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Screening of Fungi Capable of Degrading Lignocellulose from Plantation Forests

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Abstract: In an effort to prevent forest fires after the clear cutting of plantation forests, fungi capable of degrading lignocelluloses were isolated to make a fertilizer from the logging waste. Seventy five fungal species were isolated from fruiting bodies and mycelia in plantation forests of South and North Sumatera, Indonesia. Sixty three of the fungi were identified based on the appearance and morphological characteristics of their fruiting bodies and mycelia, as *Pycnoporus sanguineus*, *Dacryopinax spathularia*, *Schizophyllum commune*, *Polyporus* sp. and *Trametes* sp. Twenty fungi were categorized as white-rot fungi and 12 as brown-rot fungi. Moreover, isolates 371, 368, 265, 346, 345 and 338 were selected using indicators and tested for the ability to degrade lignin and holo-cellulose in mangium wood meal over 1 to 4 weeks. Results showed that the 6 fungi could degrade lignin and holo-cellulose in wood meal. An increase in incubation time tended to decrease the amounts of lignin and holo-cellulose. Isolate 371 was found to be best at degrading lignin and holo-cellulose in mangium wood meal.

Key words: Plantation forest, logging waste, degradation, fungi

INTRODUCTION

Logging operations in plantation forests usually generate abundant amounts of waste such as residual wood, branches/twigs, leaves and bark. The harvested wood is used for the manufacture of wood-based products. At present, pulp industries in Indonesia only use wood from tree stands with a diameter greater than 10 cm. For an industrial plantation forest of 335,000 ha, the harvest would be 7.6 million m^3 $year^{-1}$. This generates waste such as wood from residual tree stands less than 10 cm in diameter and bark, as much as 1.5 million m^3 $year^{-1}$ and 0.76-1.14 m^3 $year^{-1}$. Anshori and Supriyadi (2001) alleged that the first cutting rotation (9 years) in a mangium plantation forest for pulp (193,500 ha) generated 15.18 t ha^{-1} of bark, 31.4 t ha^{-1} of wood, 4.0 t ha^{-1} of leaves and litters and 5.84 t ha^{-1} of stumps. Further, Muladi *et al.* (2001) asserted that the total amount of wood biomass of mangium (*Acacia mangium*) stands at 5-7 years of age was about 60.5-95.8 t ha^{-1}; while the corresponding figure for eucalypt (*Eucalyptus deglupta*) stands at 5-9 years reached 45.7-116.4 t ha^{-1}. The natural decomposition of waste is usually a slow process, resulting in the accumulation of materials, which when become dry, are a potential risk for forest fires (Nakayama and Siegert, 2001).

Saprophytic fungi may accelerate the breakdown of forest waste. It is necessary to seek particular fungi capable of degrading the waste. Waste from industrial plantation forests containing ligno-cellulosic substances can provide nutrients for saprophytic fungi (Alexopoulos *et al.*, 1979). Waste-decaying fungi belong to the saprophytes, since, they live on dead or residual vegetation, degrading them into simpler molecular compounds (Dubeux *et al.*, 2006; Ohkuma *et al.*, 2001; Aumen *et al.*, 1983). Saprophytic fungi can also grow in plantation forests. The use of saprophytic fungi is expected to accelerate the decay of waste. The present study was carried out to collect, isolate and select saprophytic fungi capable of degrading logging waste containing lingo-cellulosic substances from mangium and eucalypt plantation forests in an effort to prevent forest fires by making a fertilizer from the waste in a compost box after clear cutting of plantation forests.

MATERIALS AND METHODS

This research project was conducted in the Faculty of Agriculture, Ehime University, Japan and the Forest Product Research Center in Indonesia during 2007 in an effort to find fungi to degrade lingo-cellulosic waste from mangium and eucalypt plantation forests.

Chemicals: Acetic acid, agar powder, alcohol, benzene, guaiacol, malt-extract, remazol brilliant blue-R (RBBR) and sulfuric acid were purchased from Wako Pure Chemical Company Ltd., Japan.

