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Screening and Evaluating of Wood-Rotting Fungi for Lignin Degradation and Ligninolytic Enzyme Production(I)*1

— Screening of High Active Lignin-Degrading Fungi —

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리그닌分解와 리그닌分解酵素 生産을 위한 木材腐朽菌의 選拔과 評價(I)*1

— 高活性 리그닌分解菌의 選拔 —

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要 約

Guaiacol을 첨가한 potato-dextrose agar배지와 참나무 목분 배지를 이용하여 배지의 발색 정도를 정량함으로써 리그닌 분해와 리그닌 분해효소 생산을 위한 우수 균주분리를 시도하였다. 배지의 발색정도와 리그닌 분해율과는 정의 상관을 나타내어 미지균의 리그닌 분해력 추정을 가능하게 하였으며, 버섯의 자실체와 부후재로 부터 분리한 리그닌분해균 중에서 리그닌 분해력과 laccase활성이 우수한 LKY-12, LKY-7과 *Coriolus versicolor*-13 균주를 선발하였다. 이들 균주의 리그닌 분해율은 30~35% 범위이고, glucose-peptone broth에서 리그닌 분해효소 활성이 다른 균주에 비하여 매우 높아서 우수균주의 특성을 나타냈으며, 생물학적인 펄프화 및 표백 그리고 리그닌분해효소 생산에 이용 가능한 균주로 생각되었다.

ABSTRACT

This experiment was conducted to screen a superior wood-rotting fungi for lignin degradation and ligninolytic enzyme production by evaluation of red colored zone width on potato-dextrose agar medium and oak woodmeal medium complimented guaiacol. Relationship between the red colored zone width on GU-WA medium and klason lignin loss on woodmeal medium showed the positive correlation. Thus, the potential ligninolytic activity of wood rotting fungi which are not elucidated yet may be estimated to some extent by the evaluation of the red colored zone width on GU-WA medium. Of the isolates screened from fruit bodies and decayed woods, LKY-12, LKY-7 and *C. versicolor*-13 isolates having preferential lignin degradation and laccase activity were selected. These isolates exhibited characteristics of superior wood-rotting fungi as Klason lignin loss ranged from 30% to 35%

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and ligninolytic enzyme activity of these isolates on glucose-peptone broth was higher than that of other isolates. And then, these isolates were considered to be able to use in biological pulping and bleaching and ligninolytic enzyme production.

Keywords : Wood-rotting fungi, ligninolytic activity, laccase activity, Klason lignin loss

1. INTRODUCTION

Lignin-degrading microorganizms and ligninolytic enzymes have possible industrial potential in pulping, bleaching pulps, modifying pulp surface properties, treating wastes, and converting byproduct lignins to useful chemicals. It was known that the white-rot fungi, most of which belong to the Basidiomycetes, are to the best lignin degraders among all known microorganisms, and there are several thousand species of white-rot fungi worldwide. At present, *Coriolus versicolor* and *Phanerochaete chrysosporium* of the white-rot fungi are known as the most lignin-degrading microorganisms (Arora & Sandu, 1986 ; Blanchette, 1984 ; Pasczynski *et al*, 1988).

However, there are a great variations in ligninolytic activity and extracellular enzymes characteristics among the isolates in same fungi, and their ligninolytic activities are insufficient for industrial application because they degrade the lignin porton of wood quite slowly and also degrade the carbohydrate portion. Therefore, it is important to select the best species and isolates of white-rot fungi for industrial application.

White-rot fungi degrade lignin by the action of enzymes that are secreted into the medium. Two extracellular peroxidase, lignin peroxidase and manganese peroxidase, and a laccase have been widely studied, and evidence that they participate in lignin depolymerization has accumulated. However, the mechanism and the reaction of lignin degradation by these enzymes have not been exactly elucidated yet. Only the reasons for believing that laccase or peroxidases are involved in lignin degradation were that (1) lignin contains phenolic hydroxyl groups which constitute a substrate, (2) white-rot fungi which degrade lignin also produce laccase and/or peroxidase extracellularly, (3) lignin degradation is oxidative (Eriksson *et al*, 1990).

