DIVERSITY OF APHYLLOPHORALES FUNGI ISOLATED FROM TANJUNG PUTEVG NATIONAL PARK, CENTRAL KALIMANTAN AND ITS POTENTIALITY FOR LIGNIN DECOMPOSITION

TypukArtiningsih,[1] ✉ Herwint Simbolon[1], Suhirman[2] and Mitsuru Osaki[3] >.... •*j,v\'*

'R&D Center Biology, LIPI, Indonesia;
[2]Bogor Botanic Garden, LIPI, Indonesia;
[3]Hokkaido University, Sapporo, Japan

ABSTARCT

A total of fifty-three samples of fungal basidiocarp were collected from peat area in Tanjung Puting National Park, Central Kalimantan, Indonesia. These samples are mostly determined to genus until more studies are available. The fungi were isolated on CMA medium then the isolated strains were purified on PDA medium. The fungi belong to at least 3 families of Aphyllophorales, however there were a wide diversity in the genus with respect to macroscopic examination. An agar plate screening procedures was developed for the rapid selection of fungi and estimation of their capacity for lignin-decomposer. The test is based on the visualization and interpretation of the formation of halo zone or decolourization process in well-defined agar medium containing Poly R-478. The selected isolates were also tested in to rhemazol brilliant blue R salt (RBBR) medium. Five isolates had the ability to decolourize either Poly R-478 or RBBR. These isolates showed a qualitative lignin decomposition although a comparative study of selected fungi revealed the difference potentiality. The highest loss of lignin was 28.18% and attempts have been made to determine the unidentified fungi, which is resupinate type, by direct sequencing of 18s ribosomal DNA (rDNA).

Key words: fungi, lignin, screening, decomposition, peat soil, Poly R-478, rhemazol brilliant blue, 18s rDNA

INTRODUCTION

Lignin, a major plant tree constituent, is the most abundant organic matter resource. Since lignin contains phenolic polymers, it is generally difficult to decompose. However, selected microorganisms are able to decompose high molecular weight of lignin, phenolic compounds, or its partial decomposition products. One of these called basidiomycetes has received attention because their ability to use lignin as the carbon and energy source.

Many of basidiomycetes fungi possess laccase, phenol oxidase, and peroxidase activities (unpublished data). The extracellular ligninolytic enzyme system of these fungi has been directly implicated in the degradation of a wide range of structurally different aromatic compounds, including many xenobiotics (Field *et* a/., 1993). The relatively short time required in decomposing and the presence of laccase-like activity in soil are consistent with the hypothesis that enzymatic phenol oxidation is a major driving force in decomposition of organic matter. Recently, most works have focused on the ligninolytic ability of the model white rot *Phanerochaete chrysosporium.* Several other white rot fungal strains including *Trametes versicolor* (Collins *et al.,* 1997), and *Bjerkkandera* sp. (de Jong *et al.,* 1994) have been reported to show significant levels of extracellular ligninolytic enzyme activity. Important contributions to the knowledge of Indonesian Aphyllophorales were made by Suhirman and Nunez (1998a; 1998b). However, the exploration of Aphyllophorales fungi and their ligninolytic capacity from Kalimantan peat swamp forest has not been reported. Therefore, the study will be an

urgent need before they get loss forever because of extensive fire and illegal logging.

The decolourization of polymeric dyes, like Poly R-478 or RBBR has been used to screen for white rot fungi with good peroxidative activity. It has been shown to be a good indicator of the initial transformation of xenobiotic compounds mediated by fungal peroxidative activity (de Jong *et al.,* 1992; Field *et al.,* 1993, Frietag and Morrell, 1992). Glenn and Gold (1983) used this dye as an alternative to radiolabelled lignin as a substrate in lignin biodegradation assays and suggested the application of several polymeric dyes including Poly R-478 to screen efficiently for species or strains with strong ligninolytic activity. While RBBR, an anthracene derivative, structurally resembles certain polycyclic aromatic compounds, which are substrates for peroxides (Vyas and Molitoris, 1995).

This study involved fungal exploration and collection, to find filamentous fungi with an extraordinary capability in degrading lignin compounds, as well as the isolation of fungi from natural habitat with a selective medium. Fungal identification was done according to the morphological basis and determined to genus until further studies. In order to facilitate and accelerate these tasks, we developed an agar-plate screening method by using Poly R- 478 and RBBR which enable the selection and the evaluation of lignin degrading fungi from several isolates.

