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Original Article

Catabolic Pathway of Lignin Derived-Aromatic Compounds by Whole Cell of *Phanerochaete chrysosporium* (ATCC 20696) With Reducing Agent[1]

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ABSTRACT

Whole cell of *Phanerochaete chrysosporium* with reducing agent was applied to verify the degradation mechanism of aromatic compounds derived from lignin precisely. Unlike the free-reducing agent experiment, various degraded products of aromatic compounds were detected under the fungal treatment. Our results suggested that demethoxylation, C_α oxidation and ring cleavage of aromatic compounds occurred under the catabolic system of *P. chrysosporium*. After that, degraded products stimulated the primary metabolism of fungus, so succinic acid was ultimately main degradation product of lignin derived-aromatic compounds. Especially, hydroquinone was detected as final intermediate in the degradation of aromatics and production of succinic acid. In conclusions, *P. chrysosporium* has an unique catabolic metabolism related to the production of succinic acid from lignin derived-aromatic compounds, which was meaningful in terms of lignin valorization.

Keywords : catabolic metabolism, succinic acid, lignin-derived aromatic compounds, ring cleavage, *Phanerochaete chrysosporium*

1. INTRODUCTION

Ligninolytic properties of white rot basidiomycetes allow the oxidation of heterogeneous lignin polymers. Accordingly, very recently, new concept using the ligninolytic enzyme system of basidiomycetes has emerged for lignin valorization as an attractive alternative (Beckham *et al*., 2016).

P. chrysosporium, well-studied model basidiomycete, lead to degradation of various lignin model compounds. C_α-C_β, β-ether linkages and ring fission of β-O-4 model compounds were reported to be caused by *P. chrysosporium* (Enoki *et al*., 1981; Higuchi, 1990; Weinstein *et al*., 1980). Furthermore, LiP secreted from *P.*

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chrysosporium was also reported to catalyze not only C_α-C_β cleavage of β-1 compounds (Glenn *et al*., 1983; Tien & Kirk, 1983) but also degradation of β-5 and β-β model compounds (Higuchi, 1990; Umezawa *et al*., 1982).

These days, due to the enormous progress in analytical equipments such as electrophoretic, genomic sequencing, and mass spectrometric techniques, studies on secretomes and proteomes of basidiomycetes have advanced. Among basidiomycetes, genomic annotation of *P. chrysosporium* has been sequenced by the US Department of Energy's Joint Gemone Instutite, which provides high quality sequence of basidiomycetes (Martinez *et al*., 2004). Proteomic approach also provided comprehensive identification of extra- and intra-cellular proteins related to lignin degradation like LiP, manganese peroxidase, cytochrome P450s, and benzoquinone reductase (Adav *et al*., 2012; Matsuzaki *et al*., 2008; Ravalason *et al*., 2008). These findings were indicative of multi-enzyme complex of *P. chrysosporium*.

In previous our work, we investigated the biomodification products of monolinols and synthetic lignin by *P. chrysosporium* (Hong, 2016). Whole cell of *P. chrysosporium* simultaneously induced the degradation and polymerization of monolignols and synthetic lignin composed of monolignols. With addition of reducing agents, synthetic lignin was degraded to lignin oligomer, aromatic compounds (syringic acid and 2,6-dimethoxy-benzodiol) and acid compound (succinic acid). When the identified enzymatic information of the fungus were combined, we suggested that complex enzymes system of *P. chrysosporium* induced various degraded products from lignin. Based on these results, to establish the accurate catabolic mechanism of lignin derived compounds by *P. chrysosporium*, we carried out to analyze the biodegradation products of lignin derived-aromatic compounds by *P. chrysosporium.* Coniferyl alcohol, sinapyl alcohol, syringic acid and hydroquinone were selected as lignin derived compounds. Coniferyl alcohol and sinapyl alcohol as C3C6 type were components of synthetic lignin used in our previous study, and syringic acid (C1C6 type) and hydroquinone (C6 type) were of the degradation products of synthetic lignin. Accordingly, our study aimed to demonstrate the catabolic mechanism of various types of aromatic compounds by *P. chrysosporium*.