This experiment was conducted to select high ligninolytic fungi for lignin-degrading, enzyme production and further industrial application by the evaluation of ligninolytic acctivity and the quantitation of enzyme acctivity on a lignin-degrading fungi.

2. MATERIALS & METHODS

2. 1 Isolation and screening of lignin-degrading fungi

The lignin-degrading fungi were isolated from fruit bodies of mushrooms and decayed woods gathered in forest land of eastern area of Chonnam. That is, tissues cutted from fruit bodies of mushroom and pieces of the decayed woods were inoculated on the potato dextrose agar medium complimented with 0.01% guaiacol(GU-PDA). After incubation at 28℃ for 3 days, the potential ligninolytic activity of the tested fungi were evaluated qualitatively by observing colored zones appeared on GU-PDA medium. The isolates producing higher red colored zone were selected, and were inoculated again on wood-powder agar medium(100mesh pass oak wood powder 0.2% and agar 1.6%) complimented with 0.01% guaiacol(GU-WA) as described by Nishida *et al*(1988). After incubation at 28℃ for 7 days, red colored zone width was determined, and then the selected isolates

were maintained on PDA slant at 4℃.

2.2 Ligninolytic activity and selection index of screened fungi

The selected isolates were inoculated in 50ml Erlenmeyer flask with woodmeal medium(moisture content, 300%) : one gram of *Quercus variabilis* or *Pinus densiflora* wood meal(60~80mesh) which were extracted with hot water and alcohol-benzene successively. After incubation at 28℃ for 30 days, dry weight and Klason lignin content of wood meal were determined. The selection index(SI) calculated according to Yoshihara's equation(1985). That is, SI=holocellulose content/Klason lignin content. Here, the holocellulose content was obtained by the following equation : holocellulose=dry weight of sample-Klason lignin.

2.3 Preparation of crude enzyme and enzyme assay

For extracellular lignin-degrading enzyme assay, the screened isolates were inoculated on glucose-peptone broth of 35ml as shown in table 1, and incubated at 28℃ for 7 days. The culture medium was adjusted to pH 5.0 before autoclaving.

The lignin-degrading enzyme assays were performed with the extracellular fluid, which was collected by filtration through Whatman no.4 filter paper. The filtrates were used as a crude enzyme solution.

Protein contents were determined by Lowry method, using bovine serum albumin as the standard (Bolag & Edelstein, 1991).

Laccase activity was measured by monitoring the oxidation of syringaldazine spectrophotometrically at 525nm (Bolag & Leonowicz, 1994). The reaction mixture contained 0.18M citric acid, 0.36M phosphate buffer (pH 6.0), 0.075 mM syringaldazine, and suitable amount of enzyme in total volume of 3ml. Enzyme boiled for 5 minutes was used in the control. An increase in the activity by 0.01 unit in one minute was taken as one unit.

Table 1. Composition of culture medium.

Glucose	30g
Peptone	10g
KH_2PO	41.4g
$MgSO_4 \cdot 7H_2O$	0.5g
Thiamine-HCl	2.0mg
$CuSO_4 \cdot 5H_2O$	20mg
Distilled water	1000ml

Manganese peroxidase activity was determined by measuring the changes in 465 nm with guaicol as the substrate(Pasczynski *et al*, 1988). The reaction mixture contained 0.1 M sodium tartrate buffer (pH 5.0), 0.1 mM guaicol, 0.1 mM H_2O_2, and 0.1mM $MnSO_4$. The reaction was initiated by the addition of H_2O_2. Enzyme boiled for 5 minutes was used in the control. Since laccase can also utilize guaiacol as a substrate, MnP activity was determined by subtracting the H_2O_2 and $MnSO_4$ independent activity from total activity. An increase in the activity by 0.01 unit in one minute was taken as one unit.

