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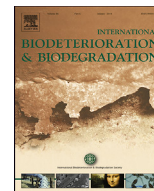

Biodegradation of eucalyptus urograndis wood by fungi

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ABSTRACT

We focused in selecting four fungi, naturally living in *Eucalyptus* sp. fields, for application in accelerating stump decay. The wood-rot fungi *Pycnoporus sanguineus* (Ps), *Lentinus bertieri* (Lb) and *Xylaria* sp. (Xa) were isolated from *Eucalyptus* sp. field and the fungus *Lentinula edodes* (Led) was obtained from a commercial strain. All fungi were studied according to their capacity to degrade eucalyptus urograndis wood. In order to evaluate mass losses of seven years old eucalyptus urograndis' wood test blocks from heartwood were prepared added to glass flasks with red clay soil. The humidity of the soil was adjusted with 50 and 100% of its water retention capacity. Mass loss evaluations occurred at 30 until 120 days after eucalyptus wood degradation. Chemical analysis and soil pH were measured only in the last evaluation. Mycelial growth assays with potato-dextrose-agar, malt-agar and sawdust-dextrose-agar at three temperatures was carried out in order to get information about the best conditions of fungi growth. On the 120th day, Ps and Lb showed good capacity of wood degradation by leading to a high mass loss in soil with highest humidity. These fungi were the best consumers of lignin, hemicellulose, cellulose and extractives, caused acidification in the soil. Ps and Lb had faster mycelial growth in sawdust-dextrose-agar, especially in high temperature, comparing to Xa and Led. Xa and Led are not good eucalyptus urograndis heartwood degraders, because they consume preferentially hemicellulose.

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1. Introduction

The *Eucalyptus* genus is the main forest specie cultivated in Brazil. In 2010, the planted area was around 4.9 million hectares (ABRAF, 2012). The most planted *Eucalyptus* tree in Brazil is the eucalyptus urograndis, generated from the crossing between *Eucalyptus grandis* × *Eucalyptus urophylla*. Because of this, eucalyptus urograndis has no scientific name and is not considered specie by geneticists. The planting of *Eucalyptus* spp. can be done in new areas, replacing the pastures, using reformed areas and also by regrowth (ABRAF, 2012). The harvesting of planted forests is usually performed after the seventh year of planting with chainsaws or mechanical harvesters. The timber is removed leaving only leaves, bark, thinner branches and the stumps, which remain fixed by their roots. Seven years after the first planting, new seedling plants are planted in between the plants that were harvested. However, with successive plantings the stumps hinder management activities, such as transit machinery for planting and fertilization.

After cutting the trees, mechanic stumps drawdown is performed to improve crop management. A complete withdrawal is required for the reuse of the area after the third crop rotation. This practice has a high cost, and usually, they are calculated by each hour worked. According to Pavan et al. (2010), for removing the stumps from 1 ha of a planted 21 year-old forest, is necessary 25 h costing approximately US$ 50.00/hr. The cost of stump removal does not vary by age, but by the density of the forest. For removing the roots, a widespread excavation is necessary, generally two or 3 m deep, to assess and remove the roots.

Besides the high costs of *Eucalyptus* spp. production for companies and smaller producers, the environment also pays with grave disturbance. The stumps and roots removal not only causes severe disruption in the microbiota, but also modifies some

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physical and chemical characteristics of the soil for several years. After one year of removing roots, several changes occur in the soil, such as decrease in the concentration of nitrogen, carbon and sulfur, and increase in concentrations of phosphorus. It can take up to 10 years for nutrient levels to be normalized in soils after removal of these roots (Hope, 2007). In an attempt to minimize these problems, the biodegradation of roots has been studied. This may provide recycling of nutrients from the wood to the soil, promoting an increase of biodiversity and richness of microorganisms, also improving physical-chemical characteristics of the soil. *Eucalyptus* spp. reforestation areas with the system that maintain the soil undisturbed have high stable carbon content, with positive effects on the reduction of CO_2 emissions. Regarding the organic matter present on the surface, the roots contribute for most stable forms of carbon in forest soils (Petersson and Melin, 2010; Kätterer et al., 2011).

In nature, many fungi can degrade wood, but the best wood-degrading fungi are the basidiomycetes, such as white-rot fungi. These fungi are able to degrade efficiently wood components, especially lignin (Rayner and Boddy, 1988). The accelerated degradation of stumps and roots with white rot fungi has been studied as a way to prevent or reduce the disturbance of soil in planted forests. The degradation of *Eucalyptus* roots in reforestation fields is termed as "biological stump removal", and consists of inoculating the stumps with wood degrading fungi. Some topics should be carefully considerate to achieve success in roots biodegradation, such as selection of the fungi according to their habitat, choosing the best season to inoculate (temperature, humidity) and confirm that the microorganism is not phytopathogenic (Alonso et al., 2007; Andrade et al., 2012). The great advantage of the "biological stump removal" application in Brazil is the high temperature in freshly cut areas of *Eucalyptus* fields, where the solar incidence is high during many months of the year. The temperature contributes to the activity of microorganisms that live in dead roots, reaching their climax in the range of 30–40 °C (Chen et al., 2000). The high frequency of rain during the summer also offers great conditions for fungi growth in *Eucalyptus* fields.

