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SECTION 5 ENVIRONMENTAL ASPECTS

Optimum Growth Conditions for the Metal-Tolerant Wood Decay Fungus, *Meruliporia incrassata* TFFH 294

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ABSTRACT

There is a worldwide need for alternative methods for the treatment and disposal of CCA-treated waste wood. Illman and Highley (IRG/WP 96-10163) reported the isolation of a unique strain of *Meruliporia incrassata* (TFFH 294) with tolerance to CCA. The strain is capable of degrading CCA treated waste wood, giving a 40% weight loss in the ASTM soil block test. The strain is an ideal candidate for degrading CCA-treated waste wood, which is accumulating in landfills at a rapid rate. In this study, we report the optimum growth conditions of the fungus, including the effect of chemically defined liquid media, growth temperature, light sensitivity, oxygen requirement and nutrient supplements. The results of a metal-tolerance test and SEM evidence of fungal growth on CCA-treated wood are given to support *M. incrassata* TFFH 294 tolerance.

Introduction

There is increasing concern worldwide about the disposal of preservative-treated wood products after use (Cooper, 1993). Preservative-treated waste wood has not been included in the wood recycling industry, basically because of technical problems and regulatory concerns. A similar situation applies to waste wood management uncertainties (Falk, 1997). The common waste disposal options for spent preserved wood are combustion and landfilling, with up to 5 million metric tons going to landfills annually. Due to environmental health and safety concerns, regulatory requirements are becoming much stricter and operational costs are increasing. For these reasons, using fungi to degrade or remediate waste wood offers an attractive alternative.

Our laboratory has developed strategies to use metal-tolerant fungi in the waste management of preservative-treated wood. Although preservative tolerant fungi have been known for years (Cowling, 1957; DaCosta, 1959; Zabel, 1954), the use of these fungi for waste management is relatively new. The long-term objectives of our work are to develop strategies for recycling, degrading and remediating preservative-treated waste wood with wood decay fungi. To this end, we have selected a metal-tolerant fungus, *Meruliporia incrassata* TFFH 294, for its capacity to degrade CCA-treated wood (Illman and Highley, 1996). Information about this metal-tolerant fungus is needed to optimize fungal growth under scale-up conditions. Growth of wood decay fungi is known to be enhanced by specific substrates and culture amendments (Akhtar et al, 1997; Kirk et al, 1986; Highley, 1973). The objectives of this study were to (a) determine the optimum growth conditions and culture medium for *Meruliporia incrassata* TFFH 294 and (b) determine the extent of growth and morphological appearance of TFFH 294 hyphae on CCA wood using scanning electron microscopy (SEM).

Materials and Methods

Culture Conditions and Storage

The brown-rot fungus used in this study was originally isolated from the USDA/FS Forest Products Laboratory research plots in Gulfport, Mississippi, identified as *Meruliporia incrassata*, and given the isolate number TFFH 294 (Illman and Highley, 1996). Cultures were stored on 2% malt extract agar (MEA) slants at 4 °C until ready for use. Prior to each experiment, cultures were transferred from storage slants to the center of petri dishes containing 2% MEA and allowed to grow in the dark at 27 °C, 70% relative humidity (RH) to obtain new hyphal growth.

Temperature

Four temperature settings, 20, 27, 32 and 37 °C, were selected for their effect on fungal growth. Four discs of fungal culture were inoculated into malt extract liquid medium. After incubation for 12 days at the various temperatures, mycelia were harvested by filtration on Whatman No.1 filter papers, air-dried and weighed to determine biomass.

Light

Light effect on growth was studied under 3 settings; 24 hours of light, 12 hours of light with 12 hours of darkness and 24 hours of darkness. Fungal inoculum was grown in malt extract liquid medium. Mycelia were harvested on day 21 for dry weight measurements.

Defined liquid medium

Two chemically defined liquid media, Bailey (Bailey, 1969; Highley, 1973) and BIII (Kirk et al, 1986) were used for the growth study of TFFH 294. Fresh fungal cultures were grown on malt extract agar plates. Four agar discs (9mm) of complete fungal growth were removed and inoculated into 25-ml of growth medium in 125-ml flasks. Flasks were kept stationary at 27 °C, 70% relative humidity (RH) for 21 days. Mycelia were harvested for biomass measurements.

Oxygen

Bailey and BIII media were inoculated with fungi as described above and stored at 27 °C for a period of 21 days. Cultures were flushed with oxygen every other day for an interval of 20 seconds. Mycelia were harvested by filtration for dry weight measurements.

