., 2012). The respective knowledge about filamentous fungi is limited (Teramoto et al., 2004; Tripathi et al., 2011; Yemendzhiev et al., 2012; Büttner et al., 2015).

White-rot basidiomycetes are capable of mineralizing even xenobiotic aromatic compounds due to their nonspecific extracellular oxidative ligninolytic enzyme system. The main extracellular enzymes participating in lignin degradation are lignin peroxidase (LiP, EC 1.11.1.14), Mn-peroxidase (MnP, EC 1.11.1.13) and copper-containing polyphenol oxidase laccase (EC 1.10.3.2). The versatile peroxidases (VP, EC 1.11.1.16) are new representatives of ligninolytic peroxidases, with hybrid functional properties of both LiPs and MnPs. In the last years novel peroxidases have been discovered, for example the dye-decolorizing peroxidase (DyP, EC 1.11.1.19). These enzymes function together with H_2O_2-producing oxidases such as glyoxal oxidase (GLOX, EC 1.2.3.1) and aryl alcohol oxidase (AAO, EC 1.1.3.7), and secondary metabolites (Liers et al., 2013).

Processes that utilize growing, immobilized cells are more

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suitable for biodegradation studies than free cells in suspension, since immobilization allows the use of fungal cells repeatedly or even continuously and it facilitates liquid–solid separation. Moreover, immobilized cells usually have a higher level of activity and are more resistant to environmental changes in pH or temperature, or to the contact with toxic chemicals (Rodríguez Couto, 2009). Immobilization of white-rot fungi has been successfully used for bioremediation approaches in different areas including wastewater treatment (Perullini et al., 2010) and remediation of toxic chemicals such as dyes (Rodríguez Couto, 2009) and phenols (Carabajal et al., 2014, in press).

Here, we used the white-rot fungus *T. versicolor*, for a nitrophenol degradation study. The capability of *T. versicolor* for xenobiotic biodegradation is widely recognized (Marco-Urrea et al., 2009; Yemendzhiev et al., 2012). *T. versicolor* BAFC 2234 was chosen among twenty five isolates of Argentinean white-rot fungi by its tolerance to grow and degrade phenolic compounds (Carabajal et al., 2014, in press). The purposes of this study were (i) to examine the ability of *T. versicolor* strain BAFC 2234 to degrade 4-nitrophenol *in vivo*, (ii) to evaluate the ability of *T. versicolor* immobilized on natural sponge to degrade 4-nitrophenol and (iii) to evaluate the degradation *in vitro* with the purified enzymes produced by this strain.

2. Materials and methods

2.1. Fungal strain

T. versicolor strain (BAFC 2234) was used for all experiments and is deposited in the Culture Collection of the Department of Biological Sciences, Faculty of Exact and Natural Sciences, University of Buenos Aires (BAFC). Stock cultures were maintained on 2% malt extract agar (MEA) at 4 °C.

2.2. In vivo transformation of nitrophenol

In vivo transformation studies of 2-, 3- and 4-nitrophenol were carried out in agitated liquid cultures (100 rpm at 24 °C) in glucose-asparagine (GA) medium (Levin and Forchiassin, 2001). Nitrophenol (0.5 mM final concentration) was added 7 days after inoculation. 250-ml Erlenmeyer flasks containing 80 ml of the medium were inoculated with mycelium pre-grown on MEA plates at 25 °C for 10–12 days. The content of an agar plate was homogenized in 80 ml of sterile water and the mycelial suspension was used to inoculate liquid cultures (5% v/v). Controls contained *T. versicolor* grown in GA medium without nitrophenol or non-inoculated GA medium with nitrophenol. Samples (1 ml of the culture liquid) were taken every 2–3 days. Each sample was centrifuged (10,000 × g for 5 min) at 4 °C and filtrated. The supernatant was used to quantify nitrophenol concentration by high performance liquid chromatography (HPLC). Cultures supplemented with 4-nitrophenol were chosen for further assays. Nitrophenol supplemented cultures were harvested after 12 days of incubation and biomasses were quantified by dry mass measurement after filtration through glass-fiber filters and drying of the mycelial pellets to a constant weight at 65 °C. In order to investigate the mechanisms involved in nitrophenol degradation, piperonyl butoxide (PB), a cytochrome P-450 inhibitor, was added to selected cultures in a final concentration of 3 mM and enzyme production and nitrophenol concentration were determined.

