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Combination of Laccase, Xylanase and Cellulase in Lignocellulose Degradation by White Rot Fungi, *Lentinus polychrous* Lev. and *L. squarrosulus* Mont.

Charida Pukahuta[1], Poonpilai Suwanarit[2], Emiko Shianagawa[3], Hisashi Hoshida[4] and Yoshinori Nishizawa[4]

ABSTRACT

The 22 isolates of white rot fungi, *Lentinus* spp. were screened for their activity of cellulase, xylanase and laccase. *L. polychrous* Lev. LP-PT-1 was cultured in 8 liquid media and 2 solid media. These 10 media were tested for lignocellulose degradation. Potato dextrose broth (PDB) and Fahraeus broth with 2 % rice straw (FRS) were selected as appropriate media. Both media were conducted to cultivate 4 isolates of *L. polychrous* Lev. LP-PT-1, LP-SW-3, LP-WR-13 and LP-UD-1 and 1 isolate of *L. squarrosulus* Mont.LS-YA. The xylanase and cellulase activities were determined at 40°C. Xylidine induced higher laccase actvity in 4 isolates, with the exception of LP-PT-1. LP-PT-1 produced three enzyme actvities in both PDB and FRS with and without xylidine addition. LP-SW-3 produced the highest activity of xylanase and laccase, 13.1 mU/ml and 3.27 U/ml, respectively, in FRS with xylidine addition. LP-SW-3, LP-WR-13 and LP-UD-1 showed the activity of three enzymes in FRS but lacked cellulase and/or xylanase activities in PDB. The production of xylanase of LP-SW-3 in FRS was induced by rice straw and of LP-UD-1 in PDB was induced by xylidine. LS-YA had the activity of all three enzymes in PDB but lacked xylanase activity in FRS without xylidine addition. FRS and xylidine addition was used to produce the culture broth for bleaching pulp. The increase in brightness of the treated pulp, 1.5-2.0 % higher than the control pulp, was correlated with the detected activity of xylanase of *L. polychrous* Lev.LP-PT-1, LP-UD-1 and *L. squarrosulus* Mont.LS-YA with low activity of laccase in each isolate. The high and moderate activity of xylanase with high and moderate activity of laccase in *L. polychrous* Lev. LP-SW-3 and LP-WR-13 did not increase the brightness of the treated pulp. It was concluded that these thermotolerant white rot fungi have the different combination of enzyme systems on lignocellulose degradation. The bleaching of pulp beneficially effected by the xylanase activity only when the activity of laccase was low.

Key words : laccase, xylanase, cellulase, lignocellulose degradation, white rot fungi, *Lentinus polychrous* Lev., *L. squarrosulus* Mont.

1 Department of Biological Sciences, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand.

2 Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

3 Ube National College of Technology, Yamaguchi 755-8555, Japan.

4 Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Yamaguchi University, Ube 755-8611, Japan.

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INTRODUCTION

White rot fungi are effective organism in lignocellulose degradation. These fungi produce lignocellulolytic enzymes, cellulase, xylanase, etc. that act cooperately to degrade the component of lignocellulose. The role of laccase has been described in ligninolysis while xylanase and cellulase involved in hemicellulose and cellulose degradation. The thermophilic and thermotolerant fungi are interesting in their metabolic actvities which can degrade lignocellulose at high temperature, 40°C, but the ability of the thermotolerant white rot fungi, *Lentinus* spp. has never been reported. This study was carried out to determine the combination of three enzyme systems in lignocellulose degradation of thermotolerant white rot fungi, *Lentinus polychrous* Lev. and *L. squarrosulus* Mont.

Lignocellulose is a widely used raw materials in industry. The structure composed of complex polymers of lignin, cellulose and hemicellulose embedded in plant cell wall (Eriksson *et al*., 1990). The degradation in nature is conducted by microorganisms of which the main degrader is white rot fungi. It was proposed by Kirk and Farrell (1987) that the hypha of the white rot fungi can penetrate inside the lignocellulosic wood and their extracellular enzymatic activities effectively catalyse the degradation. Recently, there is another propose on how the fungal degradating enzyme system works. Among the white rot fungi, *Phanerocheate chrysosporium*, *Trametes sanguinea*, *T. versicolor*, *Lentinus edodes* and *Pleurotus ostreatus* were studied (Tien and Kirk, 1984; Johansson and Nyman, 1993; Nishizawa *et al*., 1995; Buswell *et al*., 1996; Jonsson *et al.*,1998). The fungi produce ligninolytic enzymes, cellulases and xylanases that act cooperatively to degrade the component of lignocellulose. Laccase (EC 1.10.3.2), a copper containing phenoloxidase, has been described for its role in ligninolysis (Tien and Kirk, 1984) while xylanase and cellulase are involved in hemicellulose and cellulose degradation. Therefore, to depolymerize lignocellulose for pulping the synergistic interaction of cooperative enzymes system is more effective. The use of white rot fungi in biopulping is considerable not only to improve process which do not require chlorine but also to replace toxic chemicals and save on energy.