2. MATERIALS and METHODS

2.1. Materials

P. chrysosporium used as white rot fungus was obtained from the Microbiology Chemistry Laboratory of National Institute of Forest science (NIFoS). This fungus has been used for biodegradation of lignin derived compounds because lignin degradation using by *P. chrysosporium* is a well-studied model (Keyser *et al*., 1978). *P. chrysosporium* was grown on a potato dextrose agar (PDA) medium at 28℃ for one week. After 7 days of fungi inoculation on PDA medium, the mycelium had fully grown. Mycelia covering the PDA medium were sepa-

Table 1. Concentration of SSC medium components (per 1 l deionized water)

Component	Concentration	Amount (g)
Glucose ($C_6H_{12}O_6$)	1%	10
Ammonium tartrate ($C_4H_{12}N_2O_6$)	1.08 mM	0.2
Potassium phosphate (KH_2PO_4)	14.7 mM	2
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	2.03 mM	0.5
Calcium chloride ($CaCl_2$)	0.68 mM	0.1
Thiamine · HCl ($C_{12}H_{17}ClN_4OS \cdot HCl$)	2.97 mM	0.001
Trace element solution (Hong, *et al*., 2016)	10 mℓ/liter	

rated from the medium, and homogenized with 20 mℓ of distilled water at 5,000 rpm for 3 min in ice-water bath. Finally, fungal suspension was obtained. The dry weight of 1 mℓ of fungal suspension was measured after 24 hours on a dryer at 105℃.

Lignin derived compounds used in this study were coniferyl alcohol, sinapyl alcohol, syringic acid and hydroquinone. Monolignols, coniferyl alcohol and sinapyl alcohol, were synthesized from ferulic acid and sinapic acid (Sigma Aldrich Co.) by the methods of Quideau and Ralph with slight modifications (Quideau & Ralph, 1992). And syringic acid and hydroquinone were purchased from Sigma Aldrich.

2.2. Ligninolytic treatment of aromatic compounds by *P. chrysosporium*

The shallow stationary culture (SSC) medium was used as nitrogen limiting medium. SSC medium was proposed by Kirk group for the secretion of ligninolytic enzymes (Kirk & Farrell, 1987). To prepare a SSC medium, major components of medium were dissolved in 990 mℓ distilled water (Table 1). After the medium was autoclaved at 121℃ for 15 min, 10 mℓ of the trace element solution were added after filtration through 0.2 μm membrane filter (Hong *et al*., 2016). Certain volume of fungal suspension including 0.02 g of dried fungal hyphae was inoculated into 100 mℓ of SSC medium, and the flask was plugged with silistopper. After stationary pre-incubation at 28℃ for 4 days, each 40 mg of aromatic compounds was spiked in the medium. To prevent polymerization of aromatic compounds reactants by the fungus, 5 mM of ascorbic acid was added to the SSC medium as a reducing agent.

2.3. Analysis of degraded products of lignin derived-aromatic compounds by *P. chrysosporium*

The flasks were withdrawn from incubator periodically. Sample was analyzed every 5 days during 25 days. In the case of coniferyl alcohol and sinapyl alcohol experiments, each sample was centrifuged at 12,000 rpm, 15 min at 4℃ to separate mycelium. After centrifugation, the supernatant was loaded to Sep-Pak C18 cartridge (Waters) slowly. Sample loaded in car-

tridge was eluted by passing methanol, and then methanol was evaporated under vacuum condition to remove the solvent. Finally, it was dissolved in 10 mℓ of methanol and extracted with 25 mℓ of ethyl acetate with using separating funnel at 250 rpm shaker for 15 min, and this solvent extraction was carried out 3 times. Extraction using ethyl acetate was conducted with addition of sodium chloride which caused salting-out effect. Anhydrous sodium sulfate was added in liquid sample, and then the collected ethyl acetate fractions were filtrated, and evaporated under vacuum condition to remove the solvent. Finally, it was dissolved in 10 mℓ of ethyl acetate.

In the case of syringic acid and hydroquinone, the reaction mixtures were extracted with 50 mℓ of ethyl acetate, as mentioned above. The solvent extraction was carried out 3 times. After evaporation, it was dissolved in 10 mℓ of ethyl acetate.