Supplement: Corn steep liquor

Fungal inoculum was added to malt extract liquid media supplemented with 1, 2.5 and 5 % of sterile corn steep liquor (CSL, ADM Corn Processing, Cedar Rapids, IA). Cultures were grown at 27 °C, 70% RH for 30 days and mycelia were harvested for dry weight measurement. Media containing no CSL was used as a control.

Test for Metal-Tolerance: Choice test

A freshly grown fungal malt agar disc (9 mm) was placed in the center of a Petri dish (14 cm diameter) containing 12 ml water agar (Leithoff et al, 1995). No glass ring was applied to the agar disc. A CCA treated wood sample (15 mm x 30 mm) was placed at one edge of the Petri dish and an untreated wood sample was placed at the opposite side. Plates were incubated at 27°C, 70% RH and fungal growth was monitored for 14 days.

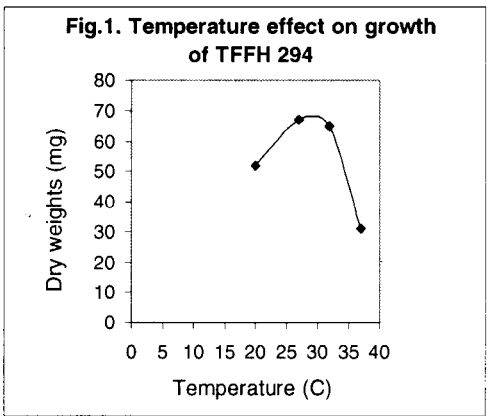
Scanning Electron Microscopy

CCA-treated and untreated Southern yellow pine wood blocks were inoculated with fungi in soil block bottles and incubated for 10 weeks at 27 °C, 70% RH. Small sections of the wood blocks were removed, attached to aluminum mounts using silver paste, and gold coated in a Denton sputter coater Desk- 1. Samples were scanned at 15KV using a JEOL 840 Scanning Electron Microscope.

Results and Discussion

Temperature

TFFH 294 cultures were grown at four different temperatures in a stationary condition. The results showed that the optimal temperature for growth was 27 °C (Figure 1). At a higher temperature of 32 °C, the growth effect is similar to 27 °C, but at 35 °C, growth declined. The results confirm that the metal tolerant wood decay fungus, TFFH294, has an optimum growth temperature range similar to that of other brown-rot wood decay fungi (Highley, 1973).



Light

The effect of light on the growth of TFFH 294 is given in Table 1. The fungus produced more cell mass under the complete dark growth condition. Cell mass measurements increased 33 % using a 24-hour dark cycle compared to the 24-hour light cycle.

Table 1. Light effect on TFFH 294 growth.

Light condition	Dry weights, mg (±S.D.)
24 hours of light	66±8
12 hours of light	70±6
24 hours of dark	88±13

Defined liquid media

Both Bailey and BIII media are chemically defined growth media for fungi. Bailey medium is commonly used for brown rot and white rot fungi and BIII medium is used for culturing the white rot fungus, *Phanaerochytes chrysosporium* (Kirk et al, 1986; Highley, 1973). More biomass growth for TFFH 294 was observed in Bailey medium than BIII medium (Table 2).

Table 2. Effect of two defined liquid media on fungal growth.

Strain	Medium	Dry weights, mg, (± S.D.)
TFFH 294	Bailey	20±10
	BIII	16±5

Oxygen

Fungal cultures were grown in two defined liquid media, Bailey and BIII, with and without oxygen added to the cultures. From the mycelia dry weight measurements, the results showed that oxygen enhanced fungal growth on both media (Table 2). This result is similar to the oxygen-enhanced growth reported for the white-rot fungus, *Phanaerochytea chrysosporium* (Kirk et al, 1986). Oxygen amendments also lowered the pH of Bailey and BIII media. Brown-rot fungi are known to secrete organic acids that lower the pH of culture medium (Highley et al, 1994). The decrease in pH values produced in the culture media is interpreted to be a result of the oxygen-enhanced growth of *M. incrassata* TFFH 294.

Table 2. Oxygen effect on fungal growth with two defined media.

