ACTIVIDAD ENZIMÁTICA LIGNINOLÍTICA DE HONGOS DE PUDRICIÓN BLANCA SOBRE PAÑAL DESECHABLE

Enzymatic ligninolytic activity of white-rot fungi on disposable diaper

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ABSTRACT
In this study, ligninolytic enzyme activities of strains native Chilean white-rot fungi were evaluated. Strains in this evaluation were: Stereum hirsutum (Ru-104), Stereum sp. (Ru-24), Trametes versicolor (Ru-107), Trametes versicolor (Ru-0030), Trametes versicolor (Tvhl), Stereum hirsutum (Sp1), Phanerochaete chrysosporium (Pn) Inonotus sp. (Sp2), Galerina patagonica (Sp3) and Anthracophyllum discolor (Sp4) on disposable diaper as substrate, because these fungi have a system extracellular enzyme system not specific, capable of degrading a variety of highly complex structures, including cellulose which is the major component of the disposable diaper. By evaluating the qualitative enzymatic activity was observed an increase of 2,2'-azino-di(3-ethylbenzthiazoline-6-sulphonic acid) medium oxidation and Reactive black 5 medium discoloration, it indicating the presence of laccase and peroxidase activity respectively. However, Inonotus sp. (Sp2) non-was capable of oxidize the 2,2'-azino-di(3-ethylbenzthiazoline-6-sulphonic acid) medium, therefore is concluded that this strain has low or no laccase activity. Enzyme activities evaluated showed a greater activity the strains that had the disposable diaper as substrate that when strains weren't with the diaper in the medium, especially the MnP activity, reaching values of up to 160 U/L by A. discolors (Sp4). With these results it is shown that white-rot fungi have enzymatic activity on disposable diapers. Therefore, these fungus will could be used as inoculum in a composting system of disposable diapers, which will could accelerating the degradation process of this residue, besides obtaining a product applicable as compost.

KeyWords: diapers, ligninolytic enzymes, white-rot fungi.

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INTRODUCTION

Accelerated modern life has caused an increase in the use of disposable products such as diapers, which are characterized by their easiness in its use and comfort for the general population.

Hygienic products are composed of polymers derived from wood, such as cellulose, and others derived from oil, such as sodium polyacrylate or superabsorbent polymers (SAP), polystyrene (PE) and polypropylene (PP), which are highly persistent in the environment. In relation to diapers, about 65% of their composition belong to these polymers (EDANA, 2008), apart from of the own residues containing the diaper after use, where urine corresponds to 58% of the total weight of the loaded diaper (Aumonier *et al.*, 2008). Only in European countries, approximately 46 million of diapers a day are used, considering under 3 years-old children (EDANA, 2008). In Chile, about 4 million diapers are used a day, resulting in a generation of approximately 540 tons d^{-1} waste. The main final disposition of this waste is landfill, whose main impacts are the use of land, gas emissions including methane which can cause explosions and contributing to global warming, and possible leachate generation that can contaminate groundwater (Ashley *et al.,* 2003). Few studies are related to degradation of diapers, SAP degradation where has been evaluated, generating much controversy, because structurally complex polymer is difficult to degrade (Stahl *et al.,* 2000). Another polymer evaluated was cellulose, which was degraded up to a 90%, where used *Pleurotus Ostreatus* as degrading microorganism (Espinosa-Valdemar *et al.,* 2011). Moreover, the quality of the compost from composting system of diapers, was evaluated (Colón *et al.,* 2011), obtaining a high quality product. However, blends of biodegradable and non-biodegradable polymers as lignin with PS have been made reducing the volume of non-biodegradable polymer up to an 80%, where a WRF was the degrading organism (Milstein *et al.,* 1992). These because they have an extracellular ligninolytic enzyme system composed mainly peroxidases and laccases able to degrade a variety of structures (Tortella *et al.,* 2008).

Based on these studies the general objective of this study is enzymatic ligninolytic activity of WRF strains on the disposable diaper.

