International Biodeterioration & Biodegradation 65 (2011) 635–643

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Potential of *Trametes trogii* culture fluids and its purified laccase for the decolorization of different types of recalcitrant dyes without the addition of redox mediators

Emanuel Grassi [a], Pablo Scodeller [b], Nestor Filiel [b], Romina Carballo [b], Laura Levin [a,*]

[a] Laboratorio de Micología Experimental, Departamento de Biodiversidad y Biologia Experimental (DBBE), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, PROPLAME-PRHIDEB-CONICET, 1428 CABA Buenos Aires, Argentina
[b] INQUIMAE-DQIAyQF, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 CABA Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 21 January 2011
Received in revised form
21 March 2011
Accepted 22 March 2011
Available online 15 April 2011

Keywords:
White-rot fungi
Laccase
Manganese peroxidase
Dye decolorization
Redox mediators

ABSTRACT

Trametes trogii BAFC 463 culture fluids (containing 110 U ml^{-1} laccase; 0.94 U ml^{-1} manganese peroxidase), as well as its purified laccase were capable of decolorizing azoic, indigoid, triphenylmethane, anthraquinonic and heterocyclic dyes, in the absence of redox mediators. Six dyes: RBBR, Indigo Carmine, Xylidine, Malachite Green, Gentian Violet and Bromophenol Blue were almost completely degraded (more than 85% decolorization after 1 d) by either laccase or *T. trogii* itself in culture, proving the role of the enzyme in dye decolorization. The purified laccase also decolorized 65% of Fast Blue RR and 30% of Azure B and Methylene Blue after 24 h. The use of redox mediators significantly increased the decolorization rates (90% decolorization of Azure B after 1 h). 1-hydroxybenzotriazole resulted the best redox mediator, but the natural mediator *p*-hydroxybenzoic acid also demonstrated its efficiency for dye decolorization. Due to their ability to decolorize recalcitrant dyes without addition of redox mediators, high laccase activities, high thermostability and efficient decolorization at 70 °C and pH 7.0, even in the presence of high concentrations of heavy metals (100 mM Cu^{+2}, Pb^{+2} or Cd^{+2}) or in a synthetic dyebath, *T. trogii* culture fluids could be effectively used to decolorize synthetic dyes from effluents.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Synthetic dyes are extensively used in several industries including textile, paper printing, food processing, cosmetics and pharmaceuticals. It is estimated that 10–15% of the dyes are lost in the effluent during dyeing process (Vaidya and Datye 1982). Wastewaters from these industries represent a serious problem all over the world. They contain different types of synthetic dyes, which are mostly toxic, mutagenic and carcinogenic. Moreover, they are very stable to light, temperature and microbial attack, making them recalcitrant compounds. Conventional wastewater treatment systems are often inefficient and existing physical and chemical technologies are expensive, time-consuming and methodologically-demanding. Currently, one of the possible alternatives for treatment of colored effluents is the use of white-rot fungi, which mediated by their extracellular ligninolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase, can oxidize a wide spectrum of organic pollutants including synthetic dyes (Pointing 2001; Kokol et al. 2007). The broad substrate specificities of laccases (*p*-diphenol:oxygen oxidoreductase; EC 1.10.3.2), together with the fact that they use molecular oxygen as the final electron acceptor instead of the hydrogen peroxide used by ligninolytic peroxidases, make these enzymes highly interesting for industrial and environmental applications. Laccases catalyze the oxidation of a wide number of phenolic compounds and aromatic amines but their substrate range has been extended to non-phenolic compounds in the presence of low molecular mass compounds acting as mediators (Bourbonnais et al. 1995; Li et al. 1999; Camarero et al. 2005). MnP (EC 1.11.1.13) is a heme-containing enzyme catalyzing the oxidation of Mn^{2+} to Mn^{3+}, which in turn can oxidize a variety of phenolic substrates (Glenn et al. 1986). As the use of whole fungal cultures for decolorization is not easily applicable in a large scale, at present, the studies of *in vitro* decolorization of synthetic dyes by crude or purified enzymes become more important (Kokol et al. 2007).