MATERIALS AND METHODS

Location

Fungal exploration was carried out in Tanjung Puting National Park, Central Kalimantan. The majority of plants belong to families Dipterocarpaceae, Annonaceae, Myrtaceae, Euphorbiaceae, Moraceae, and Clusiaceae. The National Park has been developed as conservation area of orang-utan, bekantan, and uwa-uwa, with a continuous displace of the remaining forest by agricultural land. Besides, extensive fire during the dry year of 1977 has depleted much of the area.

Isolation of fungi from natural habitat

Collecting was carried out at the end of rainy season, which involved the complete collecting of Aphyliophorales fungi found in a transect area (1 sq ha). Fungi were collected in paper bag on fallen or living, unidentified wood. Fungal specimens was sun-dried at base camp and further drying will be carried out in the laboratory, using 50 °C oven for 24-48 hour.

The isolation of fungi was carried out on corn meal agar (CMA) medium. The plates containing basidiocarps were incubated at 28 °C. The germinating spores were transferred to fresh media and well-developed colonies were picked and subjected to purification in Potato Dextrose Agar (PDA) medium. Pure cultures were obtained after successive transfers of individual colonies in the PDA medium.

Growth rate of isolated fungi

The mycelial agar of isolated fungi (l x l cm) was grown on PDA medium, incubated at 27°C for 8 days. The diameter of mycelial growth was measured everyday for 10 days. Each measurement was done in triplicate.

Identification

Macroscopic observation is generally sufficient to determine genera, except for resupinate type. Fungal identification was done according to macroscopic characteristics following the reference of Ryvarden and Gilbertson (1994); Largent (1977), Imazeki *et al:* (1988), and Breitenbach and Kranzlin (1986).

18S rDNA restriction analysis for identification of resupinate fungi

Media for culturing and harvesting the selected isolate was Czapek Dox liquid medium. After 6 days of growth, the culture was harvested into a solution of 20 mmol EDTA pH 8 and poured onto a filter-paper in a Buchner funnel. The predried cake is peeled off the filter-paper, folded, immersed into liquid nitrogen and lyophilized.

Freeze dried ground material in an Eppendorf tube are suspended in 500 ml extraction buffer by stirring with a pipette tip. The slurry is mixed with 350µl phenol. Then 150µl chloroform are added, mixed, then centrifuged for 1 h (13000 g). The upper aqueous phase is taken off immediately, transferred to tube containing 25 µl RNAse A solution (became turbid) and incubated for 5-10 min. at37°C.

DNA isolated from selected isolate (T64 and T51) was subjected to PCR amplification with NS1, NS5, NS6, and NS8 primers previously described (Raeder and Broda, 1985), using 1.25 units of AmpliTag DNA polymerase (Perkin Palmer, Norwalk, CT). The fragments consisted of 760 and 1350 bp, and then were purified using a Microspin S300 HR Columns (Pharmacia Biotech). Sequencing of the amplified DNA was performed by the dideoxy chain-terminal method provided by the manufacturer (Pharmacia Biotech) using 7-deaza-dGTP. Homology searches were carried out using the BLAST program (Gish and States, 1993) against the GenPept databases through the National Center for Biotechnology Information.

Selection of lignin-degrading fungi

After 3-6 days, those cultures that grew well were subjected to further screening in medium of Glenn and Gold (Glenn and Gold, 1983) which contained Poly R-478 (Sigma Chemical, Co.). Biological assay was started by plugging l x l *ever* mycelial agar into the medium containing Poly R-478 for 14 days in 28 °C. Each isolate was subjected to the same procedure described above and the isolate showing decolourizing ability will be selected. The decolourization of medium will indicate the fungal ligninolytic capacity. All tests were conducted in triplicate.

Physiological growth of selected fungi

Mycelia of isolated fungi, T10, T21, T25, T50, T51, and T64 was grown on sawdust-agar medium, and incubated at different temperatures: 20, 30, 40 and 50 °C for 4 days. After incubation, the mycelia growths were measured. Each measurement was done in duplicate. Similar works were done at pH 2, 3, 4, 5 and 6.