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Procurement and collection of fungal isolates: Fungi in the form of mycelia in wood material and fruiting bodies were collected from plantation forests in South and North Sumatera, Indonesia. They were placed into plastic bags and cut, cleaned with disinfectants and put into tubes containing fresh agar medium. The samples were marked with information such as number, procurement location, growth site and specific characteristics.

Isolation of fungi: Fungi were isolated using bait made of malt extract agar for the culture medium. Vegetation such as twigs, branches and leaves showing signs of attack by fungi was cut-off, cleaned with disinfectants, rinsed with distilled water or bi-distilled water and then placed on agar medium in Petri-dishes. In addition, fruiting bodies of fungi were cleaned with disinfectants and approximately 3×3 mm was placed on agar medium in Petri-dishes. Later on, when the mycelium had grown on the medium in the vicinity of the tissues, the sample was transferred to fresh agar media in tubes. This was repeatedly carried out until pure cultures could be obtained as single cultures or so-called fungal isolates.

Identification of the isolated fungi: Materials were identified by comparing their physical characteristics like the form of the fruiting bodies, the location of pileus, the hairs present on the pileus and the size and shape of pores with those of reference materials (Cunningham, 1965; Krieger, 1967; Zoberi, 1972; Laessoe, 1998; Bougher and Syme, 1998; Hall *et al.*, 2003; Suhirman, 2005).

Selection of white-rot fungi: The selection of white-rot fungi was conducted by the methods of Nishida *et al.* (1988). First, isolates were inoculated onto wood-agar medium containing gallic acid and other media containing guaiacol and then incubated at room temperature for 7 days. Observations were carried out by measuring the diameter of ring-shaped mycelia. The intensity of color in the vicinity of the mycelium was assessed as follows:

- : No changes in color
* : Slightly reddish, light brown
** : Brownish, light red
*** : Reddish, moderately brown
**** : Reddish, dark brown

Further selection was conducted by the same procedure on cellulose-agar-guaiacol medium and cellulose-agar-RBBR medium.

Decaying ability tested based on holo-cellulose and lignin content: Test fungi were selected by reference to Friedrich *et al.* (2007), Terron *et al.* (1995) and Tuomela *et al.* (2000). A test sample in the form of mangium wood meal was mixed with 1% malt extract in distilled water to a water content of about 65% and sterilized by autoclave at a temperature of 121°C and pressure of 1.5 atm, for 30 min. After the medium had cooled, it was inoculated with the test fungi and incubated for 1 to 4 weeks at 25°C, in the dark.

Determination of holo-cellulose content: Holo-cellulose content was determined with reference to ASTM 1104-56 (1978). To remove the extractives, the mangium wood meal was extracted with alcohol and benzene (2:1 v/v) for 8 h at 70°C prior to use. Initially, 1 g of oven dried sample was place in an Erlenmeyer flask (300 mL) and 150 mL of distilled water was added. While slowly shaking, 1 g of $NaClO_2$ and 0.2 mL of acetic acid were added and the flask was covered with glass and heated at 70 to 80°C for 60 min in a water bath. Again, 1 g of $NaClO_2$ and 0.2 mL of acetic acid were added and heated at 70 to 80°C for 60 min and the treatment was repeated two times. After cooling, the sample was filtered using a filter flask and washed with hot water until free of acid. Afterward, the insoluble portion was dried in an oven at 105°C for 4 h, cooled in a desiccator and weighed repeatedly until a constant weight were obtained. Holo-cellulose content was calculated as follows:

$$\text{Holo-cellulose content} = \frac{\text{o.d. weight of holo-cellulose}}{\text{o.d. weight of wood meal}} \times 100\%$$

Determination of lignin content: Lignin content was measured by the method of TAPPI Standard (T 222 os-74). About 1 g of air-dried sample was weighed accurately in a weighing bottle and transferred to a 50 mL beaker, then 10 mL of 72% sulphuric acid was added carefully with a pipette and the mixture was stirred with a small glass rod (which was left in the beaker for 4 h at room temperature). The mixture was transferred to a 500 mL round-bottle flask and diluted with water until the final volume was 300 mL. The mixture was then refluxed for 2 h, filtered in a glass filter and dried in oven at 105°C for 12 h. The crucible was cooled in desiccators for 15 min and weighed accurately. The glass filter containing the lignin was reported as a percentage by weight of the dried sample.