The thermotolerant white rot fungi are interesting because of their thermotolerant metabolic activities which can degrade lignocellulose at high temperature, 40-45°C (Cooney and Emerson, 1964). *Lentinus* spp. showed the different degradation pattern within the same genus. Some species could produce laccase and xylanase while having low activity of cellulase (Pukahuta *et al*., 2000). This property will be benefit for the removal of xylan from lignin-carbohydrate complexes which will facilitate the separation of lignin from the fiber cell wall, reducing the use of chlorine for bleaching in the brightening process and will not damage the pulp strength (Reid and Paice, 1994; Maheshwari *et al*., 2000). Therefore, this study was carried out to determine the combination between the enzyme systems in lignocellulose degradation by thermotolerant white rot fungi, *Lentinus polychrous* Lev. and *L. squarrosulus* Mont.

MATERIALS AND METHODS

Microorganisms

The fungal tissue was isolated from the fruiting bodies of identified species collected from natural and cultivated sources. *Lentinus polychrous* Lev. LP-DOA-8 was obtained from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. The fungal cultures were maintained on the potato dextrose agar (PDA) consisted of 200 ml of potato extract, 20 g of dextrose, and 20 g agar per liter. After incubation for 4 days at 45°C, the strains which showed good

growth were selected.

Media and cultivation

Cultivation for cellulose and xylan degradation was done in 30 ml of potato dextrose broth (PDB) incubated at 40°C and 250 rpm for 3 days. Inoculum was 10 mycelial disks (5 mm diameter) of four days culture grown on PDA. Cultivation for laccase activity was done in 30 ml of Fahraeus broth (Fahraeus and Reinhammer, 1968) incubated at 40°C with shaking at 250 rpm for 3 days. Inoculum was 10 mycelial disks (5 mm diameter) of four days culture grown on Fahraeus agar.

Media in the selection step were Fahraeus broth (with modification of 1 % dextrose) using as basic medium (1), supplemented with 2 % corn cob (2), 2 % wood chip (3), 2 % rice straw (4) and 2 % xylan (5). The complex media were potato dextrose broth (6), 2 % malt extract broth (7) and 2 % malt extract plus 0.1 % yeast extract (8). Volume of the liquid medium was 150 ml in 300 ml conical flask. The solid substrate fermentation was made in 25 g wood chips supplemented with 5 % rice bran and 60 % moisture adjusted with 0.1% yeast extract solution (9) and 25 g rice straw supplemented with 5 % rice bran and 60 % moisture adjusted with 0.1% yeast extract solution (10). The wood chip and rice straw used were 0.5 –1.0 cm in length. Inoculum was 20 mycelial disks (5 mm diameter) of four days culture grown on PDA. Incubation was done at 30°C with shaking at 120 rpm for 7 days. Static cultivation was made for solid substrate fermentation at 30°C. One ml of 30 mM Xylidine (Wako Pure Chemical Industries, Ltd.) in 95 % ethanol was added at day 2 and 4 to each medium of each culture.

Preparation of culture filtrate

The culture broth was filtered with filter paper (Advantec Toyo, No.1, Japan) to remove mycelium. To extract the crude enzyme from the solid cultivation, 50 ml of citrate phosphate buffer (pH 5.8) was added to cultured substrate and shaked at 30°C for 30 min.