2.3. 4-Nitrophenol transformation by T. versicolor immobilized on Luffa aegyptiaca sponge

To further evaluate fungal 4-nitrophenol transformation, cultures of *T. versicolor* immobilized on the endocarp of *Luffa aegyptiaca* as natural sponge-like carrier material were grown in a complex liquid medium TJM (tomato juice medium), which consisted of tomato juice (Albi & Co., Germany) and distilled water (50:50 v/v). To stimulate enzyme production, $MnCl_2$ and $CuSO_4$ were added prior to autoclaving (final concentration 250 μM). 500-ml Erlenmeyer flasks containing 200 ml of TJM and 5 pieces of the carrier (150 × 250 mm) were incubated on a rotary shaker at 100 rpm and 24 °C. Cultures were harvested by filtration at the 19th day of incubation and the immobilized mycelium was then transferred to a fresh nitrophenol solution (1 mM). 4-Nitrophenol transformation was followed under static conditions over a period of 5 days at 24 °C. Heat-inactivated cultures (autoclaved) served as controls to determine abiotic loss and sorption of nitrophenols to the fungal mycelium and the carrier surface.

2.4. Phytotoxicity bioassay

The phytotoxicity of 4-nitrophenol and its derivatives were determined according to the method described by Zucconi et al. (1981) using *Lepidium sativum* seeds incubated for 48 h at 25 °C with either a fresh nitrophenol solution (1 mM), or the nitrophenol solution treated for 5 days with *T. versicolor* immobilized on the sponge carrier. The germination index (GI) was calculated according to the expression: GI = [(G/Go)/(L/Lo)]*100, where Go and Lo are, respectively, the percent germination and radicle growth of the control.

2.5. Enzymatic assays

Laccase activity was determined by following the oxidation of ABTS (2,2-azino-bis 3-ethylbenzo-thiazoline-6-sulfonate) by 50 μl enzyme sample in 50 mM citrate-phosphate buffer (pH 4.5, 25 °C) at 420 nm ($\varepsilon_{420} = 36{,}000\ M^{-1}\ cm^{-1}$) (Bourbonnais et al., 1995). The activity of Mn-peroxidase (MnP) was measured at 270 nm ($\varepsilon_{270} = 11{,}590\ M^{-1}\ cm^{-1}$) by following the formation of Mn^{3+} malonate complexes in 50 mM sodium malonate buffer (pH 4.5) containing 0.5 mM $MnCl_2$, 0.2 mM H_2O_2, and 50 μl enzyme sample (culture liquid). The reaction was initiated by adding H_2O_2 (Wariishi et al., 1992). Lignin peroxidase activity (LiP) was measured following the oxidation of veratryl alcohol to veratraldehyde at 310 nm (Kirk et al., 1986; $\varepsilon_{310} = 9300\ M^{-1}\ cm^{-1}$) in 50 mM sodium tartrate buffer (pH 3.0), 5 mM veratryl alcohol, 0.2 mM H_2O_2, and 100 μl enzyme sample; the reaction was started with H_2O_2. Versatile peroxidase activity (VP) was measured at 598 nm ($\varepsilon_{598} = 47{,}600\ M^{-1}\ cm^{-1}$) by monitoring the conversion of the azo dye Reactive Black 5 in 50 mM sodium tartrate buffer (pH 3.0) containing 0.1 mM Reactive Black 5 and 0.2 mM H_2O_2 (Heinfling et al., 1998). The enzymatic activity was expressed in international units (U) defined as the amount of enzyme required to convert 1 μmol of substrate or produce 1 μmol of product per min ($\mu mol\ min^{-1}$).

