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Decolorization of Dyes by Selected Wood Degradation Fungus*1

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ABSTRACT

The objectives of this study were to select superior fungus for lignin degradation and to decolor dyes by selected fungus. Ligninolytic fungi were screened and isolated from decayed woods. Ten ligninolytic fungi were selected by ligninolytic enzyme activity on the PDA media containing rhemazol brilliant blue R, guaiacol and gallic acid. Their lignin degradation abilities were tested on the extractive-free wood powder of *Quercus acutissima* and *Pinus densiflora*. As a result, SJ-28 was selected as superior fungus for lignin degradation. Also, decolorization abilities of dyes were examined by shaking and static culture. And congo red, crystal violet, poly R-478, methylene blue used to investigate decolorization abilities of dyes. As a result, SJ-28 showed over 90% in decolorization of congo red, crystal violet, poly R-478.

Keywords : decolor dye, ligninolitic fungi, poly R-478, congo red, crystal violet, shaking culture

1. INTRODUCTION

Lignin is one of the most abundant organic polymers, second to cellulose among the renewable organic resources on the earth. Lignin is resistant to degradation by microorganisms than cellulose and hemicellulose in wood (Tien *et al.*, 1983 ; Etienne *et al.*, 1993). White-rot fungi degrade lignin by enzymes such as lignin peroxidase, manganese peroxidase and laccase. *Coriolus versicolor* and *Phanerochaete chrysosporium* of the white-rot fungi are known as the most lignin-degrading microorganisms (Orth *et al.*, 1993 ; Martin *et al.*, 1994).

Lignin is an irregular high molecular compound that is formed by dehydrogenation polymer of ρ-hydroxycinnamyl alcohol, and it is difficult to be hydrolyzed by acid. Also, decomposition of lignin is difficult because lignin is combined with cellulose and hemicellulose in wood (Cho *et al.*, 1987).

White-rot fungi degrade aromatic ring in lignin by ligninolytic enzymes. Their enzymes have been reported to decompose TNT, DDT, Dioxin (Bumpus *et al.*, 1989) and decolor dyes (Vyas and Molitoris, 1995).

Over 10,000 dyes with a total annual production in excess of 7×10^5 metric tones worldwide are commercially available, and typically 5~10% of this amount is discharged in indus-

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trial effluents (Toh *et al.*, 2002). Environmental problem by these is serious because about 24% of whole pollution load is occupied by dyes (Ministry of Environment, Korea, 1998). Dyes are used in textile dyeing, printing, color photograph and toiletry (Lim *et al.*, 2000). According to chemistry structure, dyes are classified into azo, triphenyl methane, heterocyclic and polymeric dyes (Zolliner *et al.*, 1982).

Their dyes are non-biodegradable toxicant containing functional group such as azo group, nitro group and sulfon group. Chemical or physical treatment is currently used for degrading. But research of fungi for biological treatment using microorganism has been investigated because such existing chemical and physical methods are expensive (Oh *et al.*, 1999).

Development of microorganism that decompose specific compound, non-biodegradable toxicant and heavy metal has investigated. But most of the research on dye degradation by white rot fungi has focused on *Phanerochaete chrysosporium* (Field *et al.*, 1993).

This study, therefore, was to select superior fungus for lignin degradation, and to investigate decolorization ability of several dyes by selected fungus.

2. MATERIALS and METHODS

2.1. Microorganism

White rot fungus was obtained by the isolation from fruiting bodies and decayed woods in several forest areas. And *Trametes versicolor* was provided from Korea Forest Research Institute.

2.2. Chemicals

Congo red was purchased from Junsei Co., methylene blue was purchased from Duksan Co. And crystal violet and poly R-478 were purchased from Sigma Co.

2.3. A Simple Plate-test for Lignolytic Activity

Either 0.01% guaiacol (GU), 0.1% gallic acid (GA), or 0.05% Rhemazol brilliant blue R (RBBR ; Sigma Chemical Co.) was added to a potato dextrose agar (PDA) medium with the pH of the media adjusted to 5.6. These media were inoculated with fungi and incubated at 29±1°C for 7 days. After incubation, the lignolytic activities of the fungi tested were evaluated qualitatively by observing color changes of the media (colored zones in media containing GU or GA and decolored zones in media containing RBBR) (Nishida *et al.*, 1988).

2.4. Wood Lignin Degrading Ability and Holocellulose Degrading Ability

Lignin degradation ability was investigated in wood-powder by fungi that changed all color of RBBR, GU, GA media. And decolorization ability of dye was investigated by fungus that showed the highest lignin degradation ratio among ten fungi.