MATERIALS AND METHODS

WRF strains *Stereum hirsutum* (RU-104), *Stereum sp.* (RU-24), *Trametes versicolor* (RU-107), *Trametes versicolor* (RU-0030), *Trametes versicolor* (TVHL), *Stereum hirsutum* (Sp1), *Phanerochaete chrysosporium* (PN) *Inonotus sp.* (Sp2), *Galerina patagonica* (Sp3) y *Anthracophyllum discolor* (Sp4) obtained from the strains collection of the Environmental Biotechnology Lab. (UFRO), Chile. All strains were inoculated on a piece of the central part of the diaper (1x1cm) on plates with extract malt agar to 2% for their growth and where be incubated in the dark for 7 days at 26°C. For determine the enzymatic ligninolytic activity of WRF on the diaper was performed a **Qualitative enzymatic assay** for screening of fungal ligninolytic enzymatic activity on the diaper. This was be evaluated by discoloration Reactive Black 5 (RB5) to peroxidase activity and oxidation of ABTS (ABTS (2.2 '-azino-bis (3-ethylbenzothiazolina-6-Ac. Acid) to laccase activity in petri dishes (80 mm diameter) that was contain culture medium malt extract agar MEA 2%, supplemented with RB5 or ABTS, respectively. Plates was be inoculated with the fungus over the diaper in the centre of the plate, and for 10 days at 26°C. The rate of discoloration, oxidation and mycelial growth was be measure daily (mm) from the centre of the plate to the periphery, and the colony diameter (mm). **Quantitative enzymatic assay*:*** 3 pieces of diaper (1 x 1 cm) was be taken from the growth plates with strain and was be inoculated in Erlenmeyer flasks (250 mL) that contains 100 mL of modified Kirk medium, and was be incubated at 26°C for 30 days. Furthermore, controls were performed of all strains without the presence of the diaper and a control with only the diaper under same culture conditions. The enzymatic activities were determined spectrophotometrically.

RESULTS

Qualitative enzymatic assay

The results are can observe in the Figure 1. In the Figure 1) a) is seen that all strains have laccase activity, *Inonotus sp.* (Sp2) except, that it not was capable of oxidizing medium. In Figure 1) b) is seen that all strains have peroxidase activity, highlighting *T. versicolor* (Tvhl), *Stereum sp*. (Ru-24) and *A. discolor* (Sp4), which reached a higher discoloration of medium.

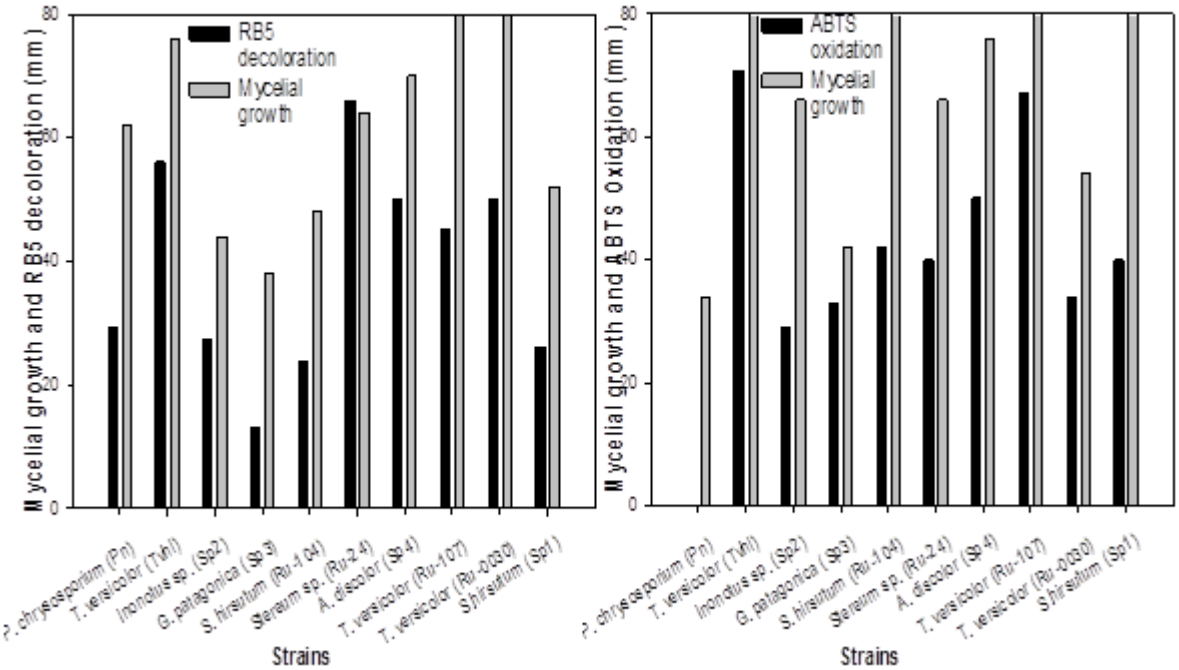

Figure 1 Enzymatic production of ten WRF strains on the diaper (Qualitative enzymatic assay) a) ABTS oxidation to laccase activity, b) RB5 discoloration to peroxidase activity.

Quantitative enzymatic assay

In general, the enzymatic activity was increasing (Lac, MnP and MiP) (Figure 2), principally when strains had the disposable diaper as substrate in the medium. *T. versicolor* (Tvhl), obtained the greatest Lac enzymatic activity when the strain had the diaper as substrate in the medium (Figure 2 b)), reaching the day 25, 160 U/L approximately, four times longer than when the strain was free in the medium. *Stereum sp*. (Ru-24) and *A. discolor* (Sp4), recorded the highest MnP activity, which reached activities of 160 and 130 U/L respectively on day 20. Finally, to the MiP activity (Figure 2e)f)), *T. versicolor* (Tvhl) was the strain that reached the highest activity on day 25 with 125 U/L (Figure 2f)), more than 50% compared with the strain without the diaper.