Ligninolytic activity

Either Poly R-478 or RBBR (Nacalai Tesque, Inc.) were added to PDA medium and to sawdust-powder agar medium containing birch wood *(Betula platyphylla* var. japonica) and agar. The pH of media was adjusted to 5.0. This work was modified from the method of Glenn and Gold (1983) and Nishida *et al.* (1988).

Mycelial agar plug of each selected fungus (l x l cm) was inoculated into those media and incubated. After 7 days incubating, the ligninolytic activity of the selected fungi was examined qualitatively by observing and measuring the decolourizing zones of the media.

RESULTS AND DISCUSSION

Forty-three samples of fungi were collected from peat-soil area in Tanjung Puting National Park. However, only 42 samples can be produced as fungal herbarium collection. This is due to contamination by *Trichoderma.*

Herbaria collection of fungi is important and helpful not only in ecological-conservation basis, but also in preservation the diversity of fungi. Since identification from mycelial cultures

remains difficult without looking at fruiting bodies. The information on identification of fungi in cultures is not much available. Tentatively identified several samples are grouped into families, which is shown in Table 1. From all samples, only 33 specimens have been identified.

In Tanjung Puting National Park, the overall diversity of fungi belonging to the Aphyllophorales likely to be high, which at least 16 different genera of fungi have been collected from approximately 1 ha of transect area. The information on this area is still lacking. A more precise resolution of species or genus diversity could be achieved by DNA characterization, because fungi having identical morphology may differ in physiological properties.

Resuspension of the DNA of T64 and T51 gave clear, colourless, often viscous solutions with spectrophotometric ratios of OD_{260}: OD_{280} between 2.22 and 2.36. The DNA is cut efficiently with restriction endonucleases (tested with EcoRI and Hindi Ilia), and is ligatable.

Table 1. Tentative identification of several fungi

No.	Family	Genus	Description
1.	Ganodermataceae	*Ganoderma* sp.l (Tl)	Frb. broadly attached, thick, whitish when fresh but turning brownish when touched.
2.		*Ganoderma* sp.2 (T8)	Frb. consists of pileus and stipe, pileus ± 4 cm across, brownish, underside cream, round, stipe eccentric.
3.		*Ganoderma* sp.3 (T12)	Frb. annual, flabelliform, long (± 100 mm), applanate with a very short stipe. Upper surface dull, brown, and soft. Pore surface whitish but changed to light brown when touched. Context whitish, thick (30 mm). White rot.
4.		*Ganoderma* sp.4 (T15)	Thick at the base (40 - 60 mm), attached broadly to the substrate but only at the center. Pores fine, the surface creamy when fresh, turning brown when touch. Upper surface smooth and soft when young, margin rounded. Causes white-rot.
5.		*Ganoderma* sp.5 (T18)	Frb. semicircular, applanate and soft when fresh. Upper surface undulating, dusty, creamy white on fresh specimen and immediately change into light brown. The pores regular.
6.		*Ganoderma* sp.6 (T53)	Dimidiate with very short and woody stipe. Stipe eccentric. Frb. rounded to oval. Upper surface rough, brown, woody with narrow and whitish margin, concentric black lines. Underside poroid, grey whitish, pores rounded. Tube layer swollen.
7.		*Ganoderma* sp.7 (T50)	Frb. rounded, divided into pileus and stipe. Stipe eccentric, cylindrical 50 mm long. Upper side undulating with distinct concentric black line, brown. Lower side brown, rounded pores. Tube ± 30 mm, long with swollen layer. The fungus is very light weight when dry. Causes white rot.
8.	Corticiaceae s.lat.	*Stereum* sp. (T25)	Frb. fiabellate to semicircular with , broadly attached, thin, upper surface concentrically zoned, yellow-orange, margin sharp. Lower surface bright yellow with pored.
9.		*Phlebia radiata* (T64)	Based on 18s RDNA direct sequencing
10.		*Phanerochaete chrysosporium* (T51)	Based on 18s RDNA direct sequencing
11.	Polyporaceae s.lat.	*Daedalea* sp. (T28)	Frb. attached strongly, hard, pileate, semicircular, 20 - 50 mm thick, broadly attached. Upper surface uneven, light brown, sharp margin. Lower surface with irregular hymenophore.
12.		*Earliella* sp. 1 (T30)	Frb. pileate to semipileate, broadly elongated attached, 5 - 15 mm thick. Upper surface undulating, rough, with narrow concentric zones, dark brown. Lower surface with the hymenophore.
13.		*Earliella* sp.2 (T43)	Frb. grow in a row, pileate, pilei semi circular to fiabellate, individual pilei, 30 - 60 mm across, 10-20 mm thick, sometimes attached to the substrate by a stipelike. Upper surface rough, white to creamy colour when fresh. Margin thin. Under side white with rounded pores. Trama white, tough. Causes white rot.

