Screening of Indonesian White Rot Fungi for *Acacia* Wood Lignin Degradation

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ABSTRACT We collected 600 samples for screening of white rot fungi from various natural sources, including fruiting bodies, rotten woods and piles of trap lumber from West Java, Central Kalimantan, South Kalimantan, Jambi, and North Sumatra in Indonesia. We obtained from 600 samples a total of 258 isolates showing positive reaction of guaiacol oxidation by laccase based on the observation of red colored zone around and beneath mycelial colony on *Acacia mangium* wood powder and agar medium containing guaiacol. Tentative identification of 45 isolates from morphologic characteristics of fruiting bodies showed that the isolates belonged to the families Ganodermataceae, Corticiaceae, Polyporaceae, and Coprinaceae.

Key words: biological pulp processing, isolation, guaiacol oxidation, screening, white rot fungi

INTRODUCTION

Wood-decay fungi are increasingly of interest to biotechnology, because their enzyme systems can detoxify pollutants (Mori et al. 2003; Wesenberg et al. 2003) and can delignify agricultural waste (Jiménez et al. 1997). The applications of wood-decay fungi to the pulp and paper industry have been recently studied (Bourbonnais and Paice, 1996; Messner and Srebotnik, 1994).

White rot fungi are a well known group of fungi belonging to Basidiomycetes and Ascomycetes and have been studied intensively on lignin degradation systems, because these organisms are able to degrade lignin to carbon dioxide and water (Boyle et al. 1992). There are several hundred species of white rot fungi belonging to a variety of fungal orders. Among the Basidiomycetes are

the Agaricales, Aphyllophorales, Auriculariales, Sclerodermatales, and Lycoperdales. There are also a few white rot fungi in the Xylariales, belonging to Ascomycetes (Kirk, 1971). Different white rot fungi vary considerably in the relative rates at which they decompose lignin and carbohydrates in woody tissue. Some selectively decompose lignin at a higher rate than carbohydrates.

Acacia mangium is a native tree species in northern Australia and Southeast Asia. It has become a major tree plantation in Southeast Asia and have chance to be main source for supplying hardwood materials in production of pulp and paper industry because of acceptable quality of wood.

Screening of white rot fungi for pulp and paper purpose has been reported. Most of the reports used subtropical wood species as substrate (Hirai et al. 1994; Takano et al. 2001). There is no report about screening on tropical wood until now, particularly on *A. mangium*. This is the first report to screen white rot fungi for *A. mangium*. Screening white rot fungi with simple substrates instead of lignin have been used to detect ligninolytic enzymes. Many white rot fungi produce extracellular enzymes in media containing phenols such as guaiacol. The oxidation of guaiacol indicates presence of phenoloxidases, specifically laccase (Nishida et al. 1988; De Jong et al. 1992). Laccase is one of the important enzymes in lignin degradation system and is able to oxidize simple phenolics to quinones which polymerize to form colored complexes (Kirk and Kelman, 1964). The reaction mechanism to form red color zone by laccase is as follows: guaiacol is transformed by laccase to be 6-methoxycyclohexa-2,4-dienone and water.

The purpose of this study was to isolate white rot fungi for their application to the biological processing of *A. mangium* pulp. We collected 600 microbial sources from several locations in Indonesia and screened.

MATERIALS AND METHODS

Sources of white rot fungi and sampling

We collected 600 samples for isolation of white rot fungi obtained from the Indonesian provinces of West Java, Central Kalimantan, South Kalimantan, Jambi, and North Sumatra. The sampling period was done on January to August 2004. The isolates were obtained from various sources including 404 fruiting bodies, 148 rotten wood, and 48 trap lumber piles. Soft fruiting bodies were immediately (around 2 days) transferred to the laboratory for isolation while hard fruiting bodies were sun-dried for 2 days and could be kept for isolation approximately for 1 month. Rotten wood and trap lumber piles had no special treatment before isolation.