The industrial use of enzymes is hampered by several factors, including the relative expense of enzymes, the cost of mediators, and lack of innovative technology, compared to that of traditional

* Corresponding author. Tel.: +54 11 47872706; fax: +54 11 45763384.
E-mail address: lale@bg.fcen.uba.ar (L. Levin).

0964-8305/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2011.03.007

processes, and thus has not been widely implemented in the industry. Several methods for improving the industrial application of enzymes exist, these include less expensive production strategies, improvement in yield from current producer organisms, or isolation of more industrially applicable forms of the enzyme that exhibit enhanced stability and superior catalytic properties through bio-prospecting (Jordaan et al. 2004). As industrial wastes are generally complex mixtures of various xenobiotics including heavy metal salts, their inhibitory effects must be taken into consideration during their bioremediation (Hatvani and Mecs 2003). The resistance of the catalytically active protein structure toward high temperature, pH and other potentially denaturing factors is one of the chief criteria for commercialization and for industrial applications of the enzymes (Papinutti et al. 2008).

Since the enzymatic system secreted by basidiomycetes depends on the kind of fungus, strain and culture conditions, more white-rot fungi have to be screened for their ability to degrade recalcitrant aromatic compounds, including dyes (Zouari-Mechichi et al. 2006). The ability of white-rot fungi to decolorize synthetic dyes has been widely studied, particularly with *Phanerochaete chrysosporium* and *Trametes versicolor* (Pointing 2001). The role of laccases and peroxidases secreted by these fungi is controversial, although in the case of *P. chysosporium* only peroxidases seem to be involved in the process (Moldes et al. 2003). In *Trametes* species laccases are the major enzymes but peroxidases are also secreted during dye decolorization (Zouari-Mechichi et al. 2006). Laccase-mediated dye decolorization has been described with crude and purified forms from many fungi; however, most of the laccases required redox mediators.

Laccase from *Trametes trogii* was reported to have one of the highest redox potential among laccases (E° 0.79 at pH 5.0) (Garzillo et al. 2001), which makes this laccase particularly interesting since high redox potentials correlate with high laccase activity (Li et al. 1999). *T. trogii* is a worldwide distributed white-rot basidiomycete. It demonstrated to be a good producer of laccases and other ligninolytic enzymes including LiP and MnP (Vares and Hatakka 1997; Garzillo et al. 1998; Levin and Forchiassin 2001; Levin et al. 2005; Zouari-Mechichi et al. 2006; Ciullini et al. 2008). Strain BAFC 463 of this fungus also proved to be an efficient tool for the degradation of several organic pollutants including nitrobenzene and anthracene, a combination of polychlorinated biphenyls (PCBs) (Aroclor 1150) and an industrial mixture of polycyclic aromatic hydrocarbons (PAHs) (10% V/V of PAHs, principal components hexaethylbenzene, naphthalene, 1-methyl naphthalene, acenaphthylene, anthracene, fluorene and phenanthrene) (Haglund et al. 2002; Levin et al. 2003). The ability of *T. trogii* to decolorize industrial dyes was confirmed by several studies (Cing and Yesilada 2004; Deveci et al. 2004; Levin et al. 2005; Mechichi et al. 2006; Zouari-Mechichi et al. 2006; Ciullini et al. 2008; Levin et al. 2010). However, the roles of single enzymes in dye decolorization have not yet been established. In this study, the decolorization of various dyes with different structural patterns was investigated using crude extracellular culture fluids and pure laccase from *T. trogii*. The main objectives of our work were: (i) to evaluate the contribution of the ligninolytic enzymes laccase and MnP of *T. trogii* to the degradation of synthetic dyes, commonly used in the textile industry (ii) to study the activity and stability of these enzymes and the ability of *T. trogii* culture fluids to decolorize different dyes under those denaturing conditions typically found in dye containing effluents (high salts and heavy metals concentrations, high temperatures, alkaline pHs) (iii) to test the possible enhancement of decolorization by the use of laccase redox mediators.