Table 1: continue

No.	Family	Genus	Description
14.		*Echinochaete* sp. (T47)	Frb. flabellate, divided into pileus and stipe. Pileus 7- - 120 mm across, individual, rounded, lateral stipe. Upper surface blackish-brown, rough. Lower surface porose, yellow, pores angular. Causes white soft-rot.
15.		*Lentinus* sp. (Ti1)	Frb. divided into pileus and stipe, pileus rounded and 50 - 150 mm across, fleshy, tough, irregularly rounded, red to black brown, undulating, stipe central. Upper surface radially wrinkled toward the margin. Lower surface black-brown.
16.		*Microporus* sp.5 (T22)	Individually frb. Consists of pileus and stipe. Pileus rounded with depressed center, margin whitish, slightly undulating, lateral stipe. Upper surface reddish brown, fine wrinkle. Lower surface white, smooth with fine pores. Corky and tough.
17.		*Polyporus* sp.l (T27)	Frb. divided into pileus and stipe, central stipe, pileus circular. Upper surface finely squamose, whitish yellow-brown, margin slightly fringed. Lower surface coarsely porose, cream-colored with elongated pores. Elastic, tough, causes white soft rot.
18.		*Pycnoporus* sp. (T19)	Frb. broadly attached sometimes resupinate, thin. Upper surface smooth, orange to orange- red, margin sharp. Lower surface deep orange-red, fine pores with angular-rounded. Causes white soft-rots.
19.		*Trametes* sp.l (T2)	White spread. Frb. semicircular, thin, mostly broadly attached, orange to orange-red, tough.
20. .		*Trametes* sp.2 (T3)	Frb. rounded, consists of pileus and stipe, pileus 30 - 40 mm across, stipe 15 - 40 mm, dark red-brown in the upper surface, and whitish in the lower surface with pores.
21.		*Trametes* sp.3 (T4)	Frb. consists of pileus, stipe 50 - 70 mm, pileus 30 - 40 mm across, individual pilei. Upperside zoned, blackish-brownish. Lower surface pinkish with round pores. Causes white soft-rots.
22.		*Trametes* sp.4 (T5)	Frb. pileate, pilei semicircular, individual pilei ± 40 mm, thin, sometimes attached to the substrate by stipe like, radially wrinkled with indistinct zones, surface white to cream. Underside white, pores rounded. Causes white rots.
23.		*Trametes* sp.5 (T6)	Frb. semicircular, broadly attached, horizontal, 20 - 40 mm across, ± 5 mm thick, cream sometimes green because of algae, margin undulating, tough. Underside fine pored, cream, often solitary, causes white rots.
24.		*Trametes* sp.6 (T49)	Similar to T2
25.		*Trichaptum* sp. (T20)	Frb. pileate or semi pileate, semicircular, individual pilei 30 - 70 mm across, thin, sometimes attached to the substrate by a stipelike. Upper surface radially wrinkled, concentrically zoned with different colour (blackish, brownish, and reddish), margin regular. Lower surface yellow with rounded pores. Causes white-rots.
26.	Unknown samples	(T26)	Attached closely to the substrate. Frb. pileate to semi pileate, semicircular, dimiate, 1 - 2 mm thick, woody. Upper surface blackish, rough, undulating, margin soft undulating. Lower surface grey, pored. Brown-rot.
27.		(T7)	Frb. divided into pileus and stipe. Pileus irregularly rounded sometimes with undulating margin, individual. The upper surface radially ribbed and concentrically indistinct zoned, dark red-brown. Stipe eccentric, fine, almost black. Lower surface pinkish with fine pores.
28.		(T29)	Frb. semicircular, broadly attached, 20 - 40 mm thick. Upper surface uneven, concentric undulating, light brown, weakly zoned, and sharp margin. Lower surface beige, lamellae, pileal trama. Corky and tough.

Frb. = fruit body

Table 2. Summary of growth rate.