Lignin peroxidase activity was measured by monitoring the oxidation of veratryl alcohol to veratraldehyde as indicated by an increase in 310nm(Tien & Kirk, 1988). The reaction mixture contained 25 mM sodium tartrate buffer (pH 2.5), 2 mM veratryl alcohol, and 0.4 mM H_2O_2. The reaction was initiated by the addition of H_2O_2. Enzyme boiled for 5min was used in the control

2.4 Physiological characteristics of lignin-degrading fungi

The lignin-degrading fungi screened were incubated on glucose-peptone broth under conditions of pH 2~9 at 28℃ and 10~50℃ at pH 5. The pH of medium was adjusted to appropriate pH with the buffer solution: 0.1M sodium tartrate buffer, pH 2~4 ; 0.1 M phosphate buffer, pH 5~6 ; 0.1 M sodium phosphate buffer, pH 7~8 ; 0.1 M Tris-HCL, pH 9. Optimal pH and temperature of fungi growth and enzyme pro-

Table 2. Number of ligninolytic fungi according to the colored zone width on GU-WA medium.

Colored zone width(mm)	NO. of fungi		Total
	fruit bodies	decayed wood	
0~10	2	20	22
11~30	6	10	16
31~50	12	8	20
51~70	6	9	15
71~90	2	1	3
Total	28	48	76

duction were determined by measuring the mycelium weight and the laccase activity after incubation for 7 days under different conditions.

3. RESULTS & DISCUSSION

3.1 Isolation and screening of lignin-degrading fungi

The Bavendam test has been used as a simple test in the detection of ligninolytic activity of fungi. However, Nisida *et al*(1988), demonstrated that the correlation between the Bavendam test and ligninolytic activity of fungi is not satisfactory, and developed a new method to detect ligninolytic activity. It is the simple plate test method which ligninolytic activities were detected by evaluating colored zones on wood meal media complimented with guaicol. To screen a superior lignin-degrading fungi, this methods were used.

Tissues cutted from fruit bodies and pieces of the decayed woods were incubated on GU-PDA medium at 28℃. In the GU-PDA medium, 105 isolates forming red colored zone were selected, which consisted of 41 isolates from fruit bodies and 64 isolates from the decayed woods.

The selected isolates with exception of isolates having slower growth rate on GU-PDA mediumwere inoculated on GU-WA medium, and incubated at 28℃ for 7 days. After incubation, the potential ligninolytic activity of the selected isolates were evaluated by observing red color change on the GU-WA medium. As shown in

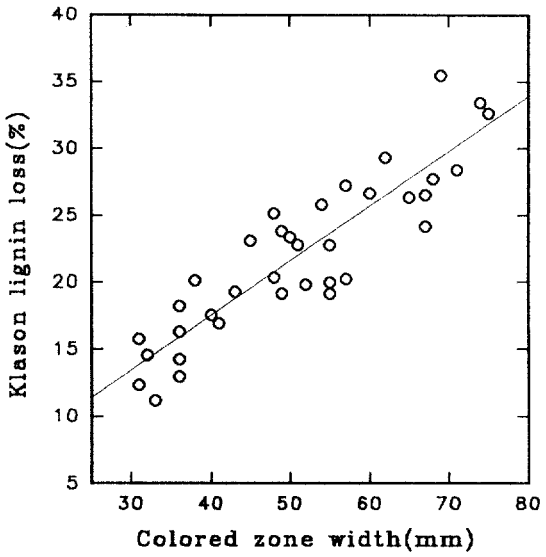

Fig. 1. Relationship of Klason lignin loss vs. colored zone width of the isolates screened.

Table 2, the selected fungi were classified into 5 groups according to the red colored zone width on GU-WA medium.

Most of the isolates from fruit bodies were shown to high growth rate on GU-WA medium, but approximately 40% of the isolates from the decayed wood showed low growth rate. The fungi having high ligninolytic avtivity in natural environment exhibit high growth rate on the same artificial conditions such as wood-meal medium. That is, fungi with low levels of color change on GU-WA medium seems to have no appreciable ligninolytic activity(Nishida *et al*, 1988). Therefore, 38 isolates exhibited relatively high levels of color change (31mm over) were selected again for further examining ligninolytic activity. All of 38 isolates that were positive on GU-WA medium were inoculated in a WA medium consisting of one gram of oak wood meal (60~80 mesh), and incubated at 28℃ for 30 days. The results indicated that all of the isolates inoculated had ligninolytic activity on WA medium, although the degrees of ligninolytic activity were different among the isolates.