Enzyme assays

Xylanase activity was measured using beechwood xylan (Sigma, USA) as a substrate. The reaction mixture consisted of 0.2 ml culture filtrate, 0.5 ml of 2 % beechwood xylan, 0.5 ml McIlvaine buffer (pH 5.0) and 0.8 ml distilled water,and incubated at 40°C for 30 min with shaking. The reaction was stopped by the addition of 1 ml of 0.3 % sodium dodecyl sulfate to the reaction mixture and the treatment was proceeded in boiling water for 10 min. After boiling the reaction mixture, the mixture was centrifuged at 800 g for 5 min. The supernatant was used for the determination of the reducing sugars by Somogyi and Nelson method (Somogyi, 1952) using 520 nm wavelength and xylose as a standard. One enzyme unit was defined as the amount of enzyme that produced one micromole of reducing sugars per min.

Cellulase activity was assayed with the same method as xylanase assay but carboxymethyl cellulose powder (CMC, Wako Pure Chemical Industries Ltd., Japan) was used as a substrate.

Laccase activity was mainly assayed by the modification method of Clutterbuck (1972). The increase of the absorbance at 550 nm caused by the oxidation of N, N'-dimethyl-p-phenylene diamine sulfate (DMP, Wako Pure Chemical Ltd., Japan) at 30°C was measured. The reaction mixture (3 ml) contained 30 mM DMP as a substrate and 40 mM sodium acetate buffer (pH 5.0). To Screen the thermotolerant strains, laccase activity was measured by monitoring the oxidation of 2, 6 dimethoxy phenol (DMOP, Fluka, Switzerland) at A_{469} (Perie and Gold, 1991). The reaction mixture (3 ml) contained 1.0 mM DMOP and 20 mM citrate phosphate buffer (pH 4.0). One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of one micromole of substrate per min.

Bleaching degree

The 2 % homogeneous pulp solution was prepared in 40 ml McIlvaine buffer (pH 5.0) and shaken for 30 min. The 160 ml culture filtrate was mixed with pulp solution and rotary shaken at 120 rpm at 35°C for 1 h. The treated pulp was vacuum-filtered, washed three times with 500 ml tap water, pressed and mounted for drying for 24 h at room temperature. The brightness of treated pulp and control pulp was measured by the brightness tester. (Elrepho-2000, Data Color Company, Switzerland) at the Department of Science Service, Bangkok (Ref. No.0307/8217). Brightness (%) was calculated from average $\pm$ t_{95}.S/$\sqrt{n}$ (n=10).

RESULTS AND DISCUSSION

The 50 fungal isolates of identified species were 36 isolates of *Lentinus polychrous* Lev., 4 isolates of *Lentinus squarrosulus* Mont.,1 isolate of *Lentinus sajor-caju*, 2 isolates of *Lentinus* spp., 5 isolates of *Pycnoporus* spp. and 2 unidentified isolates. When they were grown on PDA at 15, 20, 30, 40 and 45°C for 4 days, among 50 isolates, 25 of these could grow at five levels of temperatures. Then these 25 isolates were considered as thermotolerant species according to Cooney and Emerson (1964). However, they appeared to become larger colonies when grew at 40°C than 45°C. Another 25 isolates grew at four levels of temperature but did not show growth at 45°C. Twenty-two isolates of thermotolerant species were carried out for their lignocellulose degrading activity as shown in Table 1. The remaining three isolates with lesser growth were discarded.

The cellulase activity was 0-1.04 unit/ml and isolate LP-BR-11 was the best producer. The xylanase activity was 0-0.496 unit/ml and isolate LP-PT-1 was the best producer. The laccase activity was 0-96.6 unit/ml and isolate LP-WR-13 was the best producer. It was concluded that 14 isolates had activity of three enzymes, 5 isolates had actvity of xylanase and laccase, 2 isolates had activity of cellulase and laccase, and 1 isolate had activity of cellulase and xylanase.

All of these isolates could grow on supplemented sawdust that used for mushroom cultivation (unpublished data). In this study, one of the enzyme activity was not found in some of them. Laccase actvity was known criteria to determine lignocellulose degradation (Fukushima and Kirk, 1995, Bourbonnais *et al.*, 1997). However, this result correlated to Reid (1998) showing laccase was unaccountable for all of the lignocellulase actvity. To study the different enzyme production pattern of these species by using appropriate medium, LP-PT-1 which had three activities of enzymes with high xylanase was used.

