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

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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.

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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

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: MgSO4·7H$_2$O, 0.5 g; KH$_2$PO$_4$, 0.5 g; K$_2$HPO$_4$, 0.6 g; CuSO$_4$·5H$_2$O, 0.4 mg; MnCl$_2$·4H$_2$O, 0.09 mg; H$_3$BO$_3$, 0.07 mg; Na$_2$MoO$_4$·H$_2$O, 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}

dry mycelium); Ceq is the equilibrium concentration of GV in solution after the adsorption assay (mg L^{-1}); a and b are the Freundlich constants to be estimated, a being the maximum adsorbate uptake capacity and b the adsorption affinity constant. For plotting the isotherms qe versus Ceq, the contact time and pH values were selected on the basis of the above experiment. Due to the fact that color intensities of GV depend on pH, the fungicide concentration in the aqueous medium was calculated from calibration curves at different pH values. Experimental data points were obtained by keeping the mass of mycelium at 1 g constant and varying the concentration of the fungicide from 0 to 130 mM g^{-1}. Fungicide uptake capacity was calculated from the initial concentration in the aqueous medium. The constants were calculated by non-linear regression using STATISTICA 5.1 (StatSoft, Tulsa, OK).

2.8. Cadmium determination

Preparation of fungal biomass and culture supernatants for Cd determination: dry biomass in the range 30–100 mg and supernatants were digested with nitric acid and sulfuric acid (1:1 [vol/vol]). Complete digestion was obtained after the samples were incubated 1 h at 60 °C. Digested and sonicated (90 min at 60 °C) samples were mixed with 5% (vol/vol) Triton X-100 and supplemented with Yttrium (Y) and Strontium (Sr) as reference. Cd concentration in the samples (mg Cd g^{-1}) was measured with the method of inductively coupled plasma optical emission spectrometry at the analytical line 214.440 nm.

2.9. Statistical analyses

All statistical analyses were performed by using the software STATISTICA 5.1 (StatSoft, Tulsa, Okla). All experiments were done in triplicate. Mean values and standard deviation of the means were determined. Significant differences of means were tested by using Tukey method. The adequate fitting of the data to Freundlich model was tested with ANOVA.

3. Results

3.1. Growth in plates and liquid cultures

Cultures of the fungal strain growing on GG medium containing GV and one of the possible protective compounds showed that 4 of the 12 compounds evaluated exerted a protective effect against the toxicity of GV (Fig. 1). Colony sizes at day 6 were similar in VA, vanillin, guaiacol and ferulic acid (average rates of growth: among 0.9 and 1.1 cm day^{-1}). Fungal growth in media supplemented with these compounds plus 10 μM GV was not delayed with respect to control plates (without GV). The other compounds tested did not exhibit such a striking protective effect. Growth of G. lucidum was 90% inhibited in cultures with only GV at 10 μM but totally inhibited at higher concentrations. Regarding ligninolytic enzyme activities, neither laccase nor MnP exhibited differences in titers measured in agar plugs from plates with and without VA. In addition, laccase (0.4 U g^{-1}) and MnP (50 mU g^{-1}) were only detected after 20 d, when mycelium had completely covered the medium of plates. LiP activity was not detected in the studied strain under the assayed conditions.

The protective effect of 1 mM VA was also tested in liquid static cultures. GV exhibited a high toxicity since total inhibition of mycelial growth was observed in the entire range (1–50 μM) of the tested GV concentrations. On the other hand, the sensitivity of G. lucidum to the fungicide clotrimazole was lower, cultures with 2.5 μg mL^{-1} of the fungicide plus VA showed growth rates similar to control cultures without clotrimazole added (Fig. 2). In absence of
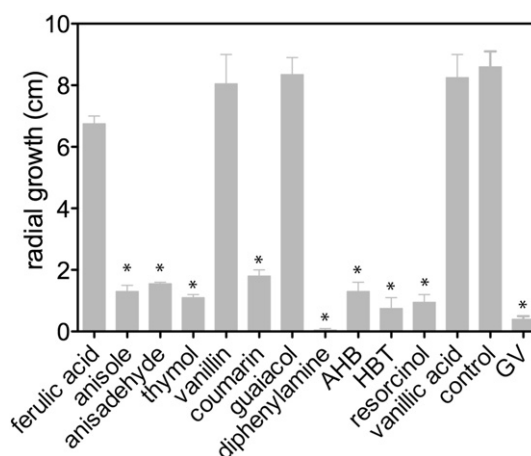

Fig. 1. Effect of different aromatic compounds on radial growth of G. lucidum E47 in plates containing MEA (malt extract 1.2%, glucose 1%, agar 2%) medium supplemented with GV (50 μM). Significant differences respect to control plates (without GV and aromatic compounds) were denoted with an asterisk (*). Bars represent standard deviation.