Isolation of fungi and screening for guaiacol oxidation

The method of Nishida et al. (1988) was modified for isolation of white rot fungi. Wood guaiacol agar (WGA) medium [*Acacia mangium* wood powder 0.2% (80 mesh pass), guaiacol 0.01% and agar 1.6%] in Petri dishes (9 cm in diameter) was used for isolation. Each sample was firstly sprayed on surface for sterilizing with 70% ethanol before isolation. For hard fruiting bodies or decayed wood, small samples (around 3 mm³) were taken from the interior of the samples, and placed on the surface of the WGA medium. For soft fruiting bodies, basidiospores were collected on sterilized filter paper and used as multispore inoculum. The cultures were incubated at 28 °C for 4 to 7 days. Fungal colonies which produced red-colored zones around and beneath the mycelia were judged as positive strains. The degree of the reaction was classified according to strength of color of the red colored zone as follows: 4+ was strong red color reaction, 3+ was medium red color reaction, 2+ was weak red color reaction, and 1+ was very weak (light) red color reaction. To obtain pure and stable cultures of the positive strains,

the mycelium was transferred to potato dextrose agar (PDA) medium and subcultured for 2 or 3 times. The plates were incubated at 28 °C for 4 days for each time. The strains obtained as above were deposited in laboratory of forest resource biology, Hokkaido University, Japan. Physiological and morphological properties were assessed, including mycelial growth rate, type of mycelium, colony formation, and color formed on the medium.

Identification

Identification was conducted according to morphological characteristics. The observation was done under microscope and compared by reference of Imazeki and Hongo (1989), Shiro (1996), and Núñez and Ryvarden (2000).

RESULTS AND DISCUSSION

We obtained from the 600 samples a total of 258 isolates that showed positive reaction on the WGA medium, indicating that these isolates had abilities to oxidise guaiacol. The number of samples collected and number of positive strains on guaiacol oxidation in the five areas is shown in Table 1. The positive strains produced red-colored zones of varying intensity around mycelia, whereas negative fungi did not show any colored zones around the inocula. The positive strains were classified by color zone and colony formation into four types: type I (14 strains) was characterized by a 3+ to 4+ color zone and colonies with thick mycelia; type II (48 strains) by a 3+ to 4+ color zone and colonies with intermediate mycelia; type III (170 strains) by a 3+ to 4+ color zone and colonies with thin mycelia; and type IV (26 strains) by a 1+ to 2+ color zone and colonies with thin mycelia. These classifications are necessary to compare the correlation between the physiological characteristics of each isolates to their ability in biobleaching for further study.

Table 1. Number of collected samples and number of positive strains on guaiacol oxidation

Location	Number of sample								
	Collected				Positive guaiacol oxidation				
	Fruit body	Rotten wood	Trap pile	Total	Fruit body	Rotten wood	Trap pile	Multispore	Total
West Java	147	82	-	229	52	43	-	7	102
Central Kalimantan	25	21	33	79	4	11	11	-	26
South Kalimantan	22	25	15	62	7	5	10	1	23
Jambi	180	-	-	180	83	-	-	-	83
North Sumatra	30	20		50	17	7	-	-	24

Table 2. Preliminary identification of 45 fungal isolates and their origins in Indonesia. These isolates were obtained from fruiting bodies and showed positive reactions of guaiacol oxidation by laccase.

Family/genus	West Java	Jambi	North Sumatra	Total
Microporus	4	3	0	7
Lentinus	2	0	0	2
Pycnoporus	1	0	1	2
Daedaleopsis	0	1	0	1
Daedalea	0	1	0	1
Trametes	3	11	1	15
Ganoderma	7	1	1	9
Amylostereum	0	0	1	1
Corticaceae	1	2	0	3
Coprinaceae	1	0	3	4
Total	19	19	7	45

Of the 258 isolates, 163 were isolated from fruiting body tissues, 66 from rotten woods, 21 from trap piles, and 8 from collected from multispores. The 163 isolates from fruiting bodies were further divided into two groups: 159 isolates from hard fruiting bodies and 4 isolates from soft fruiting bodies. We identified tentatively 45 of the 163 isolates (Table 2) from morphologic characteristics of fruiting bodies. The isolates belonged to the families Ganodermataceae, Corticiaceae, Polyporaceae, and Coprinaceae. Most of the isolates were identified as Polyporaceae, which were known as wood-decaying fungi and were able to attack and utilize wood cell wall components as a source of energy for growth and reproduction (Nuñez and Ryvarden, 2000). Of the 163 isolates from fruiting bodies, 118 fungi could not be identified because of deterioration during storage. As shown in Table 2, all strains are typical lignin degrading fungi. We identified strains belonging to the genus *Trametes* which is well-known as lignin-degraders. In this genus, for example, *Trametes versicolor* has been widely used for biological pulp processing in wood species (Kirkpatrick et al. 1989; Paice et al. 1989; Archibald, 1992).

Because our ultimate aim is to use the new selected isolates for the bleaching stage in the pulp and paper industry, the findings of the present screening which served only to isolate candidate strains are insufficient for this purpose. Further screening to select strains for *A. mangium* pulp will be necessary.

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