To select the medium for lignocellulose degradation by using *L. polychrous* Lev.LP-PT-1, the cultivation was made in eight liquid media and two solid media. Potato dextrose broth (PDB), 2% malt extract broth and 2% malt extract with 0.1% yeast extract were the complete media used because they support the growth of all fungi. Fahraeus broth (FB) with 1% dextrose was used as basal medium. 2% corn cob (FCC), 2% wood chip (FWC), 2% rice straw (FRS) and 2 % xylan (FX) were added to FB with 1% dextrose to perform the induction effect on enzyme actvity. Wood chip or rice straw supplemented with 5 % rice bran and 60 % moisture was used to compare the actvity with the liquid media. After incubation at 30°C for 7 days, LP-PT-1 grew in all media and each of the enzyme activity was shown in Table 2.

The Highest activity of xylanase was obtained in PDB at 3 and 7 days of cultivation. The Highest activity of laccase was induced in Fahraeus broth with 2 % corn cob and the highest activity of cellulase was found in Fahraeus broth with 2 % wood chip. PDB was selected to study the xylanase production. Fahraeus broth with 2 % rice straw was the appropriate medium selected to study laccase and xylanase production. Rice straw could increase xylanase together with laccase activity at

Table 1 Lignocellulose degrading activity of 22 isolates of *Lentinus* spp.[1/]

Lentinus spp.	Activity of		
	Cellulase (Unit/ml)	Xylanase (Unit/ml)	Laccase (Unit/ml)
1. *L. polychrous* Lev.LP-BR-3	0.192	0.108	5.36
2. *L. polychrous* Lev.LP-BR-4	0.175	0.244	17.8
3. *L. polychrous* Lev.LP-BR-11	1.041	0	18.4
4. *L. polychrous* Lev.LP-BR-22	0.334	0.010	38.9
5. *L. polychrous* Lev.LP-DOA-8	0.334	0.288	32.3
6. *L. polychrous* Lev.LP-DU-5	0.458	0.248	57.4
7. *L. polychrous* Lev.LP-PB-21	0.051	0.268	18.1
8. *L. polychrous* Lev.LP-PT-1	0.068	0.496	13.4
9. *L. polychrous* Lev.LP-NY-11	0.277	0.278	46.4
10. *L. polychrous* Lev.LP-SM-11	0.045	0.373	60.0
11. *L. polychrous* Lev.LP-SN-12	0	0.187	5.97
12. *L. polychrous* Lev.LP-SW-1	0	0.445	21.1
13. *L. polychrous* Lev.LP-SW-2	0	0.255	8.39
14. *L. polychrous* Lev.LP-SW-3	0.713	0	30.5
15. *L. polychrous* Lev.LP-TP-1	0	0.010	5.85
16. *L. polychrous* Lev.LP-WR-13	0.130	0.143	96.6
17. *L. polychrous* Lev.LP-WR-14	0.017	0.319	64.6
18. *L. polychrous* Lev.LP-WR-31	0.187	0.472	23.9
19. *L. polychrous* Lev.LP-UD-1	0	0.353	18.1
20. *L. squarrosulus* Mont.LS-YA	0.334	0.200	0
21. *L. squarrosulus* Mont.LS-WR	0.288	0.183	0.121
22. *L. sajor-caju* LC-YD	0.017	0.122	34.7

[1/] The cultures were incubated at 40°C at 250 rpm for 3 days. To assay for cellulase and xylanase actvity, PDB was cultivated medium and for laccase actvity, Fahraeus was cultivated medium.

day 7 while corn cob induced lesser xylanase activity. In comparison, the activity of enzyme obtained from solid culture in wood chip and rice straw was not higher than those of liquid culture. Therefore PDB and Fahraeus broth with 2% rice straw (FRS) were conducted for the cultivation of five isolates namely, *Lentinus polychrous* Lev.LP-PT-1, LP-SW-3, LP-WR-13, LP-UD-1 and *L. squarrosulus* Mont.LS-YA. These 5 isolates were selected by their pattern of enzyme production as previously shown in Table 1.