The research of wood degradation by fungi is extremely important to forestry companies, because it enables proper management of the *Eucalyptus* roots and stumps in reforestation fields. Using properly, some fungi can be considered as a sustainable way to accelerate roots and stumps waste recycling. This study aims to select fungi with high wood degradation ability, in order to apply them in accelerating biodegradation of stumps and roots of *Eucalyptus* spp.

2. Materials and methods

2.1. Wood decay fungi

During February 2009 till June 2011 we collected several fungi from *Eucalyptus* spp. fields located in southeast region of the State of São Paulo, Brazil. *Pycnoporus sanguineus*, *Lentinus bertieri* and *Xylaria* sp. were identified at Botanic Institute of São Paulo by Dr. Marina Capellari. The fungi *P. sanguineus* and *L. bertieri* were deposited in the Culture Collection of Algae, Cyanobacteria and Fungi of Botanic Institute – CCIBt, São Paulo-SP, Brazil, with respective numbers CCIBt 3817 and CCIBt 3818. *Xylaria* sp. and commercial isolate of *Lentinula edodes* (Led) are deposited in the Fungi Collection of the Mushrooms Module, Faculty of Agricultural Sciences, UNESP, Botucatu-SP, Brazil, with respective numbers BT-23 and LED 98/47.

The selection of these fungi between all those collected (around thirty isolates) was made by observations of mycelial growth in different solid medium (described below) and *Eucalyptus* sawdust (data not shown).

2.2. Mycelial growth

The mycelial growth of Ps, Lb, Xa and Led was evaluated in petri dishes with potato dextrose-agar, 2% malt extract agar (MA) and sawdust dextrose agar medium (SDA). The SDA medium was prepared according to Eira and Minhoni (1997), using *Eucalyptus* spp. sawdust, wheat and corn bran and calcium carbonate (41:1:1:1, v/v) were mixed and autoclaved for 4 h. 80 g from this mix were boiled in 1 L of distilled water. After filtration of the boiled sawdust 12 g L^{-1} agar were added to prepare the SDS solid media. All culture medium were autoclaved at 120 °C for 20 min, and placed in disposable petri dishes (90 × 15 mm). The fungal growth was measured daily, until 7.8 cm diameter. The experimental design was completely randomized factorial with 4 × 3 × 3 (fungi × culture medium × temperatures), with five replicates.

2.3. Assay of accelerating wood degradation

2.3.1. Preparation of the experimental plots

The experimental plots were based in the Standard ASTM D-2017 (1997), using 600 mL glass bottles filled with 300 g of clay texture red soil, pH 4.7, water retention capacity of 29%. The soil was screened through a 5 mm mesh and dried in green house conditions (25 °C, for 30 days). Soil humidity was obtained according to water field capacity. In the treatment with 50% (U50) 70 mL distilled water was used to get 50% of humidity and 140 mL to get 100% of soil humidity. After correction of the soil humidity, a *Pinus taeda* feeder (30 × 50 × 5 mm) was placed on the soil surface of each glass bottle and sterilized by autoclaving 121 °C for 1 h during three consecutive days. Control treatments were divided in A, B and C. Control A was prepared with bottles with not sterilized dry soil. Controls B and C, with 50 and 100% of moisture, respectively, were sterilized.

The experimental design was completely randomized in a 4 × 2 × 6 factorial (fungi × humidity × degradation evaluation time). The evaluation time of wood mass loss was at 30, 60, 75, 90, 105 and 120 days after inoculation, with eight replicates.

2.3.2. Eucalyptus wood samples

In this study, the wood samples were obtained from 13 trees of the hybrid eucalyptus urograndis (named clone 105 urograndis) seven years old, donated by the Eucatex Company S/A. Each wood sample had 50 cm of length (cut from 10 cm above the ground), and stored under green house conditions (25 °C, humidity bellow 20%) during 45 days. The bark and sapwood were removed, using only the heartwood to make the wood blocks, on dimensions of 25 × 25 × 10 mm (length, width, direction of the fibers). The inclusion criteria for the use of wood blocks were: no defects, as knots, resin or gums, and without visible evidence of fungus infection. From each tree sample were made approximately 200 wood blocks, totaling 2600 samples, which were mixed among the thirteen samples.

Each wood block was sanded (emery 20 mm), labeled and dried in the oven (102 ± 3 °C) until reached constant weight (Gehaka BG 400). The initial mass (W_1) of each sample was measured, stored in glass Petri dishes (120 by 20 mm) and sterilized by autoclaving at 120 °C for 15 min.

2.3.3. Wood inoculation

The parameters for accelerated degradation of wood by the fungi were based on Standard ASTM D-2017 (1997) and ASTM D-1413 (1994). A 7 mm diameter mycelial disc of each fungus

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