LACCASES AND MANGANESE PEROXIDASES OF *PLEUROTUS OSTREATUS* GROWN IN SOLID-STATE FERMENTATION

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ABSTRACT

Ligninolytic system of white-rot fungi is directly involved in the degradation of various xenobiotic compounds and dyes. This system could show enzymes such as laccases, manganese peroxidases (MnP), veratryl alcohol oxidase (VAO) and versatile peroxidases (VP). In this work, laccase and manganese peroxidase activities of *Pleurotus ostreatus* grown in solid-state fermentation using polyurethane foam as inert support were evaluated. Maximal laccase activity was observed at the begin of stationary phase of growth and the manganese peroxidase showed the maximal activity at the end of fermentation.

Keywords: *Pleurotus*, fermentation, enzymes, laccase, manganese peroxidase

INTRODUCTION

Over the years, have been generated products from chemicals that would be considered a necessity for daily life, but these products become a problem for the world population, because these could generate problem of pollution to the environment. These compounds are called xenobiotics, including pesticides and dyes present in waste of industrial processes such as in the effluent from the paper-producing industry and the textile industry. White rot-fungi are capable of degrading some xenobiotics compounds and different kinds of environmental pollutant due to its ligninolytic enzymatic system [1–2]. Lignin biodegradation is a key step of carbon recycling happening in almost all terrestrial ecosystems. In this process, all the white-rot basidiomycetes members (including *Pleurotus*) play an important role in the degradation of these recalcitrant woody polymers for the sustenance of microbial populations, allowing the proper utilization of the degraded cellulose [3]. Ligninolytic enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile, cosmetic industrial sectors and more. Species of *Pleurotus* genus, including *P. eryngii, P. sapidus, P. pulmonarius and P. ostreatus* produce ligninolytic enzymes such as laccases, manganese peroxidases (MnP), veratril alcohol oxidase (VAO) and versatile peroxidases (VP), in both, submerged (SF) or solid-state fermentation (SSF) [4–7].

MATERIALS AND METHODS

Microorganism: A strain of *Pleurotus ostreatus* from the American Type Culture Collection (ATCC 32783) (Manassas, Virginia, U.S.A.) was used.

Solid-state fermentation: The solid-state fermentation (SSF) was carried out in a flask of 250 ml containing 0.5 g of polyurethane foam of low density (PUF; 17 kg/m3) cubes (0.5×0.5×0.5 cm) as an inert support [8] impregnated with 15 mL of sterile culture medium (pH 6.5) with composition (in g/L): glucose, 10; yeast extract, 5; KH_2PO_4, 0.6; $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 0.4; $CuSO_4.5H_2O$, 0.25; $FeSO_4.7H_2O$, 0.05; $MnSO_4$, 0.05; $ZnSO_4.7H_2O$, 0.001 [9]. Three mycelia plugs (4 mm diameter) taken from the periphery of colonies of *Pleurotus ostreatus* grown for 7 d at 25 ºC in Petri dishes containing potato dextrose agar (DIFCO™) were used as inoculum for each flask. The cubes were washed twice with hot distilled water, oven-dried at 60 °C for 24 h, and then autoclaved at 120 °C for 15 min, before the culture. All inoculated flasks were incubated at 25 °C and samples were taken every 24 h after third day of growth.

Enzymatic extract preparation and biomass evaluation. The enzymatic extract (EE) was obtained by soft pressing the PUF cubes and the broth was filtrated using filter paper (Whatman No. 4), and the biomass (X) immobilized on PUF cubes was determined as difference of dry weight (g/L) [8].

The assay of biomass $X = X(t)$ was done using the Velhurst-Pearl logistic equation,

$$\frac{dX}{dt} = \mu\left[1 - \frac{X}{X_{\max}}\right]X \qquad (1)$$

where μ is the maximal specific growth rate and X_{max} is the maximal (or equilibrium) biomass level achieved when $dX/dt = 0$ for $X > 0$. The solution of equation 1 is as follows,

$$X = \frac{X_{\max}}{1 + Ce^{-\mu t}} \qquad (2),$$

where $C = (X_{max} - X_0)/X_0$, and $X = X_0$ is the initial biomass value.

Estimation of kinetic parameters in the above equations was performed using the non-linear least square-fitting program "Solver" (Excel, Microsoft) [8, 9].