LP-PT-1 and LP-WR-13 could produce three enzyme actvities. LP-PT-1 was the best xylanase producer and LP-WR-13 was the best laccase producer. Each of the other 3 selected isolates did not have one of the enzyme actvity. LP-SW-3 did not have xylanase actvity. LP-UD-1 did not have cellulase actvity and LS-YA did not have laccase actvity. To determine the combination between the enzyme systems in lignocellulose degradation of thermotolerant white rot fungi, *Lentinus polychrous* Lev. and *L. squarrosulus* Mont., the 5 isolates were incubated in the two liquid media at 30°C, 120 rpm for 7 days. Xylidine addition was made in day 2 and day 4. Xylidine was well known inducer for laccase production,

Table 2 The selection of medium for lignocellulose degradation by using *Lentinus polychrous* Lev.LP-PT-1.[1/]

Media	Cellulase (mU/ml)		Xylanase (mU/ml)		Laccase (U/ml)	
	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7
1. Fahraeus broth	0	4.55	0	0.045	0.077	0.43
1. Fahraeus + 2 % corn cob	5.70	1.65	1.45	0.555	0.267	3.15
2. Fahraeus + 2 % wood chip	0	6.95	0	0.060	0.045	1.77
3. Fahraeus + 2 % rice straw	0.495	4.55	0	1.40	0.128	2.42
5. Fahraeus + 2 % xylan	0	2.70	0	1.32	0.202	1.64
6. Potato dextrose broth	0	0.105	1.99	6.75	0.022	0.440
7. Malt extract broth	0	1.65	0	0.795	0.190	0.210
8. Malt extract yeast extract broth	2.60	2.60	0	0.105	0.222	0.253
9. Wood chip	Nd	3.95	Nd	0.30	Nd	0.780
10. Rice straw	Nd	3.90	Nd	0	Nd	0.155

[1/] The cultures were shaked at 120 rpm, 30Ɣ for 7 days in liquid media and static for solid state culture.
Nd : not determined

Table 3 Lignocellulose degrading activity of *Lentinus* spp. in selected media.[1/]

Lentinus spp.	Media[1/]	Cellulase (mU/ml)		Xylanase (mU/ml)		Laccase (U/ml)	
		Without xylidine	With xylidine	Without xylidine	With xylidine	Without xylidine	With xylidine
L. polychrous Lev.LP-PT-1	PDB	5.41	8.31	0.20	4.45	0.60	0.64
	FRS	6.38	7.3	2.5	12.6	1.31	0.95
L. polychrous Lev.LP-SW-3	PDB	2.3	0.90	0	0	0.20	0.83
	FRS	3.70	1.30	5.30	13.1	0.60	3.27
L. polychrous Lev.LP-WR-13	PDB	0	1.80	10.4	8.02	0.546	2.50
	FRS	12.0	5.5	1.0	6.30	0.473	1.67
L. polychrous Lev.LP-UD-1	PDB	0	1.30	0	3.72	0.253	0.993
	FRS	0.37	1.8	6.9	10.0	0.747	0.753
L. squarrosulus Mont.LS-YA	PDB	6.7	10.5	2.2	1.5	0.930	1.87
	FRS	10.6	11.2	0	3.88	0.507	0.753

[1/] PDB : Potato dextrose broth, FRS : Fahraeus broth with 2% rice straw
The cultures were incubated at 30°C for 7 days and shaked at 120 rpm. Xylidine was added at days 2 and 4.

then its addition was carried out to find the effect on the enzymes production. The results (Table 3) showed that the highest xylanase and laccase activity was obtained from LP-SW-3 in FRS with xylidine addition, 13.12 mU/ml and 3.27 U/ml, respectively. The highest cellulase activity was obtained from LP-WR-13 in FRS without xylidine addition, 12.0 mU/ml. However, to obtain the desired bleaching, the microbial enzymes should be free from cellulase activity since cellulase activity causes cellulose loss. The crude enzymes of these isolates will be treated with 2 % pulp solution in the next step, to investigate their combination between each enzyme in bleaching effect.

The culture broth was prepared by cultivation 5 selected strains, *L. polychrous* Lev. LP-PT-1, LP-SW-3, LP-WR-13, LP-UD-1 and *L. squarrosulus* Mont. LS-YA in 300 ml of FRS (Fahraeus broth with 2% rice straw) incubated at 40°C for 7 days and shaked at 250 rpm. 2 ml of 30 mM xylidine was added to the culture broth at days 2 and 4. Then the culture broth was filtered with filter paper, mixed with 2 % pulp solution, shaken, vacuum filtered, washed, pressed and mounted for drying to be treated pulp. The uninoculated FRS medium was added with xylidine and incubated under same condition as the inoculated medium of the above five cultures. The uninoculated medium or the medium without fungal degradating enzyme or the control medium was filtered. The control filtrate was mixed with 2% pulp solution and processed to be control pulp under the same method of the treated pulp. The brightness (%) of treated pulp and control pulp was measured by the brightness tester, Elrepho-2000. Testing condition was 27 ± 1°C and 65 ± 2 % relative humidity. The result was shown in table 4. The culture filtrate of *L. polychrous* Lev.LP-PT-1 and LP-UD-1 increased brightness about 2% higher than the control. *L. squarrosulus* Mont.LS-YA increased brightness about 1.5 % higher than the control. The culture filtrate of *L. polychrous* Lev.LP-SW-3 and LP-WR-13 showed the similar result to the control. The culture filtrate of *L. polychrous* Lev.LP-SW-3 showed the lower brightness of the pulp when compared to control. This may be caused by the brownish pigment of the culture filtrate of LP-SW-3 that attached to the pulp and could not be washed.