•Growth rate (diameter)	Percentage of total isolate
High growth rate : > 1.0 cm day^{-1}	78.0%
Medium growth rate : 0.5 -1.0 cm day^{-1}	12.5%
Low growth rate : < 0.5 cm day^{-1}	9.4%

*Growth was studied in petridishes containing PDA medium. Average mycelial growth was measured everyday for 9 days.

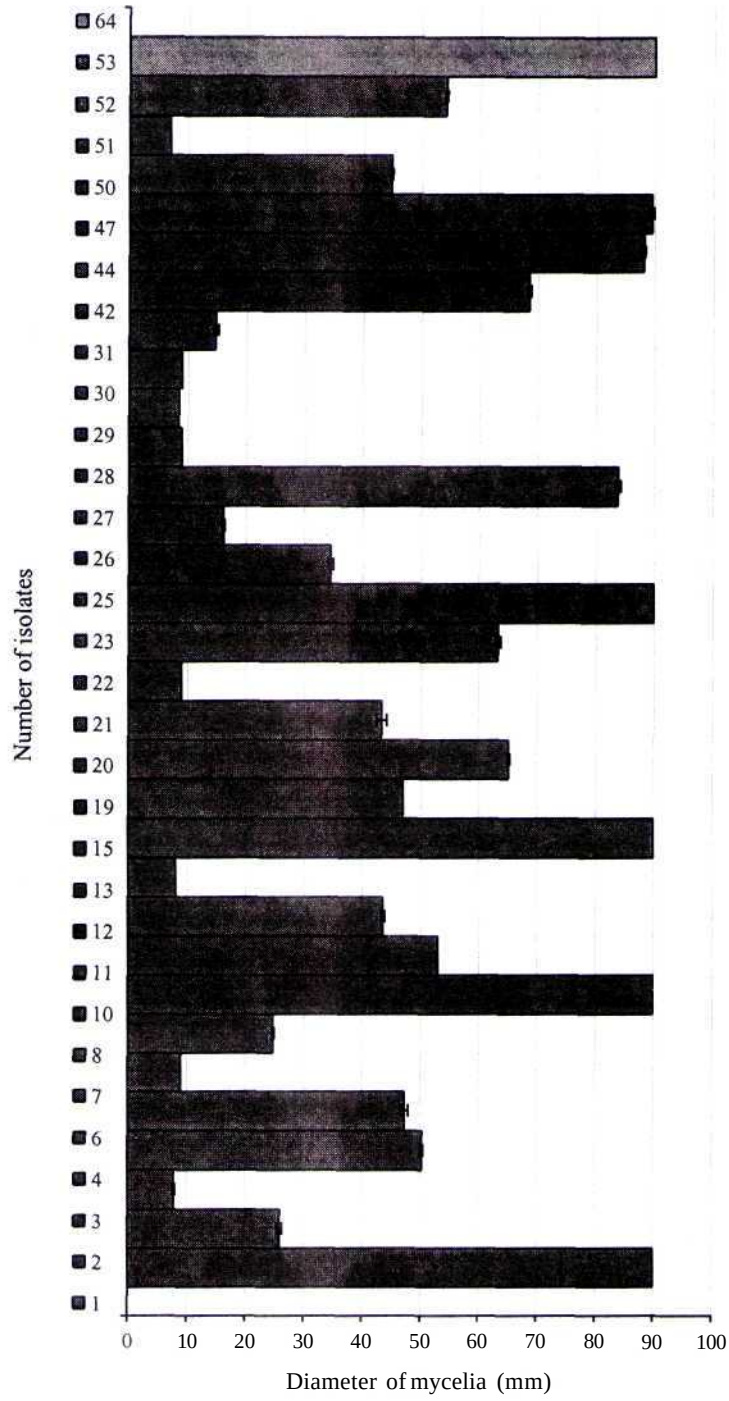

Figure 1. The mycelial growth of each culture at 4 days after incubation.