VA, none of the tested concentrations of clotrimazole allowed G. lucidum to grow at similar rates exhibited by control cultures. Although the toxicity of clotrimazole was reverted by the vanilloid, the growth response still appeared to be in an inverse relation to the fungicide concentration (Table 1).

3.2. Effect of VA concentration on the protective effect

In order to establish the concentration range of VA to protect G. lucidum against toxic effect of GV (50 μM), the fungus was cultured for 7 d at 28 °C with a range of 0.0001–10 mM of VA (Fig. 3). 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 20% higher to that obtained in control plates (without addition of GV and VA). Increased concentrations of VA from 0.01 to 5 mM enhanced the radial growth by up to 70%–80%, with further increase in VA concentration not causing further growth increase. None of the treatments exhibited the growth rate comparable with the control, suggesting that VA might exert some toxic effect besides the protective one. The decrease in mycelium size of the highest concentrations would show the toxic effect of VA. At this concentration (10 mM) the agar plates became dark probably due to the polymerization of phenolic units. Then, the effect of GV concentration in
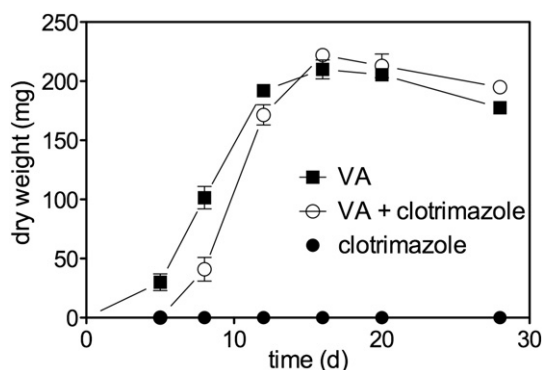

Fig. 2. Effect of clotrimazole (2.5 μg mL^{-1}) and vanillic acid (VA; 1 mM) in fungal growth (estimated as dry weight of mycelium/100 mL of culture medium) in liquid media as function of culture age. Bars represent standard deviation.

Table 1
Effect of 1 mM VA in biomass production (mg) by *G. lucidum* in liquid static cultures with variable concentrations of clotrimazole. Standard deviation (SD) was less than 5%.

	Clotrimazole concentration (μM)					
	0	0.6	1.25	2.5	5	10
With VA	197	209	195	188	140	110
Without VA	201	15	0	0	0	0

the range 5–100 μM was studied with and without vanillic acid (1 mM) showing that, regardless of the concentration of GV used, the vanilloid increased the resistance (Data not shown).

Protective effect of VA against other toxic compounds was evaluated (Fig. 4). Many toxic compounds were tested while looking for a possible explanation to the protective effect, but it was found the protection was exerted against completely unrelated compounds such as cadmium, clotrimazole, nystatin, malachite green, but not against arsenic.

3.3. Adsorption of GV onto mycelium

The GV adsorption kinetics to *G. lucidum* mycelia performed with different concentrations of GV was performed in cultivations with and without VA at pH 6. The equilibrium was attained after 4 h of contact time with the three fungicide concentrations tested. The system reached around 70% adsorption within 2 h of contact time irrespective of concentration (Data not shown).

It is well known that pH greatly influences the adsorption capacity and intensity. To study this effect, experiments were performed using citrate phosphate 0.1 M buffer at pH range 4–7. Since the color intensities of GV could be influenced by the pH values, concentrations of the dye were estimated from calibration curves performed at each pH assayed. Data obtained for qe and Ceq at different GV concentrations and pH range 4–7, were used without any prior transformation to fit the Freundlich equation. Plot of Ceq versus qe at pH 6 with VA is shown in Fig. 5. Freundlich coefficients a (capacity) and b (intensity) obtained from the experiments using mycelia produced with and without GV at different pH are depicted in Table 2. The two coefficients were estimated by non-linear regression. The high values of R^2 provide strong evidence that the model accurately reflects the process. The R^2 values were ≥ 0.95, that means that more than 95% of the variability in observed GV adsorption can be explained by the model (except at pH 4 without

Fig. 4. Effect of vanillic acid (VA; 0.1 mM) on the radial growth of *G. lucidum* E47 in MEA based media amended with different toxic compounds (50 μM). Bars represent standard deviation.