One gram of extractive-free *Quercus acutissima* and *Pinus densiflora* wood-powder (60~80 mesh) were added to weighting bottle. This was inoculated separately with each strain of fungi and incubated at 29±1°C for 30 days. After incubation, the Klason lignin content and holocellulose content of the wood powder were determined (Nishida *et al.*, 1988 ; Park *et al.*, 1993).

2.5. Decolorization of Dyes

YMG medium (yeast extract 4 g, malt extract 10 g, glucose 4 g, pH 5.5) was used for dye decolorization (Kim *et al.*, 1995). Fungi were incubated on PDA media at 29±1°C for 7 days, and maintained at 4°C. Decolorization of several dyes were examined in static (at 29±1°C) (Cripps

Table 1. Decolored zone width of screened fungi on the plate medium test

Strain	Decolored zone Diameter(mm)		
	RBBR	GU	GA
AS-4	60.76	55.39	61.92
AS-8	59.22	50.88	84.00
CTS-2	74.63	48.89	84.00
KS-1	84.00	59.51	84.00
KS-7	71.37	60.22	84.00
PC-1	72.73	66.89	84.00
SC-1	61.16	55.80	78.93
SC-8	62.54	26.44	84.00
SC-12	64.06	16.48	73.71
SJ-28	51.19	46.63	65.17
TRV	84.00	60.08	84.00

et al., 1990 ; Yoon *et al.*, 1998 ; Kim *et al.*, 1995) and shaking culture (150 rpm at 29± 1°C) (Swamy *et al.*, 1999; Yesilada *et al.*, 2002). The ability of the fungi to degrade dyes, congo red, methylene blue, crystal violet, poly R-478 were studied in YMG medium (pH 5.5). Decolorization of dyes were determined on UV-vis spectrometer. The wavelengths in nanometers used for the absorbance ratios of congo red, methylene blue, cryatal violet, poly R-478 were A_{490}/A_{434}, A_{664}/A_{482}, $A_{594}/_{460}$ and $A_{520}/_{350}$, respectively (Yoon *et al.*, 1998).

3. RESULTS and DISCUSSION

3.1. Screening of Lignin Degradation Fungi

Ligninolytic fungi were isolated from decayed woods in several forest areas. Ten ligninolytic fungi were screened by ligninolytic enzyme activity on PDA media containing RBBR, GU, GA. This method used research that was correlation between decolorization of media containing RBBR, GU, GA and ligninolytic activity of fungi (Nishida *et al.*, 1998 ; Jung *et al.*, 1995).

3.2. Wood Lignin and Holocellulose Degrading Ability by Screened Fungi

Lignin degrading abilities were tested on the extractive-free wood powder of *Q. acutissima* and *P. densiflora* using screened ten fungi. And holocellulose contents were investigated to measure other wood component except lignin.

3.2.1. Wood lignin degrading ability by screened fungi

Klason lignin loss by SJ-28 was 63.03% in

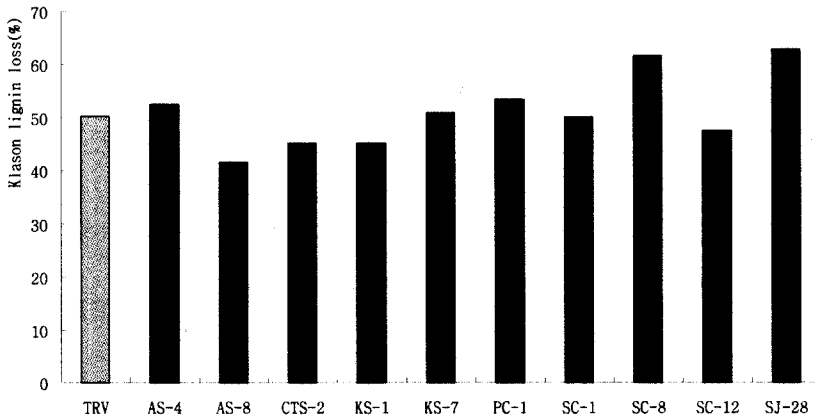

Fig. 1. Klason lignin loss of *Q. acutissima* wood powder by screened fungi.