Laccase activity assay. Laccase activity was determined in each EE by changes in the absorbance at 468 nm, using 2,6-dimethoxyphenol (DMP) as substrate. The assay mixture contained 900 µL substrate (2 mM DMP in 0.1 M acetate buffer pH 4.5) and 100 µL EE, which were incubated at 40 ºC for 1 min [9]. The activity was expressed in International Units per liter (U/l) of EE ($å_{468}$= 35645 M^{-1} cm^{-1}).

Manganese peroxidase. Manganese peroxidase (MnP) was determined by phenol red oxidation at 610 nm with extinction coefficient $å_{610}$=4460 $M^{-1}cm^{-1}$. The reaction mixture contained 20 mM succinc buffer, pH 4.5, 250 mM sodium lactate, 0.5 % bovine serum albumin, 2 mM $MnSO_4$, 0.01% phenol red, 2 mM H_2O_2 and 50 µl EE, which were incubated at 35 ºC for 1 h. The activity was expressed in U/l of EE.

RESULTS AND DISCUSSION

Fig. 1 shows the growth of *Pleurotus ostreatus*, the X_{max} was observed at the 120 h with of 5.5 g/l approximately, and a ì of 0.085 h^{-1}. The ì value was four times higher than the reported when the fungus grew in submerged culture, however, the X_{max} decreased 42% approximately [10].

The maximum laccase activity was observed at 144-168 h of fermentation with a value of about 400 U/l, after that time, the laccase activity was constant of about 100 U/l (Fig. 2). On the other hand, the MnP activity was approximately 3 U/l from the beginning of the fermentation until 456 h, peaking at 500 h with 5 U/l (Fig. 3).

Pleurotus ostreatus HAI 595 and *Pleurotus eryngii* HAI 507 were distinguished from the other species by significantly higher laccase activities reaching maxima of 4808.7 ± 164.3 U l^{-1} and 4531.0 ± 95.6 U l^{-1}, respectively, on day 14 of cultivation. The minimum MnP activity was found in *P. ostreatus* on day 7 of cultivation (12.6 ± 1.3 U l^{-1}), though with further cultivation it significantly increased to 1147.8 ± 162.5 U l^{-1}, during solid-state fermentation of wheat straw [11].

No peroxidase activity was detected during both *P. ostreatus* and *T. versicolor* fermentations. A maximum laccase activity production level of 15 U g^{-1} of dry matter (U g^{-1} d.m.) was achieved at the third to fourth day of *P. ostreatu*s fermentation, during *T. versicolor* SSF, a maximum laccase activity value of around 35 U g^{-1} d.m. was produced at the 16th day, keeping constant at least until the 22nd day [12].