2.6. Nitrophenol determination

Nitrophenol concentrations were quantified by HPLC.An Agilent HPLC system; (Agilent, 1200 series Waldbronn, Germany) equipped with a diode array detector and a diphenyl column (100 mm × 2.0 mm, Varian, Darmstadt, Germany) was used for analyses. Samples (1–1.5 ml) were centrifuged and transferred to 1.5-ml HPLC vials. The chromatography was performed under isocratic conditions using a mixture of phosphoric acid (20 mM) and acetonitrile (82:18 v/v) thermostatically controlled (50 °C) at a flow rate of 0.4 ml min^{-1}. Eluted analytes were detected at 280 nm.

2.7. 4-Nitrophenol conversion in vitro

To investigate the participation of ligninolytic oxidoreductases in the degradation of 4-nitrophenol, *in vitro* experiments were carried out in reaction mixtures (500 μl) containing 4-nitrophenol solution (0.25 mM) and sodium citrate buffer (250 μM; pH 4.5), sodium malonate buffer (250 μM; pH 4.5) or sodium tartrate buffer (50 mM; pH 3.0), for conversion with the enzymes laccase, MnP or VP respectively. The laccase reaction was started by the addition of the purified *T. versicolor* laccase (1 U ml^{-1} final concentration); and the MnP and VP reactions by adding respectively the purified enzymes (1 U ml^{-1} final concentration) and H_2O_2 (500 μM final concentration). The total enzyme activity was divided in three equal amounts; each of one was added every 3 h (as well as the H_2O_2) and stirred at room temperature. Corresponding controls did not contain enzyme. Reactions were stopped after 9 h by the addition of trichloroacetic acid (TCA) (final concentration of 5%). The reaction was analyzed by high performance liquid chromatography (HPLC) using an Agilent Series 1200 instrument equipped with a diode array detector (DAD) and negative-ion electrospray ionization mass spectrometry (ESI/MS) (Agilent Technologies Deutschland GmbH, Böblingen, Germany). Reaction mixtures were analyzed on a reverse phase Luna C18 column (150 mm × 2 mm, Phenomenex, Aschaffenburg, Germany), at 0.35 ml min^{-1} and 40 °C with aqueous 0.01% v/v ammonium formate (pH 3.5)/acetonitrile, 95:5 for 5 min, followed by a 25-min linear gradient to 100% acetonitrile. Extracellular oxidoreductases of *T. versicolor* (VP, MnP and laccase) were produced in TJM and purified by different steps of ion exchange chromatography using the strong anion exchanger Q sepharose and Mono Q (GE Healthcare, Freiburg, Germany). The isolation of proteins was performed using an ÄKTA Explorer system (GE Healthcare, Freiburg, Germany). 10 mM Na acetate with different pHs (4.5–6.0) and NaCl (1 or 2 M) were used as eluents. The purity of the enzyme preparations was proven by reducing SDS-PAGE (Invitrogen, Karlsruhe, Germany) (Carabajal et al., 2013). All chemicals used were obtained from Sigma–Aldrich (Weinheim, Germany) or Merck (Darmstadt, Germany) in highest purity available.

2.8. Statistical analysis

Experiments were carried out in triplicate. The results are expressed as mean values with standard deviation (SD); variance analysis was carried out using the ANOVA program followed by Tukey's test using the Statistica 7.0 program. Differences at $p < 0.05$ were considered to be significant.

3. Results

3.1. In vivo transformation of nitrophenol

T. versicolor BAFC 2234 was able to transform 2-, 3- and 4-nitrophenol in liquid cultures with slightly different efficiency (95%, 92% and 98.4% for 2-, 3- and 4-nitrophenol respectively). Among them, 4-nitrophenol was chosen for further investigations. The time courses of 4-nitrophenol degradation and the enzyme activities secreted in the medium supplemented with 4-nitrophenol are shown in Fig. 1A and B, respectively. *T. versicolor* grew well in the presence of 0.5 mM nitrophenol (70 mg l^{-1}) and only minor differences in mycelial biomass were found (4.19 ± 0.13 g l^{-1} vs. 3.57 ± 0.15 g l^{-1}). The strain degraded 98.0% and 98.4% of the pollutant within 48 and 96 h respectively. No changes were observed on fungal degradation ability when the cytochrome P-450 inhibitor piperonyl butoxide (3 mM) was added (Fig. 1A). Abiotic nitrophenol controls showed no significant variation in resulting nitrophenol concentration (0.5 ± 0.043 mM). The most frequent ligninolytic enzyme activity in cultures of *T. versicolor* supplemented with nitrophenol was laccase (286.5 U l^{-1}), the activity of which concomitantly increased with the decrease of nitrophenol concentration. MnP or LiP activities were not detectable in the culture liquid (data not shown). In control cultures without nitrophenol, maximum laccase activity (316 U l^{-1}) was detected in the last sampling day (Fig. 1B). VP activity was only detectable in nitrophenol supplemented cultures and reached values of 2.8 U l^{-1}.