For the analysis of extracts, trimethylsilylation (TMS) was performed. And then, gas chromatography-mass spectrometry (GC-MS) was performed on an Agilent HP7890A GC, equipped with an Agilent HP5975A mass selective detector (MSD). The stationary phase of the GC-MS was a DB-5 capillary column (30 m × 0.25 mm ID × 0.25 um coating thickness). The initial oven temperature of the GC was 50℃ for 5 min, and the temperature was then programmed to increase at a rate of 3℃/min up to 300℃, and maintained for 10 min. The temperature of injector and detector were 220℃ and 300℃, respectively, and the carrier gas was helium at a flow rate of 1 mℓ/min. Peak identification was based on comparison of the mass spectra with the NIST (National Institute of Standard and Technology) library.

To determine molecular weight distribution of the monolignols, gel permeation chromatography (GPC) was used with Shodex KF-801, KF-802, KF-802.5 and KF-803 columns (Showa Denko, Tokyo, Japan). UV detector was used at 280 nm with a solvent of tetrahydrofuran, and the flow rate was 0.7 mℓ/min.

3. RESULTS and DISCUSSION

3.1. Degradation of monolignols (C3C6 type) by *P. chrysosporium* with reducing agent

In our previous study, *P. chrysosporium* degraded coniferyl alcohol to vanillyl alcohol, 3-vanilpropanol and vanillylmandelic acid, and sinapyl alcohol to syringaldehyde and succinic acid while simultaneously producing oligomers with increase of molecular weight. Based on these results, to prevent the polymerization reaction and induce the preferentially degradation of monolignols under the fungal treatment, ascorbic acid as reducing agent was used. Resultingly, ascorbic acid affected on primarily inducing degradation reaction of monolignols by *P. chrysosporium*. Table 2 presented that molecular weight of sample treated by fungus was scarcely increased. This means ascorbic acid inhibited polymerization between unstable radicals formed by one electron oxidative system of enzymes (Kinne *et al*., 2009).

Table 2. Molecular weight of modification products of monolignols by *P. chrysosporium* with addition of ascorbic acid (Control: monolignols in medium, PCH: fungal sample treated by P. chrysosporium)

Coniferyl alcohol								
	Control				PCH			
	5d	10d	15d	20d	5d	10d	15d	20d
M_n [a] (Daltons)	354	377	423	422	343	375	371	360
M_w [b] (Daltons)	626	644	724	764	591	679	676	686
Mw/Mn	1.77	1.71	1.71	1.81	1.72	1.81	1.82	1.91
Sinapyl alcohol								
	Control				PCH			
	5d	10d	15d	20d	5d	10d	15d	20d
M_n [a] (Daltons)	353	376	390	411	380	386	398	406
M_w [b] (Daltons)	564	621	651	705	585	596	705	710
Mw/Mn	1.60	1.65	1.67	1.72	1.54	1.54	1.77	1.75

[a] number-average molecular weight
[b] weight-average molecular weight

Along with these results, various degradation products were detected through GC-MS analysis. New products were detected at 45.0, 49.4, 51.3 and 53.7 min in methanol extract on day 5 (Fig. 1(A)), and these were identified as vanillyl alcohol, vanillic acid, 3-vanilpropanol and 3-(4-hydroxy-3-methoxy phenyl) propanoic acid, respectively. In addition, analysis result of ethyl acetate extract showed succinic acid at 32.0 min and trihydroxybenzene at 43.8 min were formed from coniferyl alcohol (Fig. 1(B)). To sum it up, coniferyl alcohol was degraded to vanillyl alcohol and vannillic acid through the C_α -C_β cleavage and then, C1C6 compounds (vanillyl alcohol and vannillic acid) were degraded to C6 compound by C_α oxidation and demethoxylation. Various lignin degrdading enzymes of white rot basidiomycetes were reported to cause C_α -C_β cleavage, C_{aryl}- C_{alkyl} bond cleavage and oxidation of lignin monomers (Hofrichter, 2002; Kirk & Nakatsubo, 1983).

Sinapyl alcohol was degraded to hydroquinone and succinic acid by *P. chrysosporium* under the presence of ascorbic acid (Fig. 2). Demethoxylation and C_α oxidation were main reaction during the degradation period. These compounds were not detected in free-ascorbic acid experiment.

