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Palestra 21

PESTICIDES DEGRADATION BY ENZYMES FROM WHITE-ROT FUNGI

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ABSTRACT

White-rot fungi (WRF) growth on lignocellulosic materials causes the wood degradation. Numerous studies have demonstrated that white rot fungi not only can survive in polluted environments, but also contribute to eliminate the pollutants and remediate the polluted environment. An advantage of WRF fungi is that they do not require preconditioning to a particular pollutant because their degradation system is induced by nutrient deprivation (i.e., lack of ammonia). Furthermore, enzyme synthesis is not repressed by low chemical concentrations, even at levels ineffective for enzyme induction. Therefore, the fungi can effectively degrade very low concentrations of a pollutant at no detectable or nearly no detectable levels. Furthermore, WRF can reach the soil pollutants in ways that bacteria cannot due to its filamentous condition. Indeed, they grow by hyphal extension and extend in the soil with growth. Additionally, bacteria are much more sensitive to the toxicity of these pollutants. One of the greatest advantages of WRF is that their secreted enzymes can be purified and used as free or immobilized enzymes. All these findings support the choice of WRF as the most appropriate microorganisms for the bioremediation of soils contaminated with pesticides.

The initial attack by fungi is caused by ligninolytic system that consists of heme peroxidases, lignin peroxidase, manganese peroxidase and phenoloxidases such as laccase (Gianfreda and Rao, 2008). The ability of WRF to degrade lignin, suggests that such organisms may be useful for the biological decontamination of toxic and hazardous chemical, between them pesticides. Together with a hydrogen peroxide-generating system and cellulolytic and hemicellulolytic enzymes, WRF may act synergistically during the decay of wood. In particular, the ligninolytic enzymatic complex produced by *Phanerochaete chrysosporium* includes lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) and small amounts of laccase. LiP and MnP differ in their catalytic mechanisms. LiP acts by extracting single electrons from aromatic rings of lignin and lignin-like compounds, leading to the formation of a cation radical and subsequent cleavage reaction. MnP acts by generating Mn(II), a highly reactive intermediate, which, when stabilized by a chelator, can diffuse from the enzyme active site to attack and oxidize the lignin structure in situ (Rubilar et al., 2008).

The use of enzymes from fungi and bacteria for environmental purposes has increased with the time due to properties of this type of proteins. Enzymes are versatile, efficient and they are capable of performing specific reactions very often at so elevated rate not reachable by traditional chemical or

physical catalysis (Rao et al., 2014). Besides, enzymes in soil play an important role due to they are involved in all biochemical transformations in soil, and fungal extracellular oxidoreductases may play a particular important role in the xenobiotic degradation. Furthermore, soil enzymes activities may be used to evaluate the degree of soil degradation in natural and agro-ecosystems, being easy to measure and rapidly responding to the changes caused by both natural and anthropogenic factors (Gianfreda and Rao, 2008). Pesticides of different chemical nature and compounds like polychlorinated biphenyls (PCBs), chlorophenols and polycyclic aromatic hydrocarbons (PAHs) have been transformed or degraded by enzymes produced by fungi and bacteria (Diez et al., 2010). For example, DDT is one of the most persistent pesticides in the environment and was banned some years ago. In spite of its persistence, DDT was mineralized under nitrogen deficient conditions by ligninase system of *P. chrysosporium*.

Biological decomposition of pesticides is the most important and effective way to remove these compounds from the environment. Microorganisms have the ability to interact with substances leading to structural changes or complete degradation of the target molecule. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic generally. Then, when released into the soil, they are susceptible to further degradation by bacteria (Gianfreda and Rao, 2008).

Pesticides affect soil microbial activity, they may act at different levels modifying and regulating protein biosynthesis by repression or induction. They may have effects on membranes and other physiological effects, and may influence the dynamics of the soil populations and the soil biodiversity. The dynamics of the soil populations could be affected by the death of sensitive organisms with the consequent utilization of the organic residues by the surviving populations, the direct utilization of pesticides by the organisms that are able to degrade or to metabolize them (as C source for microbial growth), and the development of microbial populations that depend on secondary nutrient sources (metabolites produced from the decomposition of the pesticide or excreted by the proliferating microflora) (Gianfreda and Rao, 2008). The most frequently observed effects are the decrease of microorganism population and a general negative influence on some biochemical pathways of the nitrogen biological cycle such as inhibition of N-fixation or denitrifying activity. However, many negative, positive, and null effects on soil microbial activities have been observed by other authors, even when studies have been conducted on the repeated application of one or more pesticides (Gianfreda and Rao, 2008).