VA). From these results, it is evident that the maximum adsorption of dye was attained at pH range 6–7. In the treatments with VA added in these flasks, the amount of dye removed from liquid phase was more than 90% with 0.1 mM of GV.

3.4. Cadmium adsorption

Amount of Cd adsorbed to mycelia was measured by emission spectrometry in the fungus cultivated with VA 0.1 mM. The adsorption of Cd was measured by adding NO_3Cd up to 0.5 mM to 20-d-old liquid cultures with VA 0.1 mM and quantifying the remaining Cd in the supernatant after 24 h of exposure. Liquid cultivations without VA added served as controls. The mean values of removed Cd in presence of VA were 18.17 ppm, significantly different ($p < 0.05$) from that of cultivations without VA (6.92 ppm).

3.5. Adsorption capacity of ECMM

ECMM was isolated from *G. lucidum* mycelia produced with (Fig. 6a) and without (Fig. 6b) VA and used to test the adsorption of GV. Obvious differences could be observed upon comparing the two types of ECMM, assays using ECMM obtained from cultures with VA exhibited strong blue stain. The GV could only be desorbed in 1:1 ethanol:water, the GV desorbed in water was undetectable. Kinetics
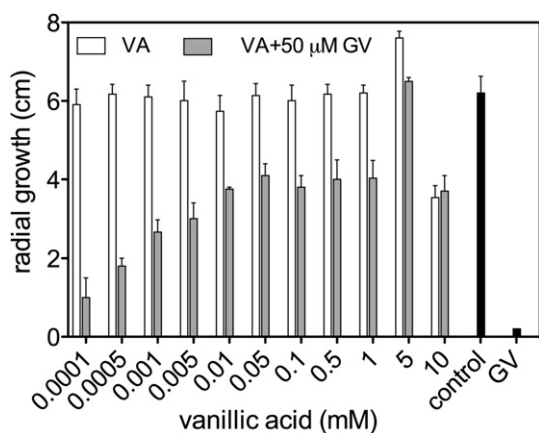

Fig. 3. Effect of vanillic acid (VA) concentration on radial growth of *G. lucidum* E47 in plates containing MEA (malt extract 1.2%, glucose 1%, agar 2%) medium and amended with 50 μM gentian violet (GV). Bars represent standard deviation.
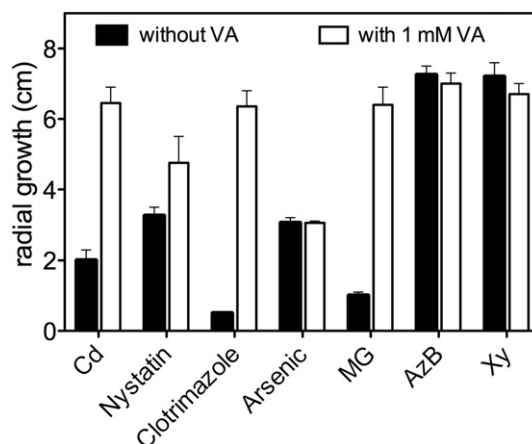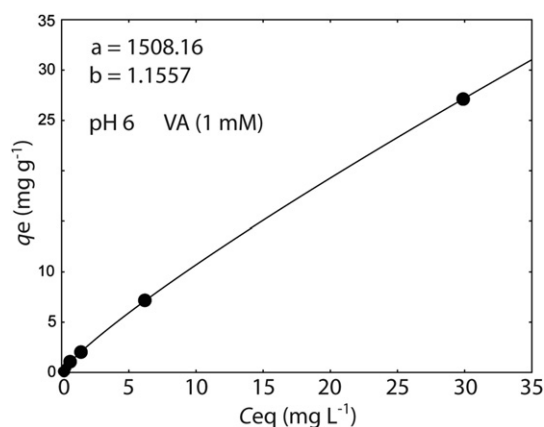

Fig. 5. Estimated Freundlich (—) isotherms fitting to experimental data (●) at pH 6 of mycelium from cultures with VA added. Concentrations of GV were determined spectrophotometrically at 584 nm.