As a result, enzyme system of *P. chrysosporium* vigorously catalyzed oxidation of monolignols while ascorbic acid played a role to block the formation of oxidative radicals from monomers. That was why degradation products were more oxidized structures. Especially, demeth(ox)ylation and hydroxylation

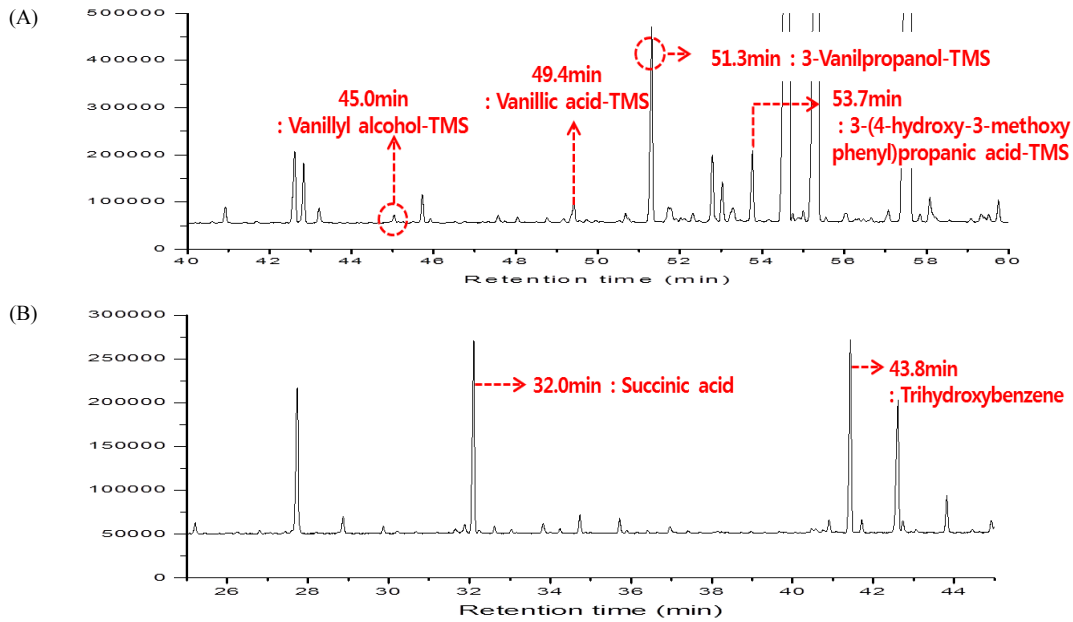

Fig. 1. Total ion chromatograms of modification products in methanol extract (A) and ethylacetate extract (B) of coniferyl alcohol by *P. chrysosporium* with ascorbic acid.