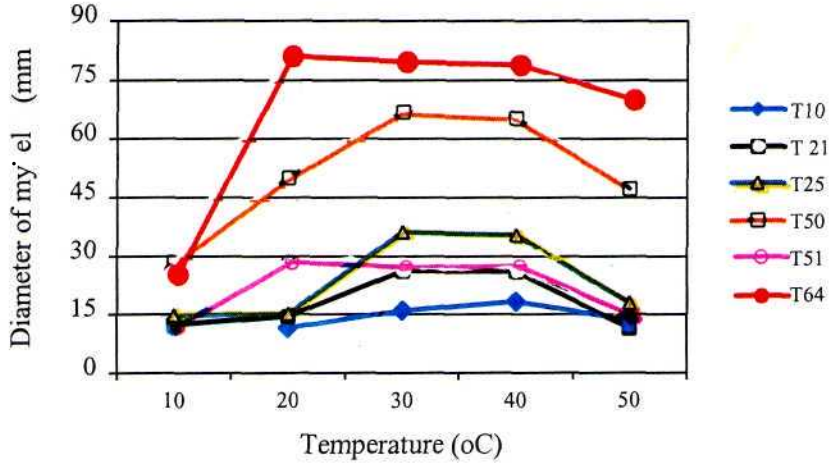

Figure 2. The effects of temperature on mycelia growth.

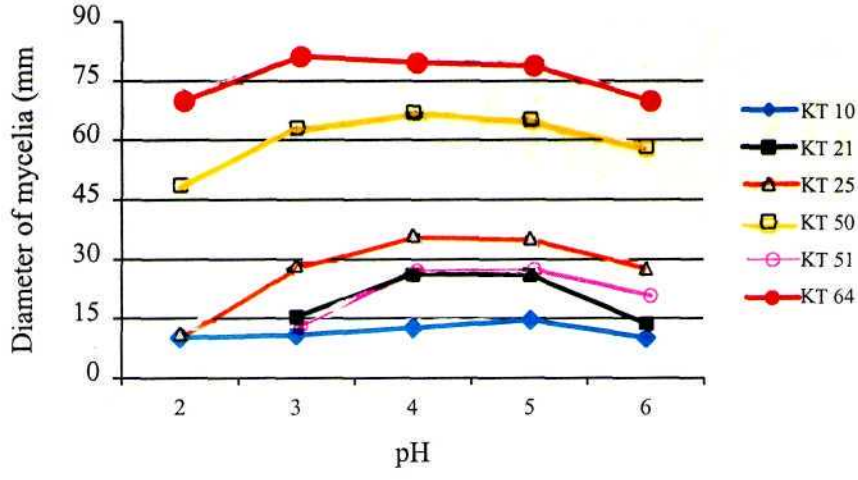

Figure 3. The effects of pH on mycelial growth

Table 3. Changes of diameter* (mm) of yellow-coloured zone by lignin-degrading isolates.

Time (day)	Lignin-degrading isolates				
	T10	T21	T25	T50	T51
Id	-	9.17 ± 0.24	+	-	+
2d	+	11.01 ± 1.41	9.00 ± 0	5.67 ± 0.47	11.67 ±0.24
3d	10.50 ± 0	13.67 ± 2.00	11.17 ±0.24	10.00 ± 0	19.83 ± 1.89
4d	11.32 ± 0.24	18.17 ± 0.24	18.67 ± 0.24	10.67 ± 0.24	23.33 ± 0.94
5d	14.00 ± 0.71	20.33 ± 0.48	19.33 ± 0.24	19.67 ±10.84	25.67 ± 1.89
6d	15.00 ± 0.71	20.33 ± 0.48	20.67 ± 0.24	28.50± 19.45	25.67 ±1.89
7d	16.00± 1.08	20.33 ± 0.48	21.16 ± 0.47	28.50 ±19.45	25.67± 1.89
8d	18.83 ± 0.63	20.33 ±0.48	21.50±0	28.50 ± 19.45	25.67 ±1.89

- : did not show colour change
+ : showed colour change, but no appreciable diameter measurement
* : indicate average of three replication of plates ± SE

Table 4. The decolourization of Poly R-478 and RBBR in PDA and sawdust-agar medium respectively at 7 days of incubation.

Isolates	Poly R-478		RBBR		Percentage of
	PDA	Sawdust-agar	PDA	Sawdust-agar	lignin loss
T10	-	+	+	42.8 ± 0.52	13.81 %
T21	-	25.4 ± 0.60	23.8 ±0.70	44.0 ± 0.03	7.08 %
T25	-	27.5 ± 0.54	53.4 ± 0.07	60.1 ±0.43	8.39 %
T50	17.1±0.25	36.4 ± 0.55	32.5 ±0.52	77.4 ± 0.62	18.69%
T51	+	15.4 ± 0.07	+	49.6 ± 0.22	• 0.16%
T64	46.3 ±0.10	83.9 ±0.33	+	87.2±0.15	28.18%

- did not show the colour-changed on the medium until 7 days.
+ showed the colour-changed on the medium but no appreciable measurement of the halo zone.
The lignin content of birch sawdust without fungal inoculation, as a control, was 31.94 %.