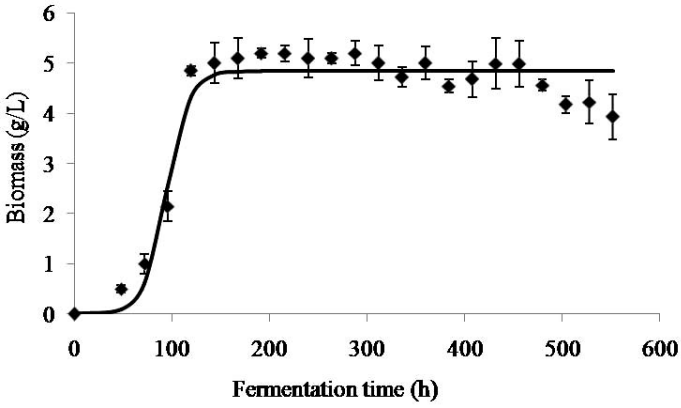

Figure 1. Biomass evolution of *Pleurotus ostreatus* grown in SSF on PUF

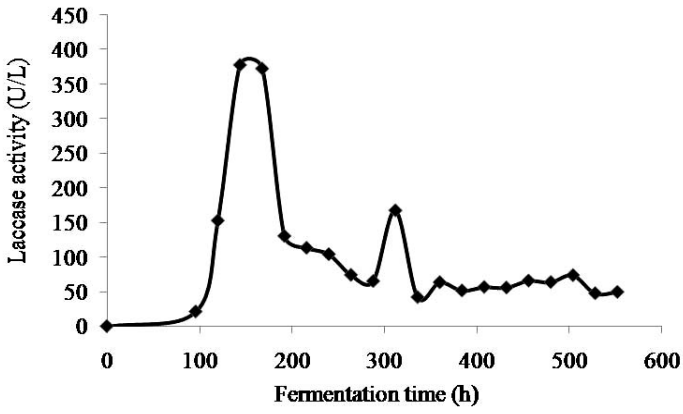

Figure 2. Laccase activity of *Pleurotus ostreatus* grown in SSF on PUF

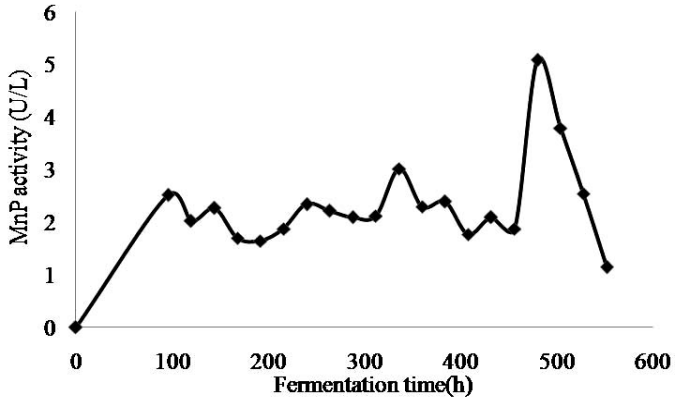

Figure 3. MnP activity of *Pleurotus ostreatus* grown in SSF on PUF

CONCLUSIONS

The strain of *Pleurotus ostreatus* used in this study showed that it can produce ligninolytic enzymes by SSF on an inert support, so it is suggested further studies to establish the optimal conditions for increase production.

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REFERENCES

[1] Kirk TK and Farreli RL. (1987). Enzymatic Combustion: The microbial degradation of lignin. *Annual Review of Microbiology.* 41: 465-505.

[2] Have R and Teunissen PJM. (2001). Oxidative Mechanisms Involved In Lignin Degradation. *Whiterot fungi. Journal Chemical Reviews.* 101: 3397-3413.

[3] Khanna PK *et al.* (1992). Evaluation of *Pleurotus* spp. for growth, nutritive value and antifungal activity. *Ind. J. Microbiol.* 32 : 197-200.

[4] Herrera-Mora JA *et al.* (2004). Efecto de las enzimas ligninolíticas de los hongos *Trametes versicolor* y *Phanerochaete chrysosporium* inmovilizados en espuma sobre la remoción de color, demanda química de oxígeno y clorofenoles en aguas residuales de industria papelera colombiana.

[5] Rothschild N *et al.* (1999). Manganese deficiency can replace high oxigen levels needed for lignin peroxidase formation by *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*. 65(2): 483-488.

[6] Ullah MA *et al.* (2000). Evaluation of solid substrates for enzymes production by *Coliorus versicolor*, for use in bioremediation of chlorophenols in aqueos effluents. *Applied Microbiology and Biotechnology.* 54: 832-837.

[7] Cullen D and Kersten P. (2004). Enzymology and molecular biology of lignin degradation. The mycota III, springer-Verlag. 13: 249-273.

[8] Díaz-Godínez G *et al.* (2001). Exopectinases produced by *Aspergillus niger* in solid-state and submerged fermentation: a comparative study. *Journal of Industrial Microbiology and Biotechnology.* 64: 271-275.

[9] Téllez-Téllez M *et al.* (2008). Growth and laccase production by *Pleurotus ostreatus* in submerged and solid-state fermentation. *Applied Microbiology and Biotechnology.* 81: 675-679.

[10] Díaz R *et al.* (2013). Influence of initial pH of the growing medium on the activity, production and genes expression profiles of laccase of *Pleurotus ostreatus* in submerged fermentation. *Electronic Journal of Biotechnology.* 16(4).

[11] Kne•eviæ A *et al.* (2013). Lignin degradation by selected fungal species. *Bioresource Technology.* 138: 117-123.

[12] Iandolo D *et al.* (2011). Enzyme production by solid substrate fermentation of *Pleurotus ostreatus* and *Trametes versicolor* on tomato pomace. *Appl Biochem Biotechnol.* 163: 40–51.