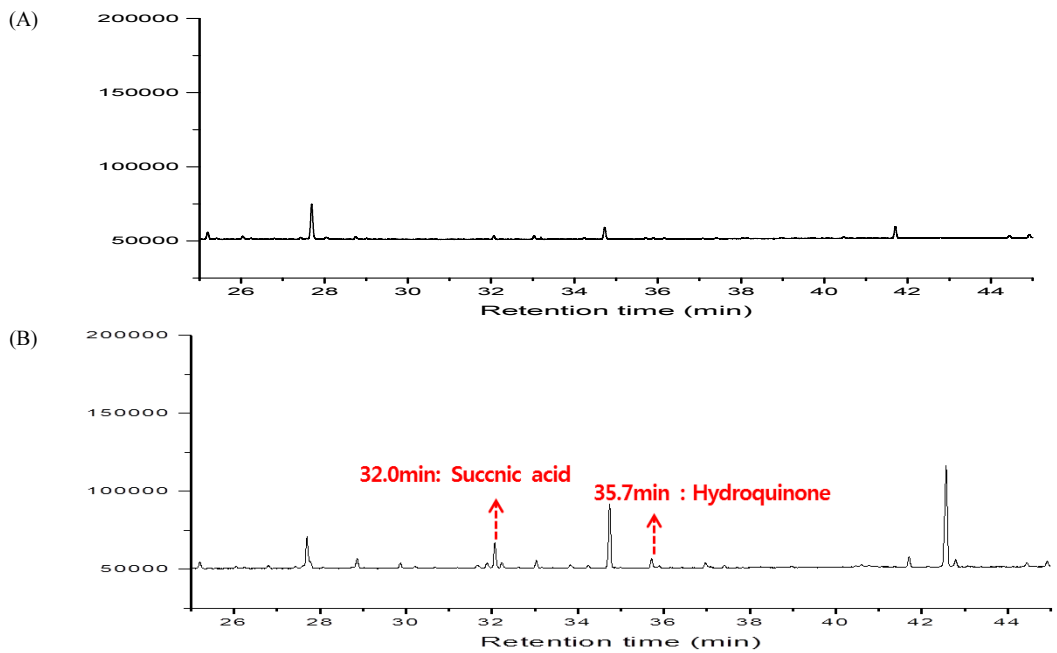

Fig. 2. Total ion chromatograms of control, only fungal medium treated with ascorbic acid (A) and modification products of sinapyl alcohol by *P. chrysosporium* with ascorbic acid (B).

within aromatics make ring fission easier, which was attributed to produce succinic acid. Hydroquinone and trihydroxybenzene were degraded to succinic acid by ring cleavage process. Dioxygenase is well known for being involved in ring cleavage. It is divided to intradiol dioxygenase and extradiol dioxygenase, and they have completely different structures and different catalytic mechanism (Harayama *et al*., 1992). Intradiol dioxygenase cleave ortho to hydroxyl substituents of aromatics. On the other hand, extradiol dioxygenase generally cleave meta to hydroxyl substituents (Lipscomb, 2008). In general, ring cleavage by dioxygenase requires catecholic substrate possessing hydroxyl substituents on two adjacent carbons. However, other researchers suggested some dioxygeases such as hydroquinone 1,2-dioxygenase and homogentisate-1,2-dioxygenase could degrade substrate which hydroxyl groups were substituted at para position such as hydroquinone and homogentisate to acid compounds (Gunsch *et al*., 2005; Miyauchi *et al*., 1999). Because *P. chrysosporium* degraded the C3C6 type of aromatic compounds to C6 type compounds such as trihydroxybenzene and hydroquinone, *P. chrysosporium* might contain the homogentisate-1,2-dioxygenase as well as intradiol dioxygenase.

Consequently, degraded intermediates such as hydroquinone and trihydroxybenzene facilitated formation of succinic acid. Finally, hydroxylated aromatics were metabolized by fungus, which means that acid compounds derived from monolignols was considered to be primary metabolites of fungus.

In conclusion, *P. chrysosporium* has catabolic ability to induce the production of acid compound from aromatics with various extra- and intra-cellular enzyme system.

3.2. Degradation products of syringic acid (C1C6 type) by *P. chrysosporium*

As mentioned in introduction section, syringic acid, 2,6-dimethoxy-1,4-benzodiol, and succinic acid were detected as the main degradation products of synthetic lignin. It was assumed that succinic acid was produced from the aromatic compounds released from synthetic lignin. Based on these results, to verify the formation mechanism of succinic acid from aromatic compounds, the degradation mechanisms of syringic acid was investigated.

Fig. 3 shows that new degraded products of syringic acid were detected at 32.0, 35.5, and 44.2 min. These peaks were identified as succinic acid, hydroquinone, and 4-hydroxybenzoate, respectively (Fig. 3). Syringic acid was transformed to 4-hydroxybenzoic acid by the demethoxylation reaction. 4-Hydroxybenzoic acid was transformed to hydroquinone by the C_{α} oxidation reaction. Next, after the ring cleavage process, succinic acid was formed through fungal metabolism. These results showed the similar degradation mechanism compared with that of monolignols. Demethoxylation and C_{α} oxidation were main reaction during degradation period of aromatics by *P. chrysosporium*. Consequently, final de-