$$\text{Lignin content} = \frac{\text{o.d. weight of lignin}}{\text{o.d. weight of wood meal}} \times 100\%$$

RESULTS AND DISCUSSION

Screening and isolation of white rot fungi from plantation forests: Fungi were collected from mangium plantation forests using purposive sampling method, at Subanjeriji, Gumawang, Sodong and Merbau in South Sumatera. From field observations, we found that most wood-decaying fungi belonged to the Basidiomycetes class. A list of fungi that grow in mangium plantation forests in South Sumatera is presented in Table 1. The fungi found in South Sumatera grew in wood trunks, stumps, branches, twigs and leaf litter of mangium species. In the Subanjeriji Region, fruiting bodies of fungi were found in particular around community-settlements, which are dominated by grasses, shrubs and clumps. Alexopoulos *et al.* (1979) mentioned that fungi can be identified based on the appearance of fruiting bodies. Around 13 species were identified based on their fruiting bodies, predominantly *Pycnoporus sanguineus* and *Schizophyllum commune* and one species (*Ganoderma* sp.) grew in living mangium trees. Moreover, in the Gumawang region, many fruiting bodies were discovered, particularly on wood waste surrounding wood-processing plants. When rain came, the waste became wet, suitable for fungal growth (Koukoura *et al.*, 2003). The dominant fungi in this area were *Polyporus* sp., *Pycnoporus sanguineus*, *Schizophyllum commune*, *Coprinus* sp. and *Dacryopinas spatthularia*.

Table 1: Wood-decaying fungi found in the mangium and eucalypt plantations

No.	Fungal species in mangium forests in South Sumatera	No.	Fungal species in eucalypt forests in North Sumatera
1	*Amauroderma conjuctus*	1	*Auricularia polytricha*
2	*Auricularia polytricha*	2	*Auricularia* sp.
3	*Auricularia* sp.	3	*Coprinus* sp. 3
4	*Coprinus* sp. 1	4	*Dacryopinax spathularia*
5	*Coprinus* sp. 2	5	*Dacryopinax* sp.
6	*Dacryopinax spathularia*	6	*Ganoderma lucidum*
7	*Fomes rheicolor*	7	*Ganoderma* sp.1
8	*Ganoderma lucidum*	8	*Ganoderma* sp.2
9	*G. colosus*	9	*Lentinus sajor-caju*
10	*G. tropicum*	10	*L. squamosus*
11	*Nameko* sp.	11	*Lentinus* sp. 2
12	*L. squamosus*	12	*Marasmius* sp. 3
13	*Lentinus* sp.1	13	*Marasmius* sp. 4
14	*Lenzites acnta*	14	*Pleurotus* sp. 3
15	*Lenzites* sp.	15	*Polyporus arcularis*
16	*Marasmius* sp. 1	16	*P. crenatoporus*
17	*Marasmius* sp. 2	17	*P. gammocephalus*
18	*Microporus xanthopus*	18	*P. pubesceus*
19	*Panus ridicus*	19	*P. sericatum*
20	*Pleurotus* sp.1	20	*Polyporus* sp.
21	*Pleurotus* sp. *2*	21	*P. sanguineus*
22	*Polyporus arcularis*	22	*Schizophyllum commune*
23	*P. crenatoporus*	23	*Trametes* sp.
24	*P. dependeus*	24	*Tremella* sp. 1
25	*P. dictyopus*	25	*Tremella* sp. 2
26	*P. gammocephalus*	26	Fungus 8
27	*P. indicus*	27	Fungus 9
28	*P. sericatum*	28	Fungus10
29	*P. supinus*	29	Fungus 11
30	*P. tenuiculus*	30	Fungus 12
31	*P. versicolor*		
32	*Polyporus* sp.		
33	*Pycnoporus puniceus*		
34	*P. sanguineus*		
35	*Schizophyllum commune*		
36	*Trametes pubesceus*		
37	*T. mimites*		
38	*Volvariella* sp.		
39	Fungus 1		
40	Fungus 2		
41	Fungus 3		
42	Fungus 4		
43	Fungus 5		
44	Fungus 6		
45	Fungus 7		

