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BIODEGRADATION OF *Triplochiton scleroxylon* K. SCHUM *IN VITRO* BY WHITE ROT FUNGI

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ABSTRACT
A study was initiated as in situ laboratory approach for screening the effects of three white rot fungi on Triplochiton scleroxylon wood blocks. The test blocks were prepared into 50x25x15mm and exposed to pure cultures of Coriolopsis polyzona; Pycnoporus sanguineus; Ganoderma lucidum and Lenzites palisoti for 16 weeks. The test blocks that were decayed for 16 weeks by the fungal organisms had averages of 49.9, 25.92, 31.73 and 21.52% weight loss respectively. The wood sample analyzed for lignin and cellulose loss as a result of attack by these fungal organisms indicated an average loss of 70.2% to 88.5% and 54.8 to 67.1% respectively. Extensive degradation of lignin was noticed throughout the secondary wall and middle lamella region between cells. In cells badly decayed by Coriolopsis polyzona, the middle lamella between cells was completely degraded.

Key words: White rot, Cellulose, hemicellulose, lignin, degradation, mycelia, and. Wood species

INTRODUCTION

Lignin, the most complex of the cell wall constituents is not readily degraded by most microorganisms (Zabel and Morrell, 1993). White rot basidiomycetes are one of the few groups of microorganisms that can degrade lignin. These organisms can degrade wood by simultaneously attacking lignin, cellulose and hemicelluloses or by specifically removing individual cell wall components (Otjen and Blanchette, 1985; Blanchette and Reid, 1986; Zabel and Morrell, 1993; Adetogun *et al*, 2009; Adetogun *et al*, 2010). Cellulose fibre, the major component of wood by weight can be used in making paper or as feed for ruminants or in chemical waste management (Otjen and Blanchette, 1985; Blanchette and Burnes, 1988; USDA, 1998; Zabel and Morrell, 1993; Scott, *et al*, 2002). However, for fungi to be considered for application to industrial biotechnology processes, it is important to understand how the degradation process takes place. According to Blanchette *et al* (1985), white rot are known to degrade lignin in two morphologically distinct ways. One type of decay is selective for lignin and hemicelluloses removal while the other type of decay is nonselective and all cell wall components are removed simultaneously either directly around fungal hyphae, causing erosion troughs or holes, or uniformly from the cell lumen outward, causing a gradual thinning of the cell walls (Wilcox, 1968). The purpose of this study was to observe at 4 week intervals, the process of selective delignification of *Triplochiton scleroxylon* K. Schum, *in vitro* by *Coriolopsis polyzona* (Pers) Ryv; *Pycnoporus sanguineus* (Lex fr) Murid; *Ganoderma lucidum* (Leysex) Karsten, and *Lenzites palisoti* Fr which cause selective delignification of tropical wood species.

MATERIAL AND METHODS

Wood specimens measuring 50 x 25 x 15 mm were prepared for decay test, from air dried sapwood of *Triplochiton scleroxylon* according to the requirements of ASTM D 1413- 76. A total of 76 wood blocks were prepared from each wood species. The blocks were sterilized in the oven for 18 hours at a temperature of 103^0C. The weight obtained after oven drying was taken as the initial dry weight (Dw1) of the individual specimen before incubation.

The blocks were conditioned in a desiccator for two months prior to the decay test. Four pairs of test blocks were aseptically placed in each Kolle-flask containing 40ml of cassava dextrose agar (CDA) (peeled cassava 200g; dextrose 12g; agar 17g; distilled water 1000ml) (Adetogun, 1998) with fully growing mycelia of a monoculture of the test fungi [*Coriolopsis polyzona*; *Pycnoporus sanguineus; Ganoderma lucidum* and *Lenzites palisoti*]. The test blocks were incubated at 70%

relative humidity and at a temperature of 25 ± 2^0C for a period of 16 weeks. Four uninoculated test blocks served as control. The experiment was replicated six times. At the end of the incubation period, they were cleaned with dry cotton wool in order to remove adherent mycelia and were thereafter oven-dried. Their weights were then taken (Dw2) so as to calculate percentage losses arising from the fungi decay of the specimens.

$$\frac{Dw1 - Dw2}{Dw1} \times \frac{100}{1}$$

Dw1: Weight of test blocks before incubation
Dw2: Weight of test blocks after incubation.

Four test blocks per fungal strain used in the study were used for lignin and cellulose determination. The decayed wood samples were milled in the hammer mill using a 40-mesh screen. The wood meals were analyzed for sulphuric acid lignin using the protocol of Effland (1977) as described by Blanchette *et al* (1985) while the hydrolysis neutralization and concentration of the decayed test blocks for cellulose analysis were done according to the procedure of Saeman *et al* (1954) as described by Blanchette *et al* (1985).