3.2 Ligninolytic activity and selection index

On oak woodmeal medium, the Klason lignin loss of each selected isolates showed a trend to increase in proportion to the colored zone width on GU-WA medium. As shown in Fig. 1, relationship between the colored zone width and Klason lignin loss showed the positive correlation. This linear relationship made it possible to use colored zone width as a basic parameter of ligninolytic activity of screening fungi in derived equation, Y=0.4088X+1.1791*.

From the results of lignin degradation on oak woodmeal medium, 19 isolates having considerably high ligninolytic activity(above 20% of klason lignin loss) were screened, and further inoculated on pine woodmeal medium. After incubation at 28℃ for 30 days, Klason lignin loss and selection index were determined. The results of the colored zone width on GU-WA medium and the Klason lignin loss and selection index on the oak and the pine woodmeal media of screened 19 isolates and 2 isolates, *Coriolus versicolor*(IFO 4942) and *Phanerochaete chrysosporium*(ATCC 34540), supplied from KCCM for comparison were shown in Table 3. Of the 19 isolates, 8 isolates from fruit bodies were identified as 3 strains of *Coriolus versicolor*, 2 strains of *Pycnoporus coccineus*, *Ganoderma lucidum* and *Schizopora paradoxa*, but 11 isolates from decayed woods not identified yet.

The colored zone width between 38mm to 82mm were found in these screened 19 isolates, the highest width being found in LJS-6 and the least in LJS-5. *P. chrysosporium* reached a maximum growth of 90mm in 5 days incubation. However, there was no significant difference in

Table 3. Colored zone width and ligninolytic activity of screened isolates.

Origin	Strains isolated	CZW (mm)	W.L(%)		K.L(%)		S.I	
			Pine	Oak	Pine	Oak	Pine	Oak
Fruit body	*C. versicolor* - 5	71	6.73	16.91	5.14	28.42	3.50	3.16
	- 8	68	7.84	20.43	6.31	27.73	3.51	3.06
	- 13	75	5.56	17.22	7.39	32.62	3.62	3.50
	G. lucidum	45	5.54	19.17	5.34	24.27	3.48	2.94
	P. coccineus - 2	49	5.27	14.81	6.42	23.84	3.73	3.24
	- 3	55	6.53	15.49	5.93	22.81	3.67	3.06
	C. hirsutus	54	5.67	12.25	7.29	25.84	3.76	3.06
	S. paradoxa	65	9.34	20.15	8.18	26.37	3.57	2.95
Decayed wood	LKY- 4	48	3.72	13.21	5.64	25.18	3.77	3.65
	LKY- 7	74	8.38	14.49	7.94	33.42	3.70	3.75
	LKY-12	69	9.19	17.29	9.12	35.48	3.67	3.67
	LSM- 1	57	4.49	15.25	6.84	27.25	3.65	3.37
	LSM-12	60	9.31	18.84	10.92	26.69	3.77	3.29
	LSM-13	67	4.83	15.28	8.92	26.55	3.58	3.49
	LSK- 8	50	5.32	13.29	5.16	23.36	3.61	3.18
	LSK-15	41	6.02	12.77	5.78	21.15	3.56	3.02
	LSK-27	51	7.28	16.22	6.17	22.81	3.62	3.17
	LJS- 5	38	6.29	15.17	5.43	23.71	3.63	2.92
	LJS- 6	82	10.21	13.68	16.72	29.34	2.95	3.56
KCCM	*C. versicolor*	71	4.59	14.39	5.58	21.34	3.78	3.69
	P. chrysosporium	90	1.23	3.76	1.58	8.15	3.77	3.81

Notes : W.L : weight losses, K.L : Klason lignin lossess, S.I : Selection index, Pine : *Pinus densiflora*, Oak : *Quercus variabilis*, CZW : colored zone width. 3. 3 Extracellular enzymes secreted by lignin-degrad

the values of the colored zone width between the isolates from fruit body and the isolates from decayed woods. When the 21 isolates were incubated on the woodmeal medium for 30 days, the weight losses varied among the isolates tested and the wood substrate used. In pine woodmeal medium, the weight losses ranged from 4.5% to 10.2%, whereas losses were considerably higher in oak wood meal, ranging from 12.3% to 20.4%. In general, white-rot fungi are commonly found on deciduous wood substrate in natural environment (Blanchette, 1984). Also in this experiment, the isolates were found to grow better on deciduous wood substrate.