In the mangium plantation forests at Merbau, fungi were discovered abundantly, including in seed orchards. From this area, around 14 species have been collected, mostly *Polyporus* sp. and *P. sanguineus*. Even in the dry season, fungi were still encountered in the Merbau area due to a sufficient amount of water. Slight rain occurred causing an increase in humidity on the forest floor, proliferating the growth of fruiting bodies at the Sodong site. Fruiting bodies grew on forest litter, predominantly white rot fungi (*Pycnoporus sanguineus*, *Schizophyllum commune*, *Panus* sp.) and other families such as *Coprinus* sp.1. The forest soil was rather humid and there was always a considerable amount of logging waste available, inducing the growth of fungi (Koukoura *et al.*, 2003).

Attempts at collecting fungi from eucalypt plantations in North Sumatera (Table 1), however, yielded almost no fungi in one-year-old forests. Fungi were only found from two years up, mostly *Lentinus*, *Ganoderma*, *Dacryopinax* and *Schizophyllum* sp. There is intentional burning in logged-over forest areas, drying out forest litter and soil. Thus, fruiting bodies as well as mycelial traces do not grow well. Further, the use of herbicides to control 1-year-old eucalypt plantations leads to the death of unwanted plants, including fungi. This is why not so few fungi were found in 1-year-old eucalypt plantations.

Among 32 isolates identified as wood-rot fungi, 20 were categorized as white-rot fungi and 12 as brown-rot fungi. In Table 2, stars indicate fungi able to degrade lingo-cellulosic substances (Friedrich *et al.*, 2007; Vane *et al.*, 2006).

Selection of white-rot fungi: To find out whether fungi in the white rot group can degrade lignin and holo-cellulose, all fungi were cultivated in cellulose-agar-RBBR medium and cellulose-agar-guaiacol medium, respectively. The coloration of the medium varied with the inoculated fungal

Table 2: Mycelial growth and color change on several media

	PDA-guaiacol medium		PDA-gallic acid medium		Wood-agar- guaiacol medium	
Fungal isolates	Growth (mm)	Color	Growth (mm)	Color	Growth (mm)	Color
HHBI-356	63±0.0	-	53±0.2	-	60±0.2	-
HHBI-353	63±0.2	-	79±0.3	-	61±0.2	-
HHBI-355	66±0.2	-	80±0.2	-	58±0.0	-
HHBI-341	24±0.2	-	30±0.2	-	55±0.2	-
HHBI-342	88±0.0	-	56±0.2	-	49±0.2	-
HHBI-298	18±0.2	*	56±0.2	***	46±0.0	***
HHBI-339	28±0.3	***	49±0.2	****	35±0.2	***
HHBI-350	39±0.0	****	82±0.2	****	51±0.2	****
HHBI-299	32±0.0	**	17±0.2	**	45±0.2	**
HHBI-343	47±0.2	****	71±0.2	****	59±0.2	****
HHBI-358	56±0.2	****	76±0.0	****	58±0.5	****
HHBI-357	55±0.2	**	54±0.2	****	31±0.2	*
HHBI-204	52±0.0	****	77±0.0	****	54±0.2	*
HHBI-359	29±0.3	****	42±0.2	****	39±0.0	****
HHBI-344	28±0.2	**	48±0.2	**	44±0.0	*
HHBI-360	34±0.2	-	88±0.0	-	42±0.2	-
HHBI-346	88±0.0	****	88±0.0	****	88±0.0	****
HHBI-340	28±0.3	***	38±0.2	***	29±0.2	**
HHBI-361	88±0.0	-	88±0.0	-	57±0.2	**
HHBI-341	12±0.3	-	14±0.2	-	41±0.2	-
HHBI-283	38±0.2	-	88±0.0	-	50±0.2	-
HHBI-354	67±0.2	****	77±0.2	****	48±0.0	*
HHBI-275	70±0.0	*	65±0.0	**	31±0.2	*
HHBI-306	36±0.0	-	29±0.2	-	36±0.0	-
HHBI-276	70±0.2	-	53±0.0	*	13±0.0	-
HHBI-304	27±0.2	***	49±0.2	****	15±0.2	**
HHBI-345	55±0.2	****	52±0.2	****	37±0.2	****
HHBI-348	52±0.2	-	55±0.2	-	40±0.2	-
HHBI-326	28±0.2	**	39±0.2	****	22±0.2	**
HHBI-338	59±0.0	***	57±0.2	****	24±0.0	**
HHBI-347	50±0.2	-	50±0.2	-	27±0.0	-
HHBI-371	88±0.0	****	88±0.0	****	nc	nc