Other sets of four decayed test blocks per fungal strain were embedded in hot distilled water prior to sectioning with slide microtome. Changes in the test blocks due to the effect of the fungus were studied by first staining with safranin red in order to stain the lignin and then with fast green to stain the cellulose. The sections were then mounted on the slide with clove oil. Filter paper was used to drain off excess clove oil. The slides were covered and then smeared with Canada balsam. The slides were heated to allow the Canada balsam to spread in order to remove all air bubbles. A light microscope was used to detect the micro morphological changes in the wood due to decay by *Coriolopsis polyzona*; *Pycnoporus sanguineus; Ganoderma lucidum* and *Lenzites palisoti*

Statistical Analysis: Data collected from the experiment were transformed using Arc sin transformation procedure. The transformed data were subsequently analyzed using 2-way analysis of variance and LSD test for mean separation [Gomez and Gomez, 1984]

RESULTS AND DISCUSSION

The percent weight loss in wood biomass and percent loss of lignin and cellulose are presented in Table 1. After 16 weeks of incubation, the test blocks had loss an average of 49.99, 25/92, 31/73 and 21.52%, respectively of their dry weight; 70.2 to 88.5% and 54.8 to 67.1% of their lignin and cellulose respectively by *Coriolopsis polyzona; Pycnoporus sanguineous*; *Ganoderma lucidum* and *Lenzites palisoti*.

The extent and rate of decay varied substantially between the different strains of the fungal organisms. *Coriolopsis polyzona* was more virulent in attack than other fungal organisms used in the study. The trends of delignification by these fungal organisms were similar. The result of this study is in consonance with the work of Blanchette *et al* (1985) that chemical analysis of decayed wood for lignin and cellulose loss is a poor indicator of the capacity for various fungi to intermittently cause selective lignin degradation. White rot fungi that attack various hardwoods, according to reports appear to preferentially degrade the syringyl lignin component (Highley, 1982; Blanchette and Reid, 1986).

Table 1: Percentage weight, lignin and cellulose loss of wood blocks of *Triplochiton scleroxylon* after 16 weeks of incubation in *Coriolopsis polyzona; Pycnoporus sanguineous*; *Ganoderma lucidum* and *Lenzites palisoti*

Fungus Species	% Weight loss	% Lignin	% Cellulose
Coriolopsis polyzona	49.99	88.5	67.1
Pycnoporus sanguineus	25.92	79.6	63.4
Ganoderma lucidum	31.73	75.8	56.7
Lenzites palisoti	21.52	70.2	54.8

Therefore, decay by these fungal organisms used in the study may also be influenced by the type of lignin found within *Triplochiton scleroxylon*.

Preferentially lignin degradation caused by *Coriolopsis polyzona; Pycnoporus sanguineous*; *Ganoderma lucidum* and *Lenzites palisoti* depletes lignin and all the associated pentosans from the lumen towards the middle lamella. According to Blanchette and Reid (1986), the various models proposed for the spatial association of various cell wall components help to explain how fungal enzymes may act on woody cell wall. Lignin and hemicellulose are associated together in a matrix that surrounds the cellulose fibrils. As this matrix is depleted, fungal enzymes can move through these spaces to reach the inner regions of the cell wall. The extensive degradation of the middle lamella region as observed with *Coriolopsis polyzona* would then be possible. The results presented here indicated that *Triplochiton scleroxylon* is highly susceptible to biodegradation by the fungal organisms used in the study and is in consonance with the works of Blanchette and Reid (1986) that wood decayed by *Phlebia tremellosus* (Schrad. Fr) Nakas and Burds indicate that digestibility increases substantially and reaffirm that lignin is removed throughout the cell wall layers.

Table2: Percentage weight loss of wood blocks of *Triplochiton scleroxylon* after 16 weeks of incubation in *C.polyzona P.sanguineus, G.lucidium,* and *L.palisoti* (mean of 6 replicates).

FUNGUS SPECIES	Initial Dry Wt DW1	Final Dry Wt DW2	DW1 – DW2	% Wt Loss	Condition of test blocks after Study
Coriolopsis polyzona	5.457	2.729	2.728	49.99	Badly decayed
Pycnoporus sanguineus	5.671	4.201	1.470	25.92	Fairly decayed
Ganoderma lucidum	5.594	3.819	1.775	31.73	Fairly decayed
Lenzites palisoti	5.594	4.390	1.204	21.52	Fairly decayed

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