2. Materials and methods

2.1. Microorganism

Strain BAFC 463 (from the Culture Collection of Universidad de Buenos Aires) of *T. trogii* (*Funalia trogii*) (Berk. in Trog.) (Polyporaceae, Aphyllophorales, Basidiomycetes) was used in these experiments. Stock cultures were maintained on malt extract agar slants at 4 °C.

2.2. Culture media

Basal culture medium (GA) contained glucose, 20 g; L-asparagine monohydrate, 3 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.6 g; $MnCl_2 \cdot 4H_2O$, 0.09 mg; H_3BO_3, 0.07 mg; $Na_2MoO_4 \cdot H_2O$, 0.02 mg; $FeCl_3$, 1 mg; $ZnCl_2$, 3.5 mg; thiamine hydrochloride, 0.1 mg; distilled water up to 1 l, supplemented with 1 mM copper sulfate. The medium was sterilized at 121 °C for 20 min, and its initial pH was adjusted to 6.5 with NaOH 1 N (Levin et al. 2002).

2.3. Culture conditions

Incubation was carried out statically at 28 ± 1 °C in 500 ml Erlenmeyer flasks with 50 ml of medium, which were inoculated with four 25-mm^2 surface agar plugs from a 7-d-old colony grown on Bacto-agar 2%. Cultures were harvested after 22 d, filtered through a filter paper using a Büchner funnel, and dried overnight at 70 °C. Dry weight of the mycelium was then determined. Culture supernatants were used as enzyme sources. All chemicals were of analytical grade and were used without further purification.

2.4. Analytical determinations

Reducing sugars remaining in the medium were determined by the Somogyi–Nelson procedure (Nelson 1944) with glucose as standard. Laccase activity was measured with 2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M sodium acetate buffer (pH 3.5) at 30 °C. Oxidation of ABTS was determined by the increase in A_{420} ($\varepsilon_{420} = 36{,}000\ M^{-1}\ cm^{-1}$) (Bourbonnais et al. 1995). MnP activity was determined using phenol red as the substrate in 0.05 M sodium succinate buffer (pH 4.5) at 30 °C ($\varepsilon_{610} = 22{,}000\ M^{-1}\ cm^{-1}$) (Glenn et al. 1986). International enzymatic units (U) were used (μmol product min^{-1}). Enzyme activity was expressed as U ml^{-1} of culture filtrate.

2.5. Effect of pH and temperature on MnP activity and stability

To test the effect of pH on MnP activity, its substrate was dissolved in 0.05 M succinate buffer of different pHs (3.8–6.2). The effect of pH on the enzyme stability was investigated by measurement of the activity remaining after incubation for up to 24 h at 30 °C in 0.05 M succinate buffer (pH 4.5) and 0.05 M phosphate buffer (pH 7.0). In the experiments testing the effect of temperature on MnP activity, the incubation temperature was in the range 30–70 °C. For temperature stability assays, aliquots of the culture filtrate were incubated at different temperatures (30–70 °C) for up to 1 d, afterwards MnP residual activity was measured.

2.6. Effect of high salts and heavy metals concentrations on laccase and MnP stability

The stability of laccase and MnP against several metal ions such as Cu^{+2}, Cd^{+2}, Pb^{+2}, Mn^{+2}, Cr^{+3}, Co^{+2} and Fe^{+2} was assessed. For this, the culture fluid was incubated with the salts of the above-mentioned

Download English Version:

https://daneshyari.com/en/article/4365319

Download Persian Version:

https://daneshyari.com/article/4365319

Daneshyari.com