Values are expressed as Mean±SD; *: Very light red/brown; **: Light red/brown, ***: Moderate red-brown; ****: Strong red-brown; -: Undetected color changes; HHBI: Hasil Hutan Bogor Indonesia (Indonesia Bogor Forest Products); nc: Not clear

species, from bright brown to violet brown (Table 2). The coloration indicated the activities of ligninolytic enzymes (phenol oxidase) produced by fungi and implied that the fungi themselves were able to degrade lignin.

White-rot fungi are considered the most promising group of microorganisms for degrading lignin because they produce extracellular polyphenol oxidases, particularly MnP and laccases, which are highly effective at degrading lignin (Dekker *et al*., 2002). Nishida *et al*. (1988) stated that discoloration could be used as an indicator of degradation in cellulose-agar-RBBR; while, coloration could be used as an indicator in cellulose-agar-guaiacol. Hartig and Lorbeer (1991) reported several fungal species able to break down lignin; *Pycnoporus sanguineus*, *Coriolus versicolor*, *Phanerochaete chrysosporium*, *Ganoderma applanatum*, *Phlebia brevispora*, *Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Schizophyllum commune* and *Chaetomum globosum*.

Among 15 fungi, 3 grew faster in both media, indicated by a rapid discoloration in the RBBR medium and coloration in the guaiacol medium. Further, 8 fungi including the 3 mentioned above grew faster in the RBBR medium, indicated by a rapid discoloration (Table 3). Three fungi that grew quickly in this experiment were identified according to the shape of their fungal bodies and morphological characteristics. Isolate 348 is a *Pycnoporus* sp.; isolate 347 is a *Polyporus* sp. and isolate 338 is a *Ganoderm*a sp. In addition, isolate 371 was identified as possibly belonging to the genus *Polyporus*. Further identification should be carried out based on the morphological characteristics of its fruiting bodies and spores.

Ability of selected fungi to break down lignin and holo-cellulose: The lignin content of the wood meal was initially 26.9%. After 1 week, it had decreased to 22.5-26.9% (Table 4). Fackler *et al*. (2006) stated that delignification was significant after 3 days treatment with fungi and the activities of extracellular ligninolytic enzymes (laccase, manganese peroxidase and/or lignin peroxidase) could be detected in fungal cultures even at low levels. Meanwhile, in this study, lignin content was reduced 0-15% relative to the control after just 1 week.

Table 3: Qualitative test for decaying activity of fungi selected from plantation forests

	Cellulose-agar-guaiacol medium, diam. of coloration (mm)					Cellulose-agar-RBBR medium, diam. of discoloration (mm)				
	1	2	3	4	5	1	2	3	4	5
Fungi	(day)					(day)				
263	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
265	14±0.5	23±0.5	27±0.5	30±0.5	30±0.5	12±0.5	12±0.5	12±0.5	12±0.5	12±0.5
276	12±0.5	12±0.5	12±0.5	12±0.5	12±0.5	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
298	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
304	20±0.5	22±0.5	22±0.5	22±0.5	22±0.5	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
306	0±0.0	0±0.0	0±0.0	00±0.0	00±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
338	15±0.5	20±0.5	22±0.5	28±0.5	32±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
345	18±0.5	23±0.6	27±0.5	34±0.5	34±0.5	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
346	17±0.5	21±0.5	30±0.5	43±0.5	43±0.5	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
352	0±0.0	0±0.0	0±0.0	0±0.0	00±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
356	0±0.0	0±0.0	0±0.0	0±0.0	00±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
361	0±0.0	0±0.0	0±0.0	0±0.0	00±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
364	0±0.0	0±0.0	0±0.0	0±0.0	00±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
368	12±0.5	16±0.5	18±0.5	20±0.5	21±0.5	0±0.0	0±0.0	0±0.0	0±0.0	20±0.5
371	17±0.5	22±0.5	30±0.5	40±0.5	42±0.5	15±0.5	27±0.5	30±0.5	40±0.5	42±0.5