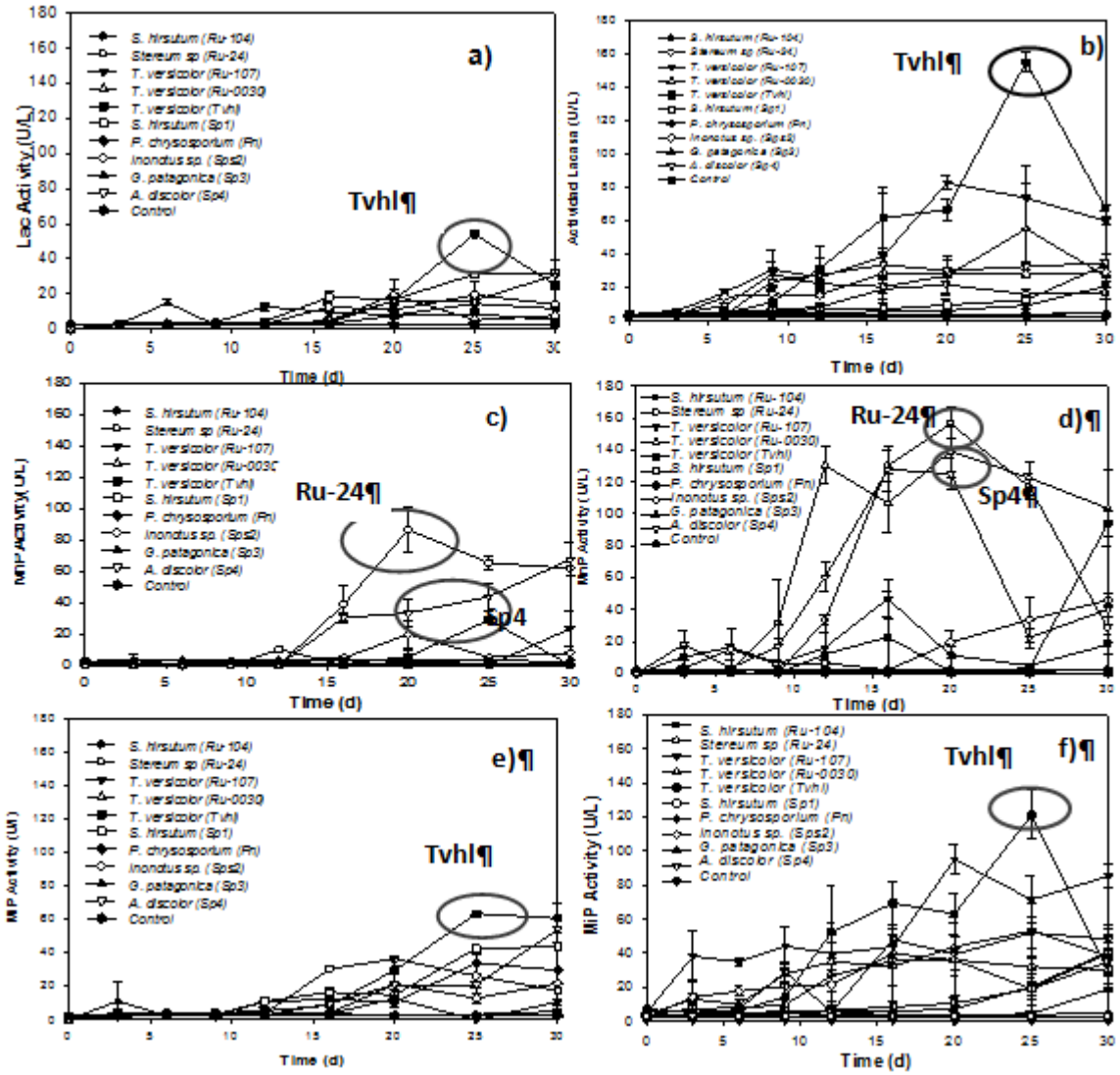

Figure 2 Profiles of ligninolytic activity of ten WRF strains in liquid medium for 30 days at 26°C (a) Lac activity of strains alone (b) Lac activity of strains +diaper (c) MnP activity of strains alone (d) MnP activity of strains + diaper (e) MiP activity of strains alone (d) MiP activity of strains + diaper.

CONCLUSIONS

These results show that white-rot fungi have a high enzymatic activity on disposable diapers. Therefore, these fungi could be a good alternative for degradation accelerate of these waste. A alternative is that they could be used as inoculum in a composting system of disposable diapers, with the purpose of accelerating the degradation of this residue and at the same time obtain a product applicable as the compost.

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