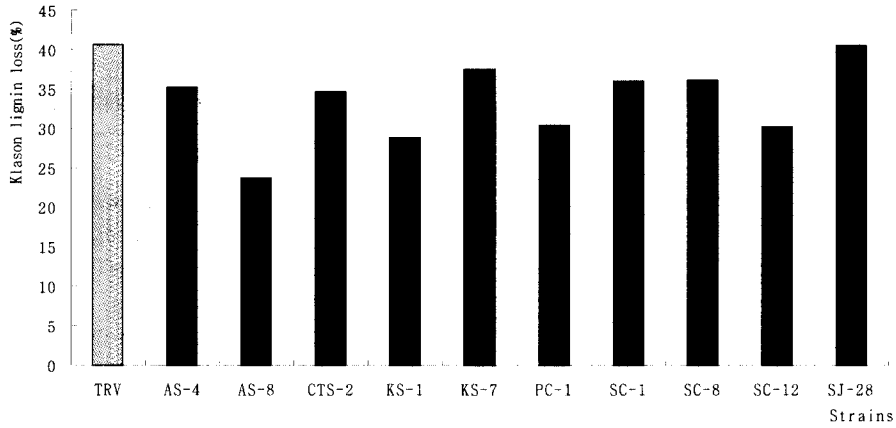

Fig. 2. Klason lignin loss of *P. densiflora* wood powder by screened fungi.

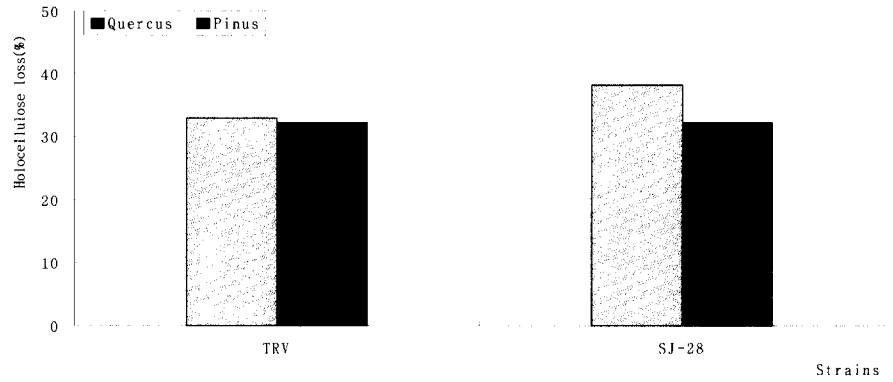

Fig. 3. Holocellulose loss by selected fungus and *T. versicolor*.

Q. acutissima wood powder medium. That was the highest among ten fungi. And Klason lignin loss by TRV was 50.30% in *Q. acutissima* wood powder medium. Lignin degrading ratios of two fungi were over 50%, and lignin degrading ratios of SJ-28 was higher than TRV that was known as superior lignin degrading fungus.

Also, Klason lignin loss by SJ-28 and *T. versicolor* were 40.56% and 40.58% in *P. densiflora* wood powder media. Lignin degrading abilities of two fungi were similar.

This results were the same as research that white rot fungi degrade more rapidly syringyl lignin consists of hardwood than guaiacyl lignin consists of softwood (Shin, 1996).

As these results, SJ-28 was selected to superior lignin degrading fungus.

3.2.2. Wood holocellulose degrading ability by screened fungi

Holocellulose content of SJ-28 was investigated to measure other wood component except lignin.

Holocellulose loss by SJ-28 and TRV were 38.19% and 32.94% in *Q. acutissima*, and were 32.18% and 32.21% in *P. densiflora*. Holocellulose loss was about 30% and was lower than Klason lignin loss.

Table 2. Decolorization rate of the dyes by selected fungus

Dye Compounds	Incubation time(day)			
	5	10	15	20
	Decolorization(%)			
Congo red	7.71	17.28	20.72	23.93
Methylene blue	5.48	8.35	13.06	14.92
Crystal violet	7.90	13.83	23.73	27.58
Poly R-478	8.65	16.80	28.68	30.56

Table 3. Decolorization rate of the dyes by *T. versicolor*

Dye Compounds	Incubation time(day)			
	5	10	15	20
	Decolorization(%)			
Congo red	5.69	10.16	16.99	18.86
Methylene blue	6.39	11.54	20.61	22.32
Crystal violet	5.31	17.69	27.34	30.79
Poly R-478	7.90	11.58	23.25	24.28

In case of holocellulose loss, SJ-28 and *T. versicolor* were similar, and this results were considered that it did not receive effects of species of trees in decompose holocellulose.

As compared lignin loss with holocellulose loss, Klason lignin loss of SJ-28 was 63.03% and 40.56% in *Q. acutissima* and *P. densiflora*, and in case of holocellulose was 38.19% and 32.18% in *Q. acutissima* and *P. densiflora*. SJ-28 was higher lignin degrading ability than holocellulose degrading ability. These results were considered that SJ-28 selectively decomposed lignin.