Values are expressed as Maen±SD

Table 4: Lignin and holo-cellulose content of mangium wood meal treated with selected fungi

	Lignin (%)		Holo-cellulose (%)	
	1	4	1	4
Fungi	(week)		(week)	
Control	26.8±0.1	26.7±0.1	68.7±0.1	66.9±0.1
371	22.5±0.2	14.6±0.4	59.2±0.1	41.8±0.4
368	24.8±0.5	23.4±0.4	65.7±0.2	53.4±0.1
265	26.9±0.4	21.8±0.1	63.7±0.2	52.2±0.2
346	24.7±0.1	24.9±0.1	65.1±0.1	52.4±0.1
345	26.8±0.2	23.1±0.1	65.5±0.1	51.3±0.2
338	26.8±0.4	22.8±0.1	65.0±0.2	45.5±0.3

Values are expressed as Mean±SD

Further, after 4 weeks, the lignin content was 14.6-24.9%. As shown in Table 4, isolate 371 was most capable of degrading lignin (44% to the control); while, isolate 346 was least capable (11% to the control). As mentioned above, isolate 371 showed the strongest coloration in the guaiacol medium (Table 3). This means that the fungus produces enzymes needed for the degradation of lignin and more of these enzymes than any of the other five fungi tested. From the results obtained here, fungus 371 selected from plantation forests is suitable for the degradation of logging waste.

Holo-cellulose (cellulose and hemicellulose) content was also measured after incubation of the 6 fungi for 1 to 4 weeks (Table 4). The holo-cellulose content of mangium wood meal was initially 68.7%. After 1 week, this had decreased to 59.2-65.7% depending on the fungus (Table 4). Isolate 371 was still powerful at degrading holo-cellulose (14%) as well as lignin. Meanwhile; isolate 368 was weakest of degrading holo-cellulose of mangium (4%). Furthermost, after 4 weeks, isolate 371 could still degrade 38% of holo-cellulose; while isolate 368 could degrade only 20%. Basidiomycetes are the most potent degraders of holo-cellulose because many species grow on dead wood or litter, in environments rich in cellulose (Baldrian and Valaskova, 2008). As mentioned earlier, fungus 371 selected from plantation forests was found to be most capable of degrading holo-cellulose as well as lignin.

Biomass which indicates large amounts of ligno-cellulosic substances discarded as wood materials, branches and roots of shade and forest trees and wood residues has been burned or degraded. Some forest fires occurring logging waste catches a light after the clear cutting of plantation forests. This causes environmental problems such as increases in CO_2, concomitantly with a rise in temperature or the accumulation of deleterious substances, leading to health concerns for humans and wild life. Making a fertilizer from the waste is an important potential use of waste biomass. Woody materials should ideally be recycled by biological degradation or removal of lignin (Watanabe *et al*., 2003). Besides lignin, the main component of biomass is holo-cellulose (cellulose and hemicellulose). The degradation of logging waste after the clear cutting of plantation forests using the fungi selected here to make a fertilizer from the waste may prevent forest fires.

CONCLUSION

Of sixty two fungi found in mangium and eucalypt plantation forests, 32 could be cultivated in MEA medium and PDA medium, including 20 white rot fungi and 12 brown rot fungi. Among the white-rot fungi, 6 were tested for activity to degrade mangium lignocellulolytic materials. Of the 6 isolates, fungus 371, genus *Polyporus,* was found to be the most effective at degrading mangium lignin and holo-cellulose.

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