3.2. 4-Nitrophenol transformation by T. versicolor immobilized on L. aegyptiaca sponge

T. versicolor immobilized on a sponge-like carrier prepared from fruits of *L. aegyptica* could remove up to 97% of 4-nitrophenol (1 mM) on the aquatic solution after 3 days and 100% after 5 days (Fig. 2). *T. versicolor* cultures produced laccase (up to 450.8 U l^{-1}) as the main ligninolytic enzyme during the conversion process. However, in this case *T. versicolor* secreted also MnP (up to 45.4 U l^{-1}) and VP (up to 2.09 U l^{-1}) but no LiP. Changes in nitrophenol concentration were not detected in heat inactivated cultures.

3.3. Phytotoxicity bioassay

The germination index was used to test the phytoxicity of nitrophenol with *L. sativum* seeds. This index was equal to zero for 1 mM nitrophenol and in control solutions (heat-inactivated cultures). *T. versicolor* was able to decrease the phytotoxicity of a nitrophenol solution after appropriate treatment. These seeds showed a germination index of 72% (±5.7).

3.4. In vitro transformation of 4-nitrophenol

4-nitrophenol transformation with the purified laccase, MnP and VP of *T. versicolor* was investigated to prove their possible involvement in nitrophenol conversion. Different degradation percentages were obtained after 9 h with the three ligninolytic enzymes. Although laccase was the main enzyme detected in liquid cultures supplemented with nitrophenol, the purified enzyme was not able to convert this pollutant. On the other hand, MnP reduced up to 40% of 4-nitrophenol concentration (data no shown). VP showed the highest efficiency in the transformation of the pollutant, it degraded 60% of 4-nitrophenol (35 mg l^{-1}) (Fig. 3). HPLC-MS studies revealed that the VP biotransformation of 4-nitrophenol resulted in formation of 2,4-dinitrophenol and a dimer, the detailed structure of which was not determined. The dimer eluted later than 2,4-dinitrophenol with retention time of 19.23 min. The respective UV–Vis spectrum shows the typical peak of nitrophenol centered around 317 nm (inset C: corresponding molecular weight of 278 *m/z*) (Fig. 3). The 2,4-dinitrophenol appeared after 4-nitrophenol, with retention time of 18.07 min (inset B:corresponding molecular weight of 185 *m/z*). In all reaction mixtures containing peroxidases, a slightly browning followed by precipitation was observed. That indicates a polymerization of primarily formed phenoxy radicals.

4. Discussion

Nitroaromatic compounds such as nitrophenols, are recalcitrant and potentially toxic to fungi (Spain, 1995; Teramoto et al., 2004). However, the relatively low concentration used here (0.5 mM) did not have a marked toxic effect on the final biomass obtained, but might have influenced ligninolytic enzyme secretion, considering