Table 2
Freundlich adsorption isotherms model coefficients (C) for GV at pH range 4–7. Assays were carried out with mycelia cultivated in media with and without vanillic acid (VA).

C	Without VA				With VA (1 mM)			
	pH 4	pH 5	pH 6	pH 7	pH 4	pH 5	pH 6	pH 7
a	1139.38	1488.86	674.02	824.35	735.85	1834.018	1508.15	2231.89
b	1.451	1.232	0.758	0.8299	1.2918	1.336	1.176	1.508
R^2	0.78	0.99	0.99	0.99	0.99	0.99	0.99	0.95
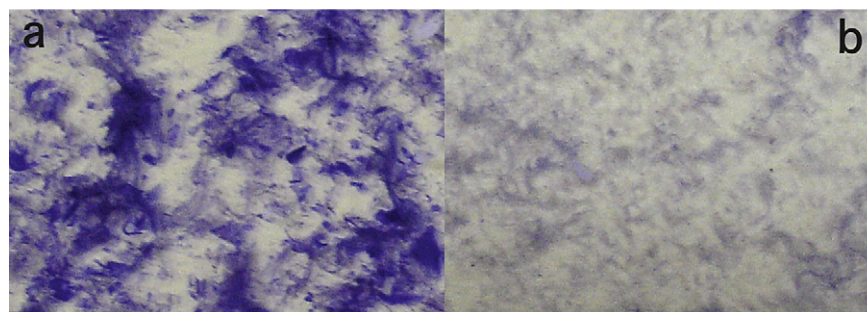
Fig. 6. The ECMM was separate from mycelia obtained from cultures **a**: with 1 mM VA and **b**: without VA, and used for GV (50 μM) adsorption.

of GV adsorption onto ECMM was performed at pH 6, using isolated ECMM from mycelia from cultivations with 1 mM VA (ECMM+) and without VA (ECMM−) (Fig. 7). Although the equilibria in both ECMM were reached simultaneously (after 5 h of contact time), the GV uptake increased from less than 10 mg g^{-1} in the ECMM− to more than 127 mg g^{-1} in the ECMM+. In terms of color removal, it was observed that reduction in absorbance was 20% and 86% using ECMM− and ECMM+, respectively.

4. Discussion

White rot fungi have been demonstrated to be efficient in transforming and mineralizing a wide range of xenobiotic organopollutants, in most cases this oxidative process is carried out by ligninolytic enzymes due to their broad substrate specificity and high redox potential (Wong, 2009). The application of white rot fungi for biodegradation of organic pollutants has already been studied for several decades, however, the problem that persists today is the slow growing rate or even the total inhibition of growth due to the sensitivity to growth conditions in contaminated environments. In order to avoid *in situ* inoculation and cultivation, the immobilization of fungi in biological reactors was also proposed

Fig. 7. Kinetics at pH 6 of GV adsorption onto isolated ECMM from mycelia produced in cultures grown with (○) and without VA (■).

(Pakshirajan and Kheria, 2012). However, a concomitant increase of costs comes with this methodology. Hence, it is not only research on ligninolytic enzymes that will contribute to bioremediation plans, but it is also necessary to conduct research on the organism's resistance mechanisms. Thermotolerance is one of the most important characteristics of fungal isolates so screening for selection of outstanding thermotolerant strains have been reported (Cruz Ramírez et al., 2012).

The protective effect was exerted by every guaiacol derivative compound (ferulic acid, vanillic acid, vanillin and guaiacol). Thus, the conclusion that the protective effect is related to the "vanilloid" structure was strongly supported by this work. Experiments in plates showed that all of the vanilloids tested inflicted a protective effect upon the fungal growth while other aromatic derivatives were unable to protect against GV toxicity.