3.3. Decolorization Ability of Dyes by Selected Fungus

Decolorization ability of SJ-28 selected as superior lignin degrading fungus was examined. Several dyes were added to YMG media, and decolorization of several dyes were examined in static (at 29±1°C) and shaking culture (150 rpm at 29±1°C).

3.3.1. Decolorization of dyes by static culture

Decolorization abilities of dyes by static culture of SJ-28 and TRV were shown in Table 2 and 3.

As a results of static culture for 20 days, decolorization ability of SJ-28 in congo red, methylene blue, cryatal violet and poly R-478 were 23.93%, 14.92%, 27.58% and 30.56%. And decolorization ability of *T. versicolor* in congo red, methylene blue, cryatal violet and poly R-478 were 18.86%, 22.32%, 30.79% and 24.28%. Decolorization abilities of two fungi by static culture were low.

3.3.2. Decolorization of dyes by shaking culture

Decolorization abilities of dyes by shaking culture of SJ-28 and TRV were shown in Fig. 4~7.

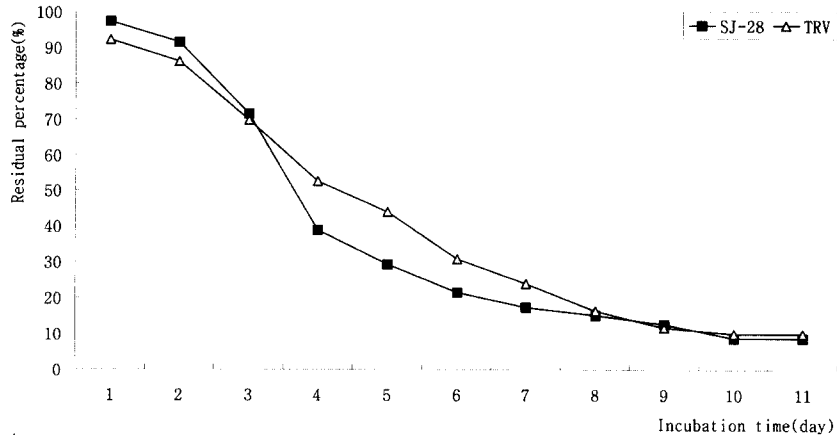

Fig. 4. Decolorization of congo red by selected fungus and *T. versicolor*

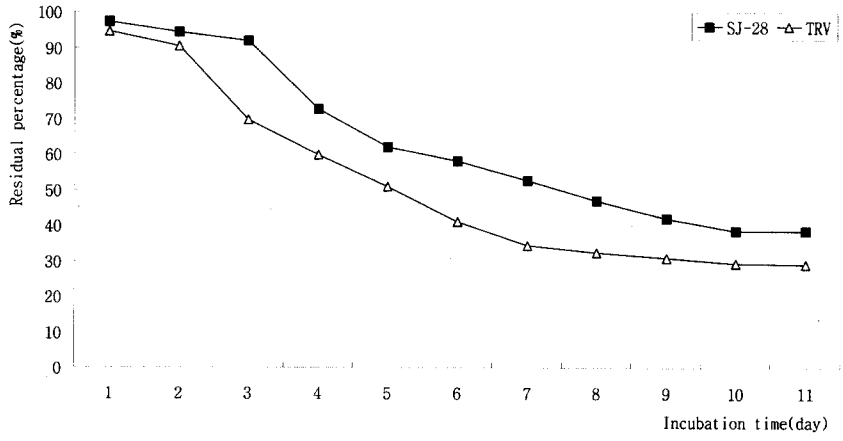

Fig. 5. Decolorization of methylene blue by selected fungus and *T. versicolor*

Decolorization ability of congo red for 11 days was shown in Fig. 4. Decolorization ratio of SJ-28 was shown higher than *T. versicolor*. Decolorization of SJ-28 was 91.31% in 11 days. In case of *T. versicolor*, decolorization ratio was 90.06% in 11 days.

Decolorization ability of methylene blue for 11 days was shown in Fig. 5. Decolorization ratio of *T. versicolor* was shown to higher than SJ-28. Decolorization of *T. versicolor* and SJ-28 were 70.96% and 61.47% in 11 days. Decolorization ratio of *T. versicolor* was higher about 9% than SJ-28.

Decolorization ability of crystal violet for 11 days was shown in Fig. 6. Decolorization ratio of SJ-28 was shown higher than *T. versicolor*. Decolorization of SJ-28 and *T. versicolor* were 90.20% and 90.09% in 11 days.