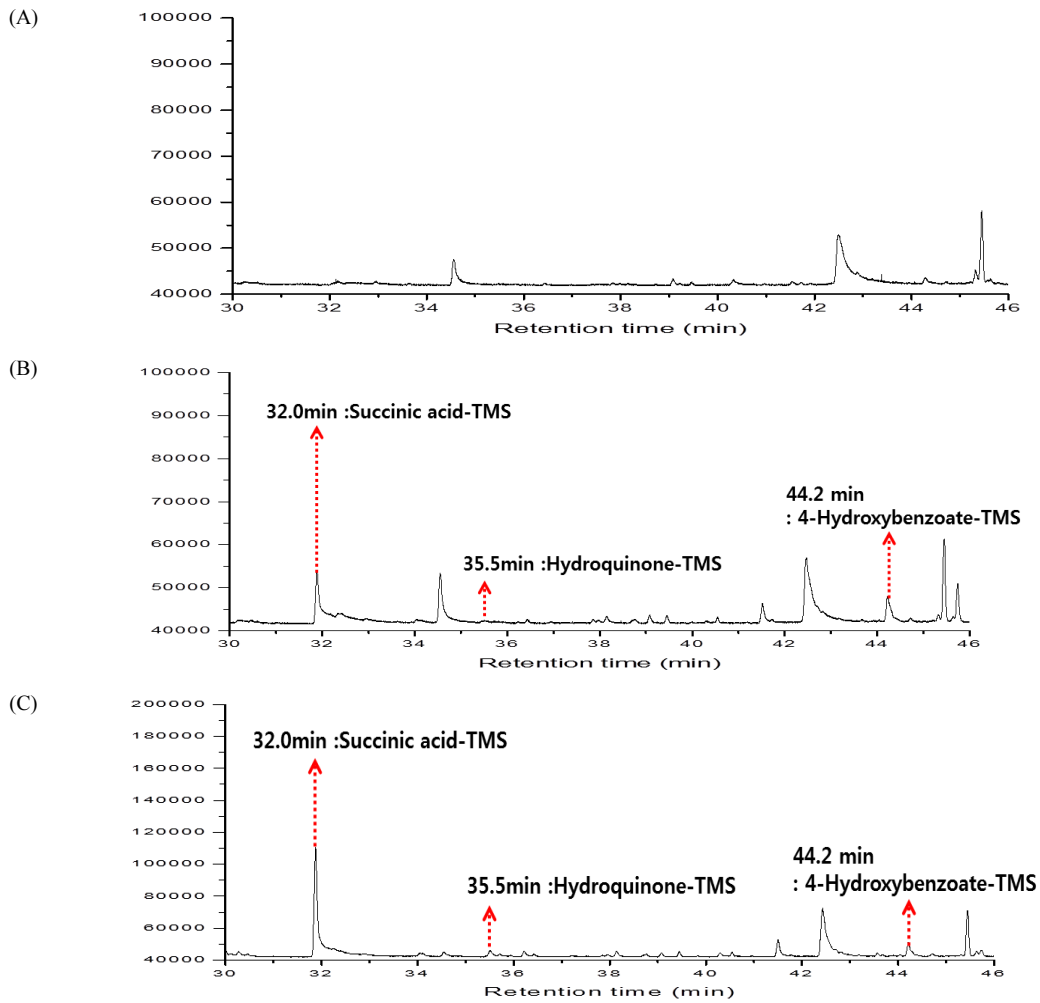

Fig. 3. Total ion chromatograms of control (A) and sample treated by *P. chrysosporium* on incubation day 10 (B) and 20 (C).

graded product of C1C6 type of aromatics was also succinic acid.

One unique phenomenon is the demethoxylation process by *P. chrysosporium* compared with that in previous studies. In general, methoxylated substrates were transformed to hydroxylated products by one electron oxidation. It was reported that peroxidases were involved in the demethoxylation of lignin-related substances (Ander & Eriksson, 1985; Ander *et al*., 1983; Ander *et al*., 1985; Kersten *et al*., 1985). This transformation was accompanied by oxidative activity as well as the demethoxylation activity of the microorganism (Lopretti *et al*., 1998). However, the results of this study indicated only the removal of methoxyl group in

aromatic compounds. This reaction could be accompanied by only demethoxylation activity without oxidative activity (Lopretti *et al*., 1998). In addition, cytochrome P 450 enzyme was reported to play a key role in the demethylation of lignin (Kelly *et al*., 2003; Warrilow *et al*., 2008). Accordingly, in the present study, *P. chrysosporium* catalyzed demeth(ox)ylation, which had an effect on the removal of methoxyl groups without accompanying oxidation. To better understand this phenomenon, more detailed studies are necessary.

