RECOMBINANT MANGANESE PEROXIDASE FROM *Phanerochaete chrysosporium* AND ITS LIGNINOLYTIC ACTIVITY ON SAWDUST FROM *Pinus spp*

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Introduction. Sawdust is the principal waste of the sawmill industry with very few uses (applications) representing an environmental problem. It is a source of cellulose which could be revalorized. Nevertheless, the main barrier in any lignocellulosic material is lignin. Due to its complexity and recalcitrant structure, its total removal is not possible with traditional chemical treatments. The use of ligninolytic enzymes for biopulping has proved successfully [1]. Fungal enzymes are the most effective ones for removing lignin due to their versatile oxidation mechanisms and *P. chrysosporium* is one of the most recognized fungi that can produce them [2]. Manganese peroxidase is thought to be the most important enzyme involved in the oxidative removal of lignin, due to its high redox potential [3]. Overexpression of this enzyme has been achieved but its activity on a lignocellulosic source such as sawdust hasn´t been evaluated [4].

The main goal of this work is to obtain a recombinant manganese peroxidase form *P. chrysosporium* and evaluate its ligninolytic activity on sawdust.

Methods. *P. chrysosporium* was cultured in Kirk's and Tien media [5] for the production of manganese peroxidase in the presence of sawdust. To determine the presence of the enzyme, its activity was measured according to the method described by Paszczinsky *et al* [6] using as criteria the increment on the absorbance at 270 nm. Total RNA extraction and cDNA synthesis were completed, and forward (5'GCGGTCTGCCCCGAC3', 5'CCCGACGGTACCAAG3', 5'GCCATAACTCGCGCCG3') and reverse degenerate (3'AKGCRGGRCCRTYGA5') primers were used to amplify the manganese peroxidase gene.

Results. Activity of manganese peroxidase was detected on day number six of the culture (Fig. 1). Amplification of the complete gene has not yet been approached (Fig. 2).

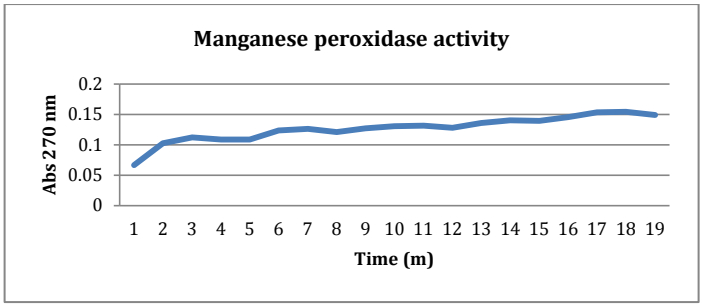

Fig.1 Determination of manganese peroxidase activity.

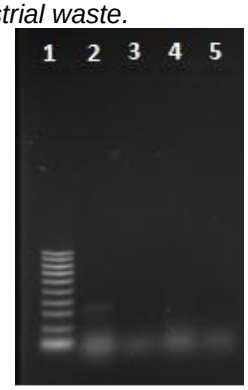

Fig.2 Electrophoresis of PCR products. Lane 1 MWM. Lane 2-3 amplification of manganese peroxidase gene using cDNA synthesized with oligodT's and its negative control. Lane 3-4 amplification of manganese peroxidase gene using cDNA synthesized with a degenerate reverse primer and its negative control.

Conclusions. A 300 bp putative manganese peroxidase gene has been amplified and currently sequencing confirmation is in progress. Activity was confirmed based on the method mentioned previously.

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