In an attempt to gain further insights into the protective effect of VA, a wide range concentration of VA was tested. It was noteworthy that an extremely low concentration of VA (0.0001 mM) inflicted a significant protective effect. This effect could not be attributable to VA *per se* (e.g. acting as an antioxidant) but it is likely that it may have triggered the production or the release of compounds that inactivate GV. Since white rot fungi are efficient in degrading aromatic xenobiotics with ligninolytic enzymes, the inactivation could have occurred through enzymatic degradation of GV, which is why the enhancement of laccase and MnP production as response to GV was evaluated. Previous reports showed that laccase induction varies across organisms and seems to be specific to certain aromatic compounds. Guaiacol and ferulic acid induced laccase production in *Trametes* sp. (Xiao et al., 2004), vanillin in *Phanerochaete flavido-alba* (de la Rubia et al., 2002) and malachite green in *Fomes sclerodermeus* (Papinutti et al., 2006). However, in *G. lucidum* it was observed that *a*) ligninolytic enzyme production was increased neither in the presence of GV nor with other xenobiotics; *b*) decolorization of GV was observed after complete growth of the fungi in plates; *c*) VA also exerted a protective effect against heavy metals but these cannot be enzymatically degraded. Therefore, fungal resistance via degradation of GV was ruled out. Another inactivation mechanism that may occur in systems involving aqueous solutions of pollutant (particularly dyes) and solid material is adsorption (Asgher, 2012). Previous reports showed that the cell wall was the primary site of biosorption in *Rhizopus* and
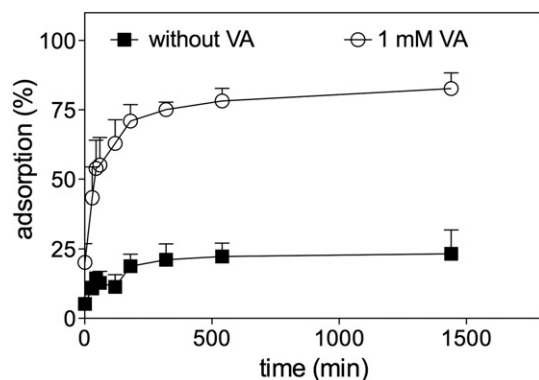
Cunninghamella elegans. Approximately 90% of the dry matter of the cell wall of these fungi contains chitin–chitosan, which has been implicated in sequestering a variety of recalcitrant aromatic substances (Ambrósio et al., 2012; Volesky, 1990).

Adsorptive capacity of the mycelia of *G. lucidum* E47 was shown to be higher in cultures in the presence of GV, mostly at pH 7, but not enough to explain the differences in growth. Nevertheless, separated ECMM from mycelium obtained in cultures grown with VA exhibited a much higher increase in the adsorptive capacity, suggesting that ECMM was the main responsible of the observed protective effect. There is strong evidence of protective effects of vanilloid compounds against oxidative stress in animal cell cultures (Rosa et al., 2008), but there is no reference in the literature to its effect on the ECMM. Nevertheless, differences in the fungal cell wall composition exerted by nutrients have been reported with the EPS being (the main component of the ECMM) the most affected components in presence of, for example, selenium (Turlo et al., 2010). The biological meaning of this effect remains unknown. Many compounds in this family are also a result of the oxidative degradation of lignin, and therefore ubiquitous in the natural environment of white rot fungi. The lack of dependence on the doses and protective effect shows the vanilloid is not consumed by the mycelium as substrate for any reaction. Instead, it seems to be acting as inducer of a particular ECMM. The positive effect on the mycelial growth could be a result of the immobilization of the toxic compounds by the ECMM matrix. It is likely that the ECMM produced in the presence of vanilloids acts as a barrier through adsorption and immobilization of the GV, thus the fungicide may be prevented from reaching the cell wall or from being translocated. Further studies in that direction may clarify the mechanism of resistance acquired by the fungus in response to vanilloids. This effect has already been observed in bacteria (González et al., 2010) in the presence of heavy metals in solution. Bioremediation by means of mycelial adsorption has been thoroughly assayed, especially for heavy metal pollution, (Colica et al., 2010; Kantar et al., 2011; Wang et al., 2011) since they cannot be degraded. The aim of the exposure to the mycelial mass is to sequestrate the pollutant from the water and concentrate it for deposition or reutilization. In addition to biosorption by dead fungi, a growing interest has been in tolerant strains with the capacity to degrade pollutants (Argumedo-Delira et al., 2012). Thus, the possibility of increasing the adsorptive capacity through the addition of small quantities of vanilloids would result in an improvement of the bioremediation process.

This study is the first report that experimentally proved that vanilloids could inflict an increased protective effect against chemical stress on the white rot fungi *G. lucidum* thereby allowing the fungus to grow and degrade the fungicide GV. This might be very useful in different bioremediation strategies.

Acknowledgments

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