High actvity of xylanase of *L. polychrous* LP-PT-1 and LP-UD-1 (12.6 mU/ml and 10.0 mU/ml) and low activity of laccase (0.95 and 0.75 U/ml) in FRS broth were correlated with the 2 % higher brightness of the treated pulp than the control pulp. Low actvity of xylanase of *L. squarrosulus* LS-YA (3.88 mU/ml) and low activity of laccase (0.755 U/ml) in FRS broth gave 1.5 %

Table 4 The brightness of the pulp treated by culture filtrate of *Lentinus* spp. and the control pulp[1/].

Treatment	Culture filtrate of					
	L. polychrous Lev.LP-PT-1	*L. polychrous* Lev.LP-SW-3	*L. polychrous* Lev.LP-WR-13	*L. polychrous* Lev.LP-UD-1	*L. polychrous* Mont.LS-YA	Control filtrate
Brightness (%) of pulp	60.4 ± 0.2	58.8 ± 0.2	58.3 ± 0.4	60.4 ± 0.3	59.9± 0.2	58.4 ± 0.2

1/ Control pulp was the pulp treated by uninoculated medium or control filtrate under the same condition as the pulp treated by each of the culture filtrate of *Lentinus* spp. 40 ml of 2% homogenous pulp solution prepared in McIlvaine buffer pH 5.0 was mixed with 160 ml of control filtrate and shaken at 120 rpm at 35°C for 1h. Then it was vacuum filtered, washed three times with 500 ml tap water, pressed and mounted for drying for 24 h at room temperature. The brightness of treated pulp and control pulp was measured by the brightness tester. The basic solution was not involved in the bleaching process.

higher brightness of the treated pulp than the control pulp. When the treated pulp of *L. polychrous* LP-SW-3 was compared to LP-WR-13 and the control pulp, the high activity of xylanase (13.1 mU/ml) and high activity of laccase (3.27 U/ml) showed similar brightness to the moderate actvity of both xylanase (6.3 mU/ml) and of laccase (1.67 U/ml). In addition, it seemed that cellulase activity was not directly related to the brightness of the pulp. The high, moderate and low activity of cellulase (11.2, 7.3 and 1.8 mU/ml) of *L. squarrosulus* LS-YA, *L. polychrous* LP-PT-1 and LP-UD-1 resulted in high percentage of brightness in treated pulp. The moderate and low activity of cellulase (5.5 and 1.3 mU/ml) of *L. polychrous* LP-WR-13 and LP-SW-3 did not increase brightness.

Unlike *Phanerocheate chrysosporium*, *Pleurotus ostreatus* and *Ganoderma lucidum* whose optimum temperature for growth and production of lignocellulose degrading enzymes was 25-35°C (Punnapayak *et al*., 2002), *Lentinus* spp. grew well at 35-40°C (Pukahuta *et al*., 2000) and their enzymes worked at 30-40°C in this study. The thermotolerant white rot fungi, *Lentinus* spp. has the advantage of being easier to application in pulp and paper industry and enzymes fermentation. However, we need to characterize these enzymes and to know the operating conditions.

CONCLUSION

Xylanase activity (low, moderate and high) of *Lentinus* spp. in this study increased the brightness of the treated pulp whenever the activity of laccase was low. The moderate and high activity of laccase effected the dark pigment in the broth and caused the lower brightness which was similar to the control pulp. Cellulase activity was not related to the brightness of the pulp. These thermotolerant white rot fungi, *Lentinus* spp. have the different combination of enzyme systems on lignocellulose degradation.

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