3.3. Degradation products of hydroquinone (C6 type) by *P. chrysosporium*

Above observation suggested that hydroquinone as a degradation product of both C3C6 and C1C6 type compounds was metabolized intracellularly by *P. chrysosporium* and succinic acid was formed finally. Accordingly, the degradation mechanism of hydroquinone by *P. chrysosporium* was examined to establish precise degradation mechanism of aromatic compounds.

Fig. 4 shows that new peak was detected at 32.0 min in the fungal sample. *P. chrysosporium* converted hydroquinone to succinic acid, directly. Compared with control, hydroquinone in medium (Fig. 4(A)), succinic acid was detected in fungal sample on day 15 (Fig. 4(B)). With increase of incubation time, amount of succinic acid derived from hydroquinone increased on day 25 (Fig. 4(C)).

Succinic acid is a major degradation product of hydroquinone. From incubation day 5, the amount of succinic acid increased, and the concentration of succinate on incubation day 20 reached 56.8 mg/g $_{\text{of the substrate}}$ (Fig. 5). The amount of succinic acid formed from hydroquinone by the fungus was much more than that from syringic acid. This result suggested that hydroquinone was final intermediate in the degradation process of aromatics by *P. chrysosporium*. Although no intermediates formed by ring fission to enter the metabolic pathway were detected, a unique aromatic metabolism in the fungal system was observed in this study.

To form succinic acid from hydroquinone, various processes were required. Cytochrome P 450 monooxygenase reportedly plays a role in the intracellular monooxygenation reaction in the biodegradation and bioremediation of aromatic compounds (Hirosue *et al*., 2011; Wong, 1998; Yadav *et al*., 2003). Generally, ring fission of hydroxylated aromatic compounds occurred by dioxygenase (Eltis & Bolin, 1996; Masai *et al*., 1999; Rieble *et al*., 1994). In particular, hydroquinone which hydroxyl groups were positioned at *para* could be catalyzed by as hydroquinone 1,2-dioxygenase and homogentisate-1,2-dioxygenase (Gunsch *et al*., 2005; Miyauchi *et al*., 1999). In our other study, enzymatic information of *P. chrysosporium* (ATCC 0696) was provided to have a specific catabolic enzyme system based on cytochrome P450 monooxygenase and homogentisate-1,2-dioxygenase. Accordingly, hydroquinone was oxidized by intracellular enzymes, and then, de-