As part of our works on the lignin degrading fungi, we have attempted to use an extremely easy and rapid DNA isolation procedure which has shown to be applicable for identification especially for resupinate type. The phylogenetic analysis of sequencing data was done by searching homology on databases (data bank) and assessing phylogenetic relationships among datas. Isolates T64 and T51 belongs to *Phlebia radiata* (Corticiaceae) and *Phanerochaete chrysosporium* (Corticiaceae) respectively.

In order to examine the growth rate, each of isolated fungi was grown on PDA medium then measured the mycelial growth. The results in Figure 1 showed that until 4 days incubation, T10 was the fastest growing, followed by the isolate of T1, T15, T 25, T47 and T50; meanwhile T3 and T52 were the slowest growing in PDA medium. Isolate T52 did not even show a reasonable growth until day 6 (data is not shown). According to Setliff and Eudy (1980), the growth of isolated fungi could be classified into high, medium, and slow growth rate. Table 2 shows higher proportion of the strains isolated from Tanjung Puting National Park that grew rapidly in PDA medium (about 78%). Of the isolates, 12.5% grew slower than 0.5 cm/day, whereas only 4 isolates had low growth rate. These results suggest that high growth rate of fungi can not be assumed to indicate the ability to utilize lignin.

Report by Buckley and Dobson (1998) showed that decolourization of Poly R-478 by C. *lignorum* correlated well with lignin peroxidase and mangan peroxidase. From this study, 5 isolates produced yellow-colored zones in the media, but 27 isolates showed negative activity on the same media. Those isolates respond differently on Poly R-478 media, which indicate the ability of lignin decomposition (Table 3). The isolates degraded lignin selectively compared with other isolates that did not show the colour change of Poly R-478 medium. Among these, T50 showed the highest rate of decolourization and at 6 days incubation was eventually decolourized completely. The fungus was unidentified yet. Although the mycelia were grown, 27 isolates did not produce yellow-coloured zone on Poly R-478 (data not shown) indicating the isolates did not produce ligninolytic activity.

The degree of decolourization, particularly those by T21 and T51, was high, but remained unchanged even after 12 days. These isolates may be dependent on a readily available co-substrate for the depolimerization of lignin compounds. As reported by Crawford *et al.* (1981), Glenn and Gold (1983), Bumpuss *et al.* (1985), ligninolytic fungi mostly needed co-substrate to degrade lignin. Consequently, there is a variation within isolates in capacity to decolourize Poly R-478, as expected. Therefore, growth and decolourization of selected fungi in Glenn-Gold medium containing Poly R-478 were strongly correlated with their capacity to degrade lignin.

The effects of temperature and pH on the growth of mycelia were done for the selected isolates (presented in Figure 2 and Figure 3). All isolates mostly grew better at temperature between 20 and 40°C. The mycelial growth of T50 and T64 only slightly decreased at 50°C, eventhough T64 showed sharp increase at 20°C.

T21 and T51 did not show the growth at pH 2. On the other hand, T50 and T64 showed the widest range of pH as compared to other isolates. An examination of the physiology of these selected fungi will be beneficial to understand fungal behavior and provides basic information for further studies.

Screening test of fungi with lignin-degrading capacity by using agar plates has been used worldwide (Nishida *et al.,* 1988; Vyas and Molitoris, 1995), because this method can be used to select fungi from large numbers of colonies. However, the principle behind the method, some possible variations or their specific significance and the efficiency of the method are the important tasks to be performed. In the present studies, all the isolates that have the ability to decolourize Poly R-478 in medium Glenn-Gold were used to study the influence of the medium composition on the reliability of the lignin-degrading capacity.

All isolates grown in sawdust-agar medium showed the decolourization of Poly R-478 or RBBR although the diameter of halo zone is variable among the isolates (Table 4.) For example, isolate T10 started to decolourize the media but no appreciable measurement of halo zone at 7 days incubation, while T50 and T64 showed the highest decolourization. These may explain the variation in the time course of decolourization relating to the capacity of each isolate. On the other hand, the isolates grown on PDA containing Poly R-478 or RBBR showed lower ability of decolourization than those on sawdust-agar medium. Even the decolourization of Poly R-478 by T10, T21, and T25 at 7 days of incubation was not observed.