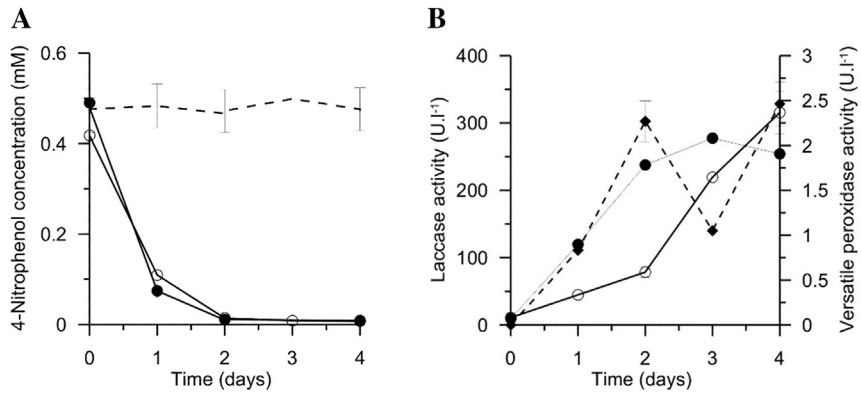

Fig. 1. A: *In vivo* degradation of 4-nitrophenol. Filled circles, 4-nitrophenol; blank circles, 4-nitrophenol with piperonyl butoxide; dashed line, control. **1 B**: Enzyme activities in the culture medium. Filled circles, laccase activity in the culture medium; blank circles, laccase activity in the control; dashed line, versatile peroxidase activity.

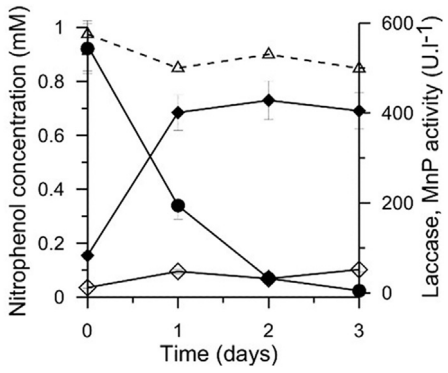

Fig. 2. *In vivo* degradation of 4-nitrophenol with *T. versicolor* immobilized on *L. aegyptica*. Filled circles 4-nitrophenol; blank triangles dashed line control (inactivated culture); filled diamonds laccase activity; blank diamonds MnP activity measured on the nitrophenol solution.

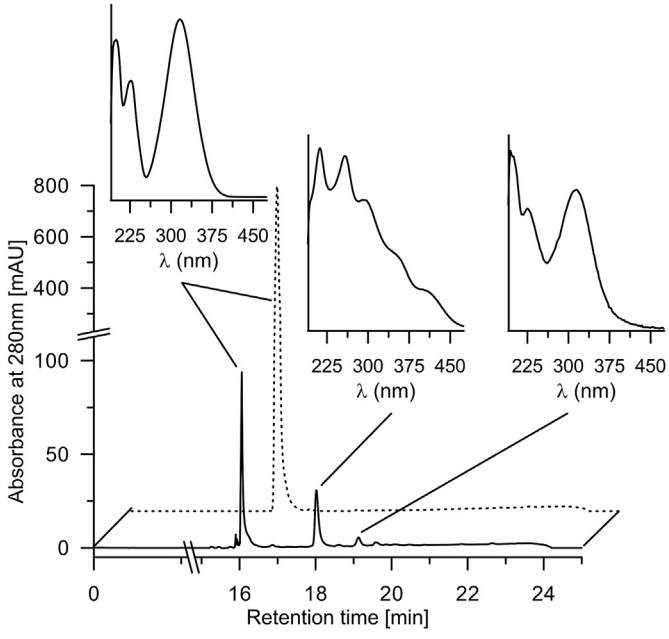

Fig. 3. HPLC elution profiles of *in vitro* reaction of *T. versicolor* versatile peroxidase with 0.5 mM nitrophenol at time 0 h (dashed line) and after 9 h (solid line). Insets of UV–Vis spectra showing nitrophenol (A), 2,4-dinitrophenol (B) and the dimerized product obtained (C).