Delignification showed higher level on oak woodmeal substrate than on pine woodmeal. Klason lignin loss caused by 21 isolates ranged from 5.1% to 16.7% in pine woodmeal medium, and from 21.2% to 35.5% in oak woodmeal. The greatest Klason lignin loss was caused by LKY-12 isolate with 9.1% and 35.5% on pine and oak woodmeal respectively, and followed by LKY-7 with 7.9% and 33.4%, and *C. versicolor*-13 with 7.4% and 32.6%. These isolates seemed to delignify to a higher degree than these of *C. versicolor* and *P. chrysosporium* supplied from KCCM. Especially, Klason lignin loss of *P. chrysosporium* was found to be a low level in this experiment. Exceptionally, only LJS-6 strain exhibited relatively high level of Klason lignin loss on pine woodmeal.

Selection index which indicated delignification selectivity of lignin-degrading fungi was about 2.9~3.8, and there was no significant difference among each isolates.

3.3 Extracellular enzymes secreted by lignin-degrading fungi

A total of 21 isolates were analyzed for their extracellular enzymes after incubations of 7 days on glucose-peptone broth. The most abundant activity found in glucose-peptone broth was laccase activity. The laccase activities and the manganese peroxidase activities of extracellular enzymes are shown in Table 4. Of the 21 isolates, 19 isolates except for LSK-27 isolate and *P. chrysosporium* exhibited laccase activity, and the highest level of activity was found in the LKY-12 isolate with 91.5 U/mg of protein, followed by *C. versicolor*-13 with 78.1 U/mg, and LKY-7 with 68.4 U/mg. These isolates exhibited higher levels of laccase activity than *C. versicolor* supplied from KCCM under same condition. However, *G. lucidum* and *P. coccineus* exhibited much lower levels of laccase activity than the other isolates.

Because of the ability of laccase to utilize guaiacol as substrate, the manganese peroxidase activity with guaiacol as the substrate was necessary to subtract the activity which was not dependent on the presence of H_2O_2 and manganese(Pasczynski *et al*, 1988). The each isolates exhibited much less manganese peroxidase activity than laccase activity in glucose-peptone broth. The manganese peroxidase activity of the isolates ranged from 2.2 unit to 14.0 unit per mg of protein. However, LSK-27 isolate exhibited no laccase activity showed relatively high level of manganese peroxidase (22.92 unit/mg of protein). And, although it was low levels, the manganese peroxidase activity of the isolates of LKY-12, LKY-7, and *C. versicolor*-13 which exhibited the highest level of laccase activity was greater than that of other isolates. In many wood-rotting fungi, the production of laccase corresponds with the presence of ligninolytic activity, thus suggesting a important role for this enzyme in lignin biodegradation(Bolag & Leonowicz, 1994). Also in this experiment, the three isolates of LKY-12, LKY-7 and *C. versicolor*-13 exhibited high activity of laccase showed preferential lignin degradation (Table 3). But this higher ligninolytic activity is not likely to be a result of the laccase activity, since the ligninolytic activity was detected in case of the LSK-27 isolate having no laccase activity.

To detect lignin-peroxidase, the veratryl alco-

Table 4. Extracellular enzyme activity of screened strains.