Strain	Medium	O_2	Dry weights, mg (± S.D.)	PH
TFFH 294	Bailey	+	23±1	2.73
		–	20±1	3.04
	BIII	+	21±2	2.96
		–	18±2	3.61

Supplement: Corn steep liquor

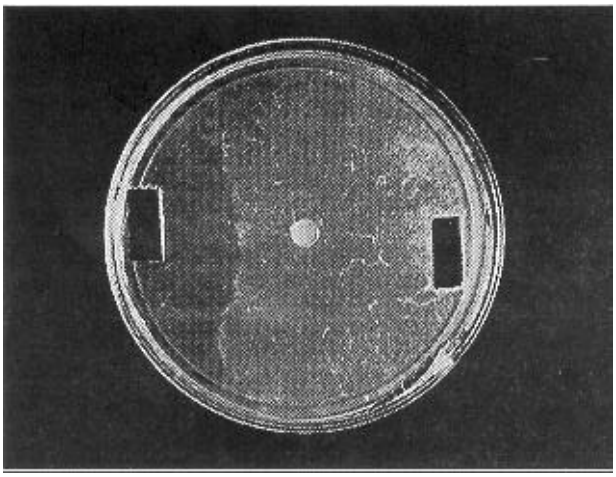
The addition of corn steep liquor (CSL) to growth medium has been shown to have a profound effect on fungal growth (Akhtar et al, 1997). Several concentrations of CSL were used in TFFH-294 media and the results are given in Table 4. The highest enhancement effect was obtained by adding 1% CSL in malt extract medium. The growth of some fungal species was not enhanced by the CSL-amendment, producing minor or no growth effect (unpublished data). Without CSL, the isolate TFFH 294 has a slower growth rate in MEA than other brown-rot fungi, such as *Postia placenta* and *Gloeophyllum trabeum* (unpublished data). By adding CSL to the culture medium, TFFH 294 can be given an advantage over other competitive decay fungi. Corn steep liquor is a by-product of the production of cornstarch, making it relatively economical. The commercial availability of CSL has also made it a select nutrient supplement in feed stock. Currently CSL is used as a liquid supplement for ruminants, nutrient source for poultry and protein source and binding agent for cattle range blocks. The composition of commercial CSL products can vary slightly. A typical composition is 50.7% dry substances, 40.8% protein, 16% lactic acid and 12.8% reducing sugars with a pH of 3.9.

Table 4. Effect of corn steep liquor on fungal growth.

Strain	CSL concentration	% Dry weights
TFFH 294	0	100
	1.0 %	321
	2.5 %	256
	5.0 %	196

Test for Metal-Tolerance: Choice test

The tolerance of metal preservatives is indicated clearly by the Choice test. In the choice test, TFFH 294 had a primary growth preference toward untreated wood (Figure 2). However, TFFH 294 also grew toward the CCA-treated wood block (Figure 2), an indication that it is a metal tolerant fungus. This method has provided a rapid approach to screening large numbers of fungal strains. Among hundred of strains we screened, the majority of the fungi grew towards the untreated wood only and no growth was observed towards treated wood. Interestingly, some strains had a growth preference towards both the untreated and the treated blocks, similar to that observed with TFFH 294, while a few grew preferentially towards the treated wood sample (manuscript in preparation).



+ CCA - CCA

Figure 2

Scanning electron microscopy

The SEM data demonstrated that no morphological changes occur in TFFH 294 hyphae during degradation of CCA-treated wood. Hyphal morphology and growth patterns were similar on all wood samples. The difference in hyphae on non-treated vs treated wood was one of degree, with less hyphae on the treated. A representative micrograph of TFFH 294 growing on CCA-treated wood is given in Figure 3.

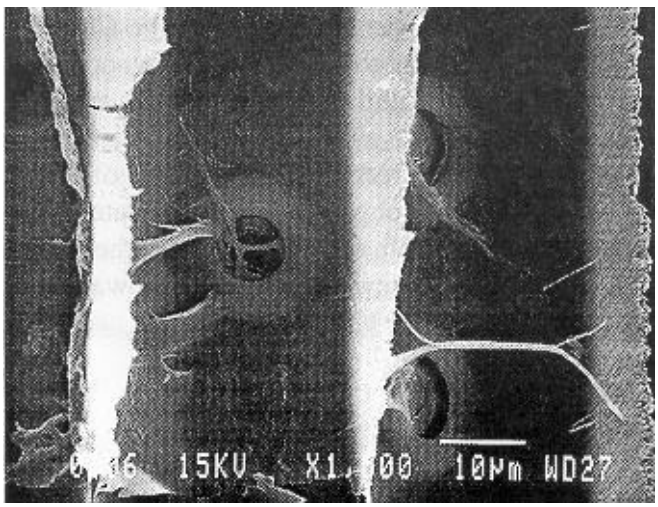


Figure 3

Summary

Optimum growth conditions for the unique *Meruliporia incrassata* isolate TFFH 294 have been identified in this study. The temperature and light requirements were found to be similar to other brown-rot wood decay fungi. A simple micronutrient medium (Baily Medium) sustained biomass production. An economical culture amendment was identified for enhancing growth. Aeration was shown to enhance growth and acid production. Employing these results may provide optimized conditions for utilizing *M. incrassata* TFFH 294 in waste management systems.

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