that VP activity was detected only after nitrophenol supplementation. Laccase was the main ligninolytic enzyme produced by *T. versicolor* BAFC 2234 cultures. The titers were higher in nitrophenol supplemented cultures than in non-supplemented ones, during the first days of incubation. Aromatic compounds are known to act as elicitors/inducers of laccase production by diverse fungi (Elisashvili et al., 2010). In cultures of white-rot fungi, such as *Pleurotus* spp., *Phanerochaete laevis* or *T. versicolor*, laccase activity increased in response to the addition of phenolic compounds (Tripathi et al., 2011). Here, nitrophenol addition might have stimulated the secretion of laccase by *T. versicolor*. Inactivation/inhibition of peroxidases could explain why these enzyme activities were not detected during *in vivo* degradation (Sáez-Jiménez et al., 2015). The secretome of *T. versicolor* BAFC 2234 cultivated on tomato juice medium with copper and manganese, was recently explored (Carabajal et al., 2013). This medium, rich in phenolic compounds, stimulated the production of MnP and laccase by several agaric fungi (Ullrich et al., 2004). Under these conditions, 2 laccases, 5 MnPs and a VP were identified in the secretome, being one of the laccases, the most abundant enzyme secreted, followed by the group of MnPs. DyP was also found to be produced by *T. versicolor* 2234 in this medium, while LiP was not detected. Among the enzymes secreted, 1 laccase, 1 VP and 3 MnP, could be purified (Carabajal et al., 2013). Ligninolytic enzymes, such as MnP, LiP, laccase, are thought to be involved in oxidation and even mineralization of nitroaromatic compounds (Tripathi et al., 2011). Recently a DyP of *Auricularia auricula-judae* was found to be capable of acting on nitrophenol (Büttner et al., 2015). Additionally, peroxidases may play an indirect role in the degradation process, by producing free radicals, which can attack very recalcitrant molecules usually not directly attacked by enzymes (Anastasi et al., 2010).

T. versicolor was already successfully used to degrade diverse aromatic compounds such as trichlorobenzenes (Marco-Urrea et al., 2009), *p*-cresol (Ryan et al., 2007) and nitro- and hydroxylphenol derivatives (Yemendzhiev et al., 2012). Possibly *T. versicolor* 2234 is able to degrade nitrophenol via two different pathways. One of which might occur extracellularly by ligninolytic enzymes (Scheibner et al., 1997) and the other one intracellularly converting nitrophenol, for example, by cytochrome P-450s, unspecific phenol hydroxylases and/or nitroreductases (Scheibner et al., 1997; Teramoto et al., 2004; Yemendzhiev et al., 2012).

In the present work we showed that purified MnP as well as the VP of *T. versicolor* were capable of degrading 4-nitrophenol *in vitro*. The fungus secreted laccase as the main ligninolytic enzyme, but the purified enzyme was incapable of degrading the pollutant. Nevertheless, the synergistic action of laccase, MnP and VP in 4-

nitrophenol degradation *in vivo* cannot be ruled out, considering that natural mediators may be present in the extracellular fluids of the fungus and play a role in nitrophenol degradation by laccase. Laccases oxidize phenolic compounds but also non-phenolic compounds in the presence of mediators (Bourbonnais et al., 1995), but the addition of laccase mediators was not attempted in this work during *in vitro* assays. Moreover, laccase may oxidize nitrocatechol that is produced intracellularly by monooxygenases (Ullrich and Hofrichter, 2007). The laccase of *Trametes trogii* catalyzed the oxidation of 4-nitrocatechol (Garzillo et al., 1998). 4-Nitrophenol degradation was demonstrated by a laccase-copper synergistic system (Lu et al., 2012). Although the involvement of cytochrome P-450 in the metabolism of 4-nitrophenol has been reported in other ligninolytic white-rot fungus, *Phanerochaete chrysosporium* (Teramoto et al., 2004), the transformation of 4-nitrophenol by *T. versicolor* was not affected by exogenously added piperonyl butoxide, a cytochrome P-450 inhibitor, suggesting that this enzymatic system is not involved in nitrophenol degradation by *T. versicolor* or can bypassed by uninhibited enzymes. However, non-specific phenol hydroxylases and/or nitroreductases, might be involved in the process, but their role was not investigated in this study. *T. versicolor* is known to produce high levels of different lignin-modifying enzymes, but each one may differently contribute to the final degradation of diverse pollutants. In a recent study this strain showed potential to remove high concentrations of phenol, being oxidized *in vitro* by purified laccase and MnP (Carabajal et al., 2014, in press).