Origin	Strains	L.A	P.C	Sp.L	Mn.P	Sp.Mn
Fruit body	*C. versicolor*- 5	65.43	1.67	39.24	7.54	4.52
	- 8	33.20	1.79	18.55	7.87	4.40
	-13	117.22	1.50	78.14	9.26	6.17
	G.lucidum	T	1.65	-	3.59	2.18
	P. coccineus- 2	15.18	1.81	8.37	T	-
	- 3	10.26	1.51	6.80	T	-
	C. hirsutus	38.15	1.65	23.12	6.89	4.18
	S. paradoxa	23.93	1.70	14.08	7.75	4.56
Decayed wood	LKY- 4	29.19	1.51	19.33	6.34	4.20
	LKY- 7	125.87	1.84	68.41	9.25	5.02
	LKY-12	136.33	1.49	91.49	13.24	8.89
	LSM- 1	35.40	1.84	22.48	8.26	4.49
	LSM-12	32.66	1.75	18.66	16.31	9.32
	SM- 13	29.45	1.73	17.02	7.78	4.50
	LSK- 8	24.27	1.35	17.90	4.92	3.64
	LSK- 15	17.80	1.57	11.33	4.32	2.75
	LSK- 27	-	1.64	-	22.92	13.97
	LJS- 5	19.66	1.55	12.69	3.69	2.38
	LJS- 6	42.19	1.74	24.25	8.03	4.61
KCCM	*C. versicolor*	47.54	1.51	31.48	6.43	4.26
	P. chrysosporium	-	1.68	-	T	T

Notes : L.A : laccase activity(unit/ml), P.C : protein concentration(mg/ml), Sp.L : specific activity of laccase(unit/mg of protein), Mn.P : manganese peroxidase activity(unit/ml), Sp.Mn : specific activity of manganese peroxidase(unit/mg of protein), T : trace.

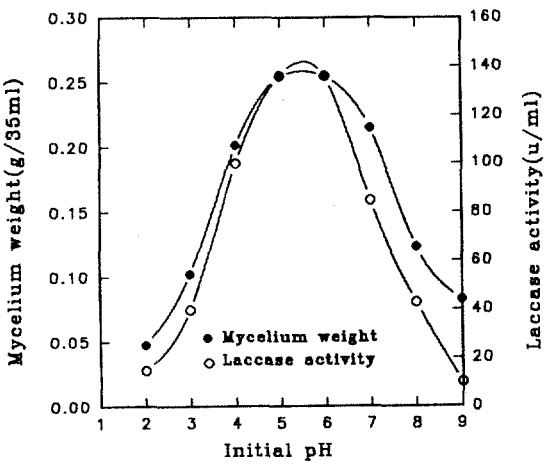

Fig. 2. Optimal pH of medium on mycelial growth and laccase activity of LKY-12 isolate.

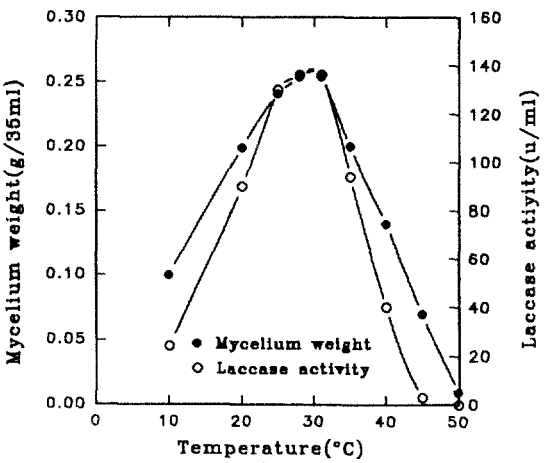

Fig. 3. Optimal temperature of incubation on mycelium growth and laccase activity of LKY-12 isolate.

hol was used as the substrate. However, it was unable to detect LP activity in any of the isolates under glucose-peptone broth used in these experiments. Orth and Royse(1993) demonstrated that the potential to produce lignin-degrading peroxidases is present in nearly all

ligninolytic fungi. Thus, these isolates appear to be capable of producing lignin-peroxidase under the proper conditions.

3.4 Physiological characteristics of lignin-degrading fungus, LKY-12

The effect of pH and temperature on growth rate and laccase activity of LKY-12 isolate were shown in Fig. 2 and 3. Even though absolute, exact evaluations of physiological characteristics could not be made because of effect of buffer solution on mycelial growth, LKY-12 isolate was seemed to optimally grow between pH 5 to pH 6 and between 25℃ to 31℃. This optimal growth conditions of LKY-12 isolate was similar with those of most Basidiomycetes. But the range of optimal condition for laccase activity secreted on medium was exhibited to more or less narrow in comparison with the mycelial growth. This results were considered due to the low stability of enzyme on pH and temperature. The optimal pH and temperature for laccase production of LKY-12 isolate was pH 5 and 31℃ respectively.