Decolorization ability of poly R-478 for 11 days was shown in Fig. 7. Decolorization ratio of SJ-28 was shown higher than *T. versicolor*. Decolorization of SJ-28 and *T. versicolor* were 96.44% and 95.81% in 11 days.

As compared lignin degrading ability with

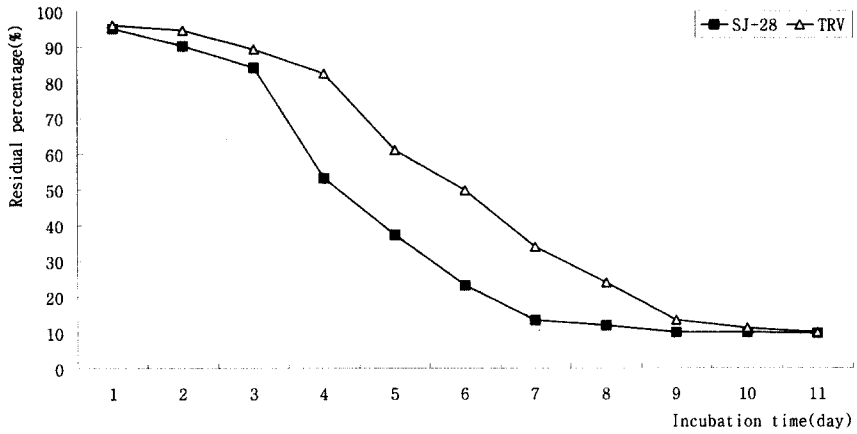

Fig. 6. Decolorization of crystal violet by selected fungus and *T. versicolor*

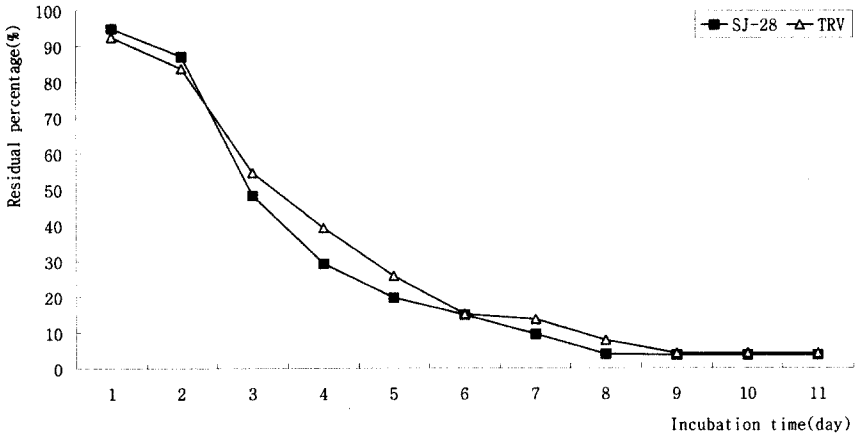

Fig. 7. Decolorization of poly R-478 by selected fungus and *T. versicolor*

decolorization ability of poly R-48, lignin degrading abilities of SJ-28 and *T. versicolor* were the highest as 63.03%, 50.30%, and also decolorization abilities of SJ-28 and *T. versicolor* in poly R-478 were the highest as 96.44%, 95.81%. These results were the same as research that was correlation between decolorization of poly R-478 and lignin degrading ability (Glenn *et al.*, 1983 ; de Jong *et al.*, 1992).

As these results, decolorization ratios of SJ-28 and *T. versicolor* were 14.92~30.56%, 18.86~30.79% in static culture and were 61.47~96.44%, 70.96~95.90% in shaking culture. Decolorization abilities of SJ-28 and *T. versicolor* were higher in shaking culture. These results were the same as research that was higher decolorization ratio in shaking culture than decolorization ratio in static culture (Swamy *et al.*, 1999).

Decolorization abilities of congo red, crystal violet, poly R-478 by SJ-28 and *T. versicolor* were over 90% in 11 days, whereas methylene blue was low in 61.47%, 70.96%. Specially, as compared decolorization of poly R-478 with

decolorization of congo red, methylene blue and crystal violet, poly R-478 showed the fastest and the highest decolorization.

Selected fungus, SJ-28 showed higher decolorization ability than *T. versicolor* in congo red, crystal violet, poly R-478 except methylene blue.

Based on these results, it can be considered that SJ-28 fungus selected may be effective in decolorization of dyes as well as lignin degradation.

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