VP oxidizes Mn^{2+} (as MnP), and also high redox-potential aromatic compounds (as LiP), and does not require mediators to oxidize phenolic and non-phenolic compounds including lignin, pesticides, high redox-potential and polymeric dyes and polycyclic aromatic hydrocarbons (Ruiz-Dueñas et al., 2009). In this work we demonstrated for the first time the ability of *T. versicolor* VP to transform 4-nitrophenol. The fact that *T. versicolor* VP is able to catalyze this reaction is further evidence of its high-redox potential character. After a reaction time of 9 h two main products were detectable 2,4-dinitrophenol and a dimer, the detailed structure of which was not determined. Following the dimerization, further oxidations and polymerization are likely, but the products are insoluble and thereby not detectable with RP-HPLC (Kobayashia and Higashimura, 2003). Nitrophenol degradation by VP of *T. versicolor* may result in the formation of dimers, resembling the action of laccases on different phenol compounds. Liquid chromatography-mass spectrometry analysis showed recently that laccases from *T. trogii* catalyzed the transformation of two phenolic compounds: *p*-coumaric acid and tyrosol, resulting also in the formation of phenolic dimers (Chakroun et al., 2012). The capacity of phenoloxidases to polymerize phenols and facilitate their covalent binding to humic compounds is widely documented (for both free and immobilized laccase) (Ahn et al., 2002). Recently Büttner et al. (2015) investigated the action of *A. auricula-judae* DyP on nitrophenol conversion, in the case of 2-nitrophenol and 4-nitrophenol, dinitrophenols (2,4-DNP and 2,6-DNP) were identified as products, and for 4-nitrophenol also *p*-benzoquinone. In addition, some findings suggested the formation of random polymerization products derived from phenoxy radical intermediates. 4-nitrophenol oxidation and consequent formation of 2,4-DNP has also been described for LiP from *P. chrysosporium* (Massey et al., 1994).

The use of immobilized cells has shown potential in various bioprocesses among them wastewater treatment (Rodríguez Couto et al., 2004). Particularly, *L. aegyptica* sponge is a very convenient material for immobilization of fungal strains; as a natural support, it is cheap and once it is no longer suitable as carrier, it can be composted. *T. versicolor* immobilized on natural supports as jute, straw and hemp fiber was successfully applied in Amaranth decolorization (Shin et al., 2002). In this work, *T. versicolor* immobilized on *L. aegyptica* sponge could remove 97% of 1 mM 4-nitrophenol within 72 h. Moreover, after treatment with *T. versicolor*, phytotoxicity of the treated nitrophenol solution decreased noticeably and led to a germination index of 72% (±5.7) evaluated with *L. sativum* seeds. Immobilized white-rot fungal cultures in trickling packed-bed reactors removed phenol and 2,4,6-trichlorophenol (800 and 85 mg l^{-1} concentrations) by greater than 98% in 24–30 h batch cycles (Ehlers and Rose, 2005). The incubation of dry olive mill residue with peroxidase-producing basidiomycetes also led to a decrease in its phytotoxicity and phenol depletion caused by polymerization (Reina et al., 2013).

5. Conclusions

This study shows the ability of an Argentinean *T. versicolor* strain, immobilized and non-immobilized, to degrade 4-nitrophenol. Considering nitrophenol as comparatively resistant to microbial degradation (Teramoto et al., 2004), *T. versicolor* BAFC 2234 immobilized on natural plant sponge was found to degrade 97% of 1 mM 4-nitrophenol within 72 h, and decreased the phytotoxicity of respectively treated samples. This strain produced laccase as the main ligninolytic enzyme accompanied by lower titers of MnP and VP. Its purified MnP and VP, but not laccase, were able to transform 4-nitrophenol. Substantial conversion of nitrophenol, a typical high-redox potential phenolic substrate, is reported for VP for the first time. No changes were observed on fungal degradation capacity when the cytochrome P-450 inhibitor piperonyl butoxide was added. Immobilization on sponge-like carriers allows combining a high rate of nitrophenol degradation with the re-utilization of the biomass in several cycles. Based on these results, the investigated strain may have good prospects for an application in detoxification of industrial wastewaters polluted with nitrophenol.

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