Several works have reported that native microorganisms from soil and sediment are capable of degrading pesticides. In this work, we will report the use of chilean native WRF and its enzymes in the degradation of some pesticides, considering the following aspects:

1. Selection of native fungi. Evaluation of ligninolytic enzymes
2. Optimization of the growth conditions and ligninolytic enzymes production
3. Extraction, purification , characterization and immobilization in nanoclays of MnP
4. Evaluation of different Technologies.
5. Immobilization of white–rot fungi (ligninolytic supports)

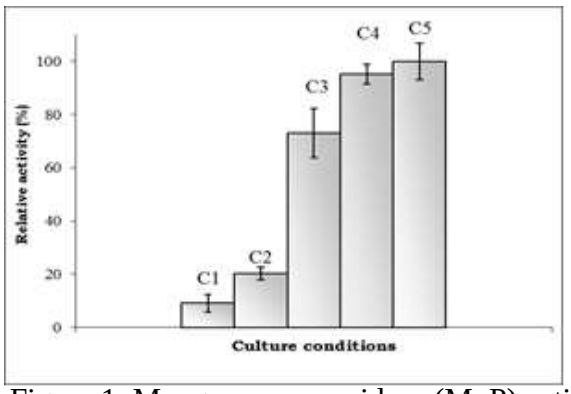
In our research group, the main activities have been focused on the search of native fungi in forests of the south of Chile with high ligninolytic capacity (Tortella et al., 2008), the optimization of the growth conditions and enzymes production (Bustamante et al., 2011) the characterization of the MnP enzyme (Acevedo et al., 2011), the evaluation of diverse supports (lignocellulosic wastes, nanoclays) for the immobilization both the fungi and the MnP, for the degradation of organic compounds such as chlorophenols, PAHs and some pesticides (Acevedo et al., 2010; Elgueta et al., 2016). The degradation of contaminants by *A. discolor* using different technologies, has been studied,

as well as, the comparison with other WRF fungi (*P. chrysosporium* and *Bjerkandera adusta*) (Rubilar et al., 2007). The degradation of chlorophenols using columns packed with allophanic soil inoculated with the fungus, and fixed-bed reactor (biobeds) for pesticides degradation (Diez et al., 2012; Diez et al 2013) was also studied.

Of the above mentioned studies, the principal results shows that some of native WRF of forests of the south of Chile present high ligninolytic potential and therefore aptitude to be evaluated for the bioremediation of contaminated environments. Fungal isolates obtained from various geographic sites in South of Chile (native Chilean forests) were obtained, including those most frequently used in biotechnology studies as *Trametes versicolor*, *Stereum hirsutum* or *Schyzophyllum commune*. A total of 48 fungi were collected, but not all did grow in in vitro conditions. The most representative isolated genera of fungi were *Trametes* (17%), *Ganoderma* (14%), *Stereum* 12%, *Neoclitocybe* 8%, *Anthracophyllum* 6% and of *Schyzophyllum* and *Tremella* 4%. Qualitative screening of twenty eight strains of native fungal Basidiomycetes of Chile indicated variation in the lignocellulolytic activities among these strains. Results indicated that most of the strains presented laccase activity (73%), whereas peroxidase and cellulose activities were presented in 40% and 37% of the strains, respectively. Other enzymatic activities were obtained at a lower percentage (xylanase 28% and Tyrosinase 7%). The control strain, *P. chrysosporium* CECT-2798, only presented peroxidase and xylanase activity. *Anthracophyllum discolor* Sp4, *Lenzites betulina* Ru-30, *Stereum hirsutum* Sp1 and *Trametes versicolor* Ru-0030 presented all enzymatic activities evaluated, except tyrosinase, which was only detected in the two *S. hirsutum* strains (Tortella et al., 2008). Eleven selected strains presented high concentrations of lignin peroxidase (Lip) and manganese peroxidase (MnP). *A. discolor* (Sp4), produced high LiP and MnP activity compared to the control fungus *P. chrysosporium* CECT-2798.

