Journal of Environmental Management 124 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Journal of Environmental Management

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

Protective effect of vanilloids against chemical stress on the white-rot fungus *Ganoderma lucidum*

Francisco Kuhar, Leandro Papinutti*

Laboratorio de Micología Experimental, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, C1428EHA Ciudad Universitaria, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 14 November 2012
Received in revised form
10 March 2013
Accepted 19 March 2013
Available online 11 April 2013

Keywords:
Bioremediation
Biosorption
Exopolysaccharide
Fungal growth
Ligninolytic enzymes
White rot fungi

ABSTRACT

Bioremediation of contaminated sites by biosorption of pollutants onto a wide range of materials has emerged as a promising treatment for recalcitrant aromatic compounds or heavy metals. When adsorption occurs on living white-rot fungi mycelia, the pollutants may be degraded by ligninolytic enzymes. However, the survival of mycelia in harsh conditions is one of the drawbacks of those methodologies. In this study, it was demonstrated that culture media supplemented with several guaiacol derivatives (vanilloids) increased the resistance of *Ganoderma lucidum* E47 cultures to chemical stress by enhancing the adsorptive capacity of the extracellular mucilaginous material (ECMM). The toxicity of the fungicides gentian violet (GV), malachite green (MG) and clotrimazole, and the heavy metal Cadmium was noticeably diminished in fungal cultures supplemented with the guaiacol derivative vanillic acid (VA). No degradation of the tested compounds was detected. The activity of the oxidative enzymatic systems like laccase, a well-known oxidase associated to dye degradation, was only detectable after complete growth on plates. Extremely low concentrations of VA caused a significant protective effect, radial extension of the growth halo in plates supplemented with 0.0001 mM of VA plus GV was up to 20% to that obtained in control plates (without addition of GV and VA). Therefore, the protective effect could not be attributable to VA *per se*. ECMM separated from the mycelium exhibited a much higher increase in the adsorptive capacity when isolated from liquid cultures containing VA, while that obtained from unsupplemented cultures showed an almost null adsorptive capacity.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Ganoderma lucidum belongs to the ecological group of the white rot fungi due to its ability to preferentially degrade lignin, which is a recalcitrant heteropolymer of phenylpropanoid unities. Lignin degradation by white rot fungi has been extensively studied, and results revealed that at least three extracellular oxidoreductases, namely, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac), are responsible for initiating the depolymerization of lignin (Cullen and Kersten, 2004). Not only do these enzymes attack lignin, but it has also been demonstrated that their substrates include a wide range of pollutants, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and synthetic dyes. The ability of white rot fungi to degrade environmental pollutants has been widely studied (Gao et al., 2010; Pointing, 2001). Particularly, *G. lucidum* was demonstrated to be able to degrade phenanthrene and pyrene (Ting et al., 2011) and decolorize textile dyes (Asgher et al., 2010; Bibi et al., 2009). Although wood is the natural habitat of this ecophysiological group of fungi, it is important to investigate ways to improve the survival of white rot fungi in polluted environments, an unusual habitat for such organisms. In fact, an important problem of *in situ* bioremediation processes involving white-rot fungi is the lost of viability of inoculum after it is introduced to the remediation site due to toxicity of the contaminant.

Adsorption of persistent pollutants is another application of fungal cultures to bioremediation, especially for elemental substances that cannot be degraded, such as heavy metals (Cerino-Córdova et al., 2012; Srinivasan and Viraraghavan, 2010) or also radioactive isotopes (Kulshresta and Venkobachar, 2008). Although aromatic pollutants may also be adsorbed, these compounds could not be degraded as long as dead fungi was used (Maurya and Mittal, 2011).

Characterization of fungal growth and exoenzyme production is fundamentally important to develop environmental biotechnology

* Corresponding author. Tel.: +54 4576 3300; fax: +54 4576 3384.
E-mail addresses: leandropapinutti@gmail.com, leandru@bg.fcen.uba.ar (L. Papinutti).

0301-4797/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jenvman.2013.03.040

for biodegradation of recalcitrant organic pollutants and waste treatment. Generally speaking, white rot fungi are introduced into contaminated soils as pre-grown inoculum based on lignocellulosic substrates, such as sawdust, wood chips and wheat straw, which are subsequently mixed in with the polluted soil (Barr and Aust, 1994). It is usually the case that with a larger inoculum biomass, a faster and more successful establishment of the fungus in the soil is obtained (Gao et al., 2008; Lestan et al., 1996). Special care is required when balancing the carbon and nitrogen ratio in the substrates, which has a significant influence on the degrading performance of white rot fungi (Borrás et al., 2011). The selection of a suitable inoculum carrier can easily overcome the lack of nutrients and allow soil colonization (Lang et al., 1998; Mougin et al., 1997). One of the main constraints of the empirical application of those organisms is the toxicity exerted by the pollutants on the growing mycelium. A possible solution to this problem would be to screen not only for organisms able to perform the degradation and detoxification but also for the ability of the organism to adapt to higher concentrations of these compounds.

While studying the inductive effect of aromatic compounds on ligninolytic enzyme activities, we found a protective effect against gentian violet. The aim of this work is to explore this effect inflicted by guaiacol derivatives such as vanillic acid, ferulic acid or vanillin toward chemical stress in the white rot fungus *G. lucidum*.