Based on the screening of decolourizing ability in sawdust-agar medium, T64 showed the highest activity. The decolourization rate in sawdust-agar medium was generally 2-3 times higher than the one in PDA medium. The results indicated that the fungi grown in lignin medium produced high peroxidative activities. The lignin content in sawdust may induce the activity. As reported by Glenn and Gold (1988), ligninolysis is usually induced by low concentration of nitrogen, carbon, and sulfur.

CONCLUSION

The results clearly show that nature offers a wide variation in specificities of fungi and suggest that tropical peat soils are a rich source of highly ligninolytic strains. They should be examined more closely. Besides several other applications, these fungi might also be employed (1) for producing clean product such as biopulp, (2) to manipulate the flux rate and magnitude of the organic component of peat soil, one of the larger organic carbon reservoirs.

REFERENCES

Breitenbach J and Kranzlin F. 1986. *Fungi of Switzerland Vol. 2. Non Gilled Fungi.* Verlag Mykologia, Lucerne.

Buckley KF and Dobson ADW. 1998. Extracellular ligninolytic enzyme production and polymeric dye decolourization in immobilized cultures of *Chrysosporium lignorum* Cll. *Biotechnol. Let.* **20,** 301-306.

Bumpuss JA, Tien M, Wright DS and Aust SD. 1985. Oxidation of Persistent Environmental Pollutants by White Rot Fungus. *Science.* **228,** 1434-1436.

Coulter JK. 1950. Peat Formations in Malaya. *Mai. Agr.J.* 33, 63-81.

de Jong E, Field JA and de Bont JAM. 1994. Aryl Alcohols in the physiology of Ligninolytic Fungi. *FEMS Microbiol. Rev.* 13, 153-188.

de Jong E, de Vries FP, Field JA, Van der Zwan RP and de Bont JAM. 1992. Isolation and screening of basidiomycetes with high

peroxidative activity. *Mycol. Res.* 96:1098-1104.

Field JA, de Jong E, Feijoo Costa G and de Bont JAM. 1993. Screening for Ligninolytic Fungi Applicable to the Biodegradation of Xenobiotics. *Trends Biotechnol.* **11,** 44-49.

Gish W. **and States** DJ. 1993. Identification of protein Coding Regions by Database Similarity Search. *Nat. Genet.* 3, 266-272.

Glenn JK and Gold MH. 1983. Decolourization of Several Polymeric Dyes by The Lignin-Degrading Basidiomycete *Phanerochaete chrysosporium. Appl. Environ. Microbiol.* 45, 1741-1747.

Imazeki R, Otani Y and Hongo T. 1988. *Fungi of Japan.* Yama-kei, Tokyo.

Largent DL. 1977. *How to Identify Mushrooms to Genus I: Macroscopic features.* Mad River, Eureka.

Nishida T, Kashino Y, **Mimura** A **and Takahara Y. 1988.** Lignin Biodegradation by Fungi. I. Screening of Lignin Degrading Fungi. *Mokuzai Gakkaishi* 34, 530-536.

Raeder U and Broda P. 1985. Rapid Preparation of DNA from Filamentous Fungi. *Letters in Applied Microbiology* 1, 17-20.

Ryvarden L and Gilbertson RL. 1993. *European Polypores.* Fungiflora, Oslo, Norway.

Setliff EC and Eudy, WW. 1980. Screening White-Rot Fungi for Their Capacity to Delignify Wood. Dalam: *Lignin Biodegradation: Microbiology, Chemistry and Potential Applications.* **Vol.** I. Kirk TK, Higuchi T and Ching H (Editor). CRC Press.

Stalpers JA. 1978. Identification of Wood-Inhabiting Fungi (Aphyllophorales) in Pure Culture. *Stud. Mycol.* **16,**1-248.

Vyas BRM and Molitoris HP. 1995. Involvement of an Extracellular H_2O_2-Dependent Ligninolytic Activity of the White Rot Fungus *Pleurotus Ostreatus* in the Decolourization **of** Remazol Brilliant Blue R. *Appl. Environ. Microbiol.* 61, 3919-3927.