The major growth of *A. discolor* and enzymatic production was in medium containing glucose, low concentration of nitrogen, in static conditions and immobilized on wheat grains. The best conditions for ligninolytic activity were pH 5.5, temperature of 26ºC, and C/N ratio of 250 (Bustamante et al., 2011). The culture conditions largely influences the MnP production from the fungus *A. discolor* (Figure 1). The MnP enzyme from *A. discolor* was partially purified and characterized showed thermal stability between 40 and 50 ºC, the Km values for 2,6-DMP and H_2O_2 were 24.83 µM y 36.98 µM, respectively (Acevedo et al., 2011). Manganese peroxidase (MnP) produced by *A. discolor*, was immobilized on nanoclay and its ability to degrade polycyclic aromatic hydrocarbons (PAHs) compared with the free enzyme was evaluated. At the same time, nanoclay characterization was performed. Results indicated that 75% of the enzyme was immobilized on the nanoclay through physical adsorption. As compared to the free enzyme, immobilized MnP from *A. discolor* achieved an improved stability to temperature and pH. The immobilized enzyme was able to degrade pyrene (> 86%), anthracene (> 65%), alone or in mixture, and to a less extent fluoranthene (< 15.2%) and phenanthrene (< 8.6%). Compared to free MnP from A. discolor, the enzyme immobilized on nanoclay enhanced the enzymatic transformation of anthracene in soil (Acevedo et al., 2010).

The degradation of pentachlorophenol (PCP) in soil slurry cultures by *B. adusta* and *A. discolor* was evaluated. In this case, the high degradation rates obtained during the fungal cultures are due to the agitation imposed to maintain the slurry phase, which, in turn, increased the bioavailability of the pollutant to the fungi. From the two fungal strains evaluated, *A. discolor* presented the best results of ligninolytic activity and PCP degradation (> 90%). Similar result is observed in the degradation of lignin in reactor airlift using pelletized *A. discolor* as inoculum (Rubilar et al., 2007).



	Culture Conditions	MnP (U/L)
C1	Agitated conditions	130
C2	Static conditions	290
C3	C2 + wheat straw	1040
C4	C3 + inducer ($MnSO_4$)	1367
C5	C4 + surfactant	1436

Figure 1. Manganese peroxidase (MnP) activity associated with different culture conditions (C1 to C5) of the white-rot fungus *Anthracopyllum discolor* Sp4 (adapted from Acevedo et al., 2011)

The degradation of chlorophenols (2,4-DCF, 2,4,6-TCF and PCF) in columns packed with allophanic soil inoculated with the *A. discolor* immobilized in wheat grains, in continuous process was evaluated. The chlorophenols were added alone and in mixtures concentrations between 100 and 300 mg/L. The inoculated columns increased significantly the useful life and the efficiency of degradation of the chlorophenols. In fixed-bed reactor contaminated with PCF (100 to 300 mg/L) and inoculated with *A. discolor* immobilized on wheat grains, the major removal of the chlorophenol was observed in comparison with non-inoculated beds (Diez et al., 2012).

The formulation of pelletized support to immobilize the WRF *A. discolor* to evaluate its capability to degrade the atrazine using a biopurification system (BS), was also studied. Different proportions of sawdust, starch, corn meal and flaxseed were used to generate three supports (F1, F2 and F3). Immobilizations with coated and uncoated pelletized support (CPS and UPS, respectively) were assessed. UPS-F1 was selected as the most effective system to the fungal inoculation in the BS. The immobilized A. discolor was efficient at colonizing the support formulated. The highest atrazine degradation was observed after 30 days. The result obtained for phenoloxidase and respiration activity indicates that inoculated and non-inoculated BS were biologically active over the incubation time. The fungal inoculation might improve the atrazine degradation in the BS (Elgueta et al., 2016).

In conclusion, the white-rot fungi have high degradative and ligninolytic capacity to eliminate organic recalcitrant compounds as pesticides, chlorophenols, PAHs and other organic recalcitrant molecules, for which its utilization in biorremediación of contaminated environments is promissory.

Keywords: Pesticides, Enzymes, fungi.

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