2. Materials and methods

2.1. Fungal strains and culture conditions

G. lucidum E47 strain (University of Guelph, Guelph, Canada) was used. The fungi were maintained in MEA (malt extract 1.2%, glucose 1%, agar 2%) medium at 4 °C. Inoculum consisted of a 25-mm^2 surface agar plug from a 12-day-old culture grown on MEA. The defined media (GG) contained: $MgSO4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.6 g; $CuSO_4 \cdot 5H_2O$, 0.4 mg; $MnCl_2 \cdot 4H_2O$, 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; glutamic acid, 9 g; glucose 10 g; and distilled water up to 1 L, pH was adjusted to 6.0. Solid GG medium contained agar 20 g L^{-1}. Liquid cultures were performed in 125 mL Erlenmeyer flasks containing 25 mL of medium at 28 °C. All chemicals were of analytical grade and were used without further purification.

2.2. Screening of aromatic compounds in agar plates

Effect of several aromatic compounds on the growth of *G. lucidum* in the presence of high concentration of the fungicide gentian violet (GV) was tested. To screen the protective effect of aromatic compounds, the fungus was inoculated on 9 cm diam plates containing 20 mL of solid GG supplemented with GV 50 μM and aromatic compounds of different chemical structure: Four guaiacol derivatives (vanilloids): guaiacol (1 mM), ferulic acid (1 mM), vanillic acid (VA; 1 mM), vanillin (0.5 mM), and eight aromatic compounds unrelated to guaiacol: resorcinol (1 mM), coumarin (0.5 mM), diphenylamine (0.1 mM), anisaldehyde (1 mM), anisole (1 mM), thymol (1 mM), hydroxybenzoic acid (HBA; 1 mM), and 1-hydroxybenzotriazole (HBT; 1 mM). Plates without GV served as control. Radial growth was measured in two perpendicular directions from the edge of the, inoculum to the advancing margin of the colony.

2.3. Assays of toxicity on plates

In order to determine the effect of VA concentration on the protective effect, plates containing solid GG and different concentrations of VA (from 0.1 μM to 10 mM) were inoculated with the fungus and radial growth was measured daily. The chemical stressors were evaluated as before, in agarized media with or without vanillic acid. The following toxic substances were tested: GV 50 μM, malachite green 50 μM, clotrimazole 25 μg mL^{-1}, nystatin, arsenic and cadmium.

2.4. Enzyme assays

Ligninolytic activity was determined on solid media from plates with and without VA 0.1 mM and GV 50 μM. Laccase and manganese peroxidase activity was spectrophotometrically determined according to the method of Levin et al. (2004): Agar plugs with mycelium from the growing margin were added to 2.5 mL of the reaction buffer containing the enzyme substrate (at a ratio of 20 mg plug mL^{-1} reaction buffer). Lignin Peroxidase activity was measured following Archibald (1992). One unit of enzyme activity (U) was defined as the amount of enzyme required to oxidize 1 μmol of substrate in 1 min. Activity in plates was expressed as U g^{-1} of solid medium.

2.5. Liquid cultures

Batch cultures of *G. lucidum* were grown in 250-mL Erlenmeyer flasks containing 25 mL of GG medium at 28 °C. The growth capacity of the fungi in the presence of GV, clotrimazole and VA was evaluated. Mycelial dry weight was measured periodically for growth estimation.

Extracellular mucilaginous material (ECMM) was separated from the liquid cultures by centrifugation at 3000 × g 10 min. After removal of the ECMM from mycelium, it was re-suspended in distilled water and centrifuged again at 3000 × g 10 min in order to wash away the remaining culture medium.

2.6. Adsorption

Gentian violet was obtained from Sigma (St. Louis, USA), and used without further purification. Stock solution of dye was prepared using GV salt in double distilled water. Adsorption of GV (0–130 mM) was investigated in a batch system to obtain rate and equilibrium data. Effects of initial concentrations, contact time and pH of the medium on the adsorption rate and capacity were studied. The pH of the media was adjusted over a range of 4–7 using acetate buffer (10 mM final concentration). The pH of the solutions was measured with a pH meter (HI 9321, Hanna Instruments). The adsorption of GV to mycelia and ECMM was monitored spectrophotometrically at 584 nm in the aqueous solution and the values were expressed as A_t $100/A_0$ where: A_t = absorbance at time t and A_0 = absorbance at time 0. Gentian violet concentrations (mg L^{-1}) were correlated with the absorbance values. A control flask containing only distilled water and ECMM was also used to determine the zero level absorbance. Aliquots of supernatants were collected at predetermined time intervals to determine the residual dye concentration in the solution. Before analysis, the samples were centrifuged at 3000 × g for 10 min, and the absorbance of supernatant liquid was measured. Gentian violet adsorbed by the biomass was calculated according to a material balance.

2.7. Kinetic study of GV adsorption to mycelium

Due to its empirical character and applicability on heterogeneous systems, Freundlich model equation was used to describe adsorption isotherms of GV to mycelium. Freundlich equation: $qe = aCeq^{1/b}$ where qe is the adsorbate uptake capacity (mg GV g^{-1}

Download English Version:

https://daneshyari.com/en/article/1056256

Download Persian Version:

https://daneshyari.com/article/1056256

Daneshyari.com