4. CONCLUSIONS

To screen the superior wood-rotting fungi for lignin degradation and ligninolytic enzyme production, tissues from fruit bodies of mushrooms and pieces of decayed woods were incubated on potato-dextrose agar (GU-PDA) medium and oak woodmeal agar (GU-WA) medium complimenting 0.01% guaicol. The isolates which may have high potential ligninolytic activity were selected by evaluations of red colored zone width on GU-PDA and GU-WA medium. These results were confirmed by determination of Klason lignin loss on oak woodmeal(WA) medium. Relationship between the red colored zone width on GU-WA medium and klason lignin loss on oak woodmeal medium showed the positive correlation. Thus, the potential ligninolytic activity of wood-rotting fungi which are not elucidated yet may be estimated to some extent by the evaluation of the red colored zone width on GU-WA medium.

Exceedingly the variation of lignin degradation exists among selected isolates. The greatest Klason lignin loss was caused by the isolate of LKY-12 with 9.1% and 35.5% on pine and oak woodmeal respectively, and followed by LKY-7 with 7.9% and 33.4%, and *C. versicolor*-13 with 7.4% and 32.6%. And also these 3 strains exhibited preferential laccase activity on glucose-peptone broth as compared to the other isolates. Generally manganese peroxidase activity was found to be a much low level in same condition.

Based on these results, it can be considered that the isolates of LKY-12, LKY-7, and *C. versicolor*-13 may be used for potential lignin degradation and ligninolytic enzyme production. Thus, production and purification of the laccase and the manganese peroxidase, isolation of respective genes, and identification of other enzymes involved in lignin degradation will be important for further characterization of the lignin-degrading system of these isolates.

REFERENCES

1. Arora, D.S. and D.K. Sandu. 1986. Degradation of lignocellulosic residues by polyporus versicolor and the effect of moisture contents and phenolic compounds. ***Acta. Biotechnol*** 6 : 293~297
2. Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. Appl. Environ. ***Microbiol***. 48 : 647~653
3. Bolag, J.M. and A.Leonowicz. 1994. Comparative studies of extracellular fungal laccases. ***Appl. Environ. Microbiol***, 48 : 849~854
4. Bolag, D. M. and S. J. Edelstein. 1991. Protein methods. Wiley-liss : 56~59
5. Davidson, R.W., L.E. Campbell and D.J. Blaisdell. 1983. Differentiation of wood-decay

fungi by their reactions on gallic or tannic acid medium. *J. Agric. Res.* 57:683~695.
6. Eriksson, K.E.L., R.A.Blanchette and P. Ander. 1990. Microbial and enzymatic degradation of wood and wood components. Springer-Verlag : 225~324
7. Konishi,K., Y.Inoue and T.Higuchi.1974. Decomposition of lignin by *Coriolus versicolor*. 4. Effect of laccase type enzyme on the inter phenylpropane linkage of lignin. *Mokuzai Gakkaishi* 18 : 571~576
8. Nishida, T., Y.Kashino, A.Mimura and Y. Takahara. 1988. Lignin biodegradation by wood-rotting fungi I. Screening of lignin-degrading fungi. *Mokuzai Gakkashi* 34 : 530~536
9. Orth, A.B., D.J. Royse and M. Tien. 1993. Ubiquity of lignin degrading peroxidases among various wood-degrading fungi. *Appl. Environ. Microbiol.* 59 : 4017~4023
10. Paszczynski, A., R. L. Crawford and V. B. Huynh. 1988. Manganese peroxidase of Phanerochaete chrysosporium : purification. *Methods Enzymol.* 161 : 264~270
11. Tien, M. and T.K. Kirk. 1988. Lignin peroxidase of Phanerochaete chrysosporium. *Methods in Enzymol.* 161 : 238~249
12. Yoshihara, K., I.Akamatsu and H.Kamishima. 1985. Screening of a white-rot fungus with strong lignin degrading activity. *Japan Tappi* 39(7) : 65~74