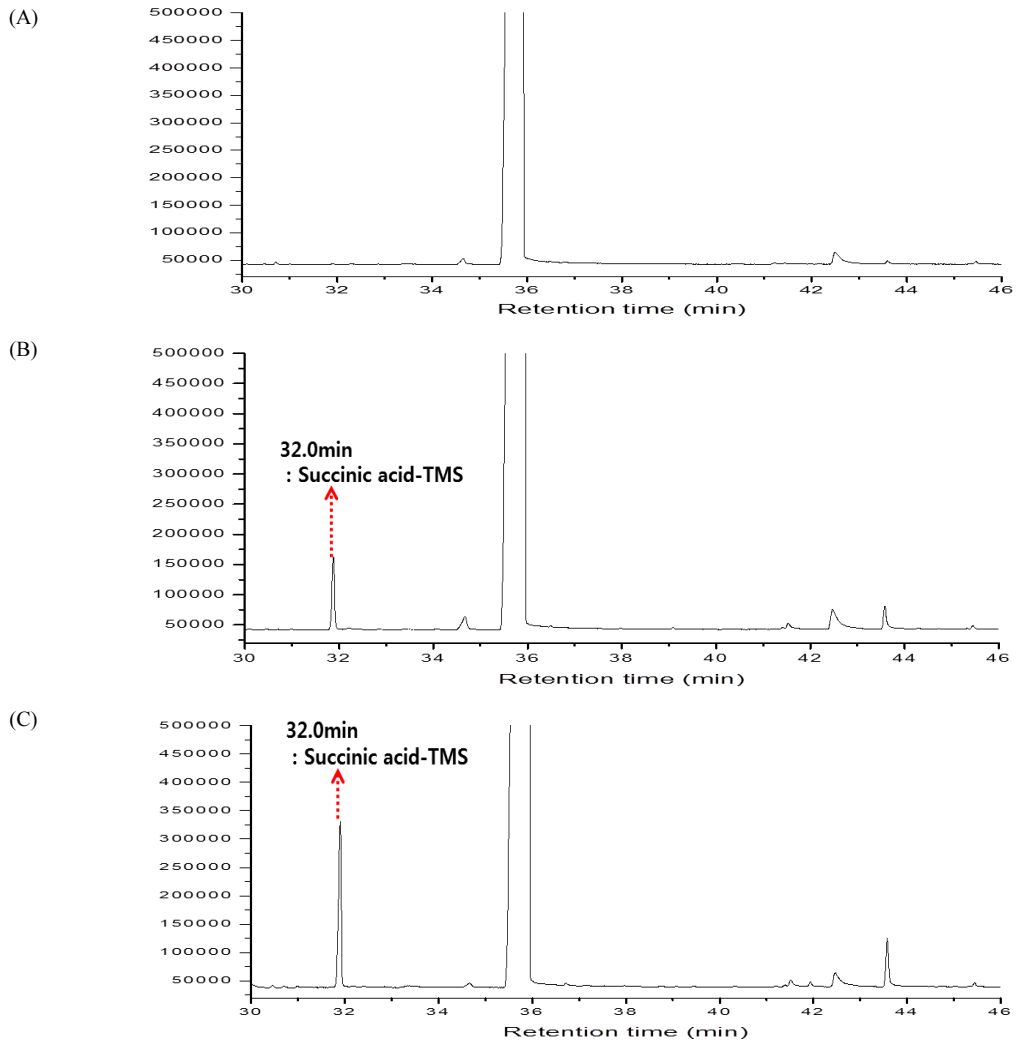

Fig. 4. Total ion chromatograms of control (A) and sample treated by *P. chrysosporium* on incubation day 15 (B) and 25 (C).

graded products formed during secondary metabolism were assumed to enter the primary metabolism of fungus, resulting in the production of the acid compound. Primary metabolism related to production of succinic acid is TCA cycle and glyoxylate metabolism. Basidiomycetes were reported to have a unique metabolic system, a short-cut TCA/glyoxylate bicycle system (Munir *et al*., 2001; Shimizu *et al*., 2005). *P. chrysosporium* exposed to aromatic compounds such as vanillin and benzoic acid was reported that metabolic systems such as tricarboxylic acid cycle and glyoxylate cycle were activated during aromatic compounds deg-

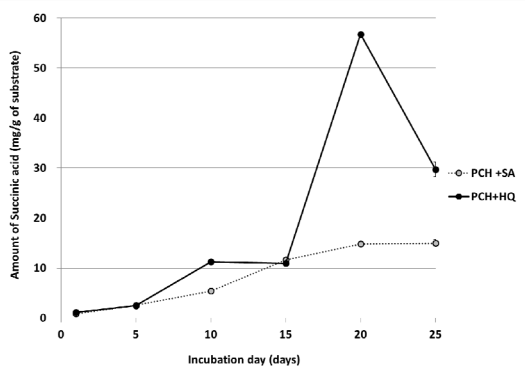

Fig. 5. Quantitative analysis of succinic acid from syringic acid and hydroquinone under the ligninolytic treatment by P. chrysosporium (PCH+SA: succinic acid derived from syringic acid by fungus, PCH+HQ: succinic acid derived from hydroquinone by fungus).

radation (Matsuzaki *et al*., 2008; Shimizu *et al*., 2005). Consequently, above observations established that the formation of succinic acid was enhanced with the metabolism of aromatic compounds by the fungus. It was concluded that *P. chrysosporium* has unique catabolic metabolism related to production of succinic acid from aromatic compounds (Fig. 6).

4. CONCLUSIONS

The catalytic enzyme system of *P. chrysosporium* induced the production of succinic acid from lignin-derived aromatic compounds with addition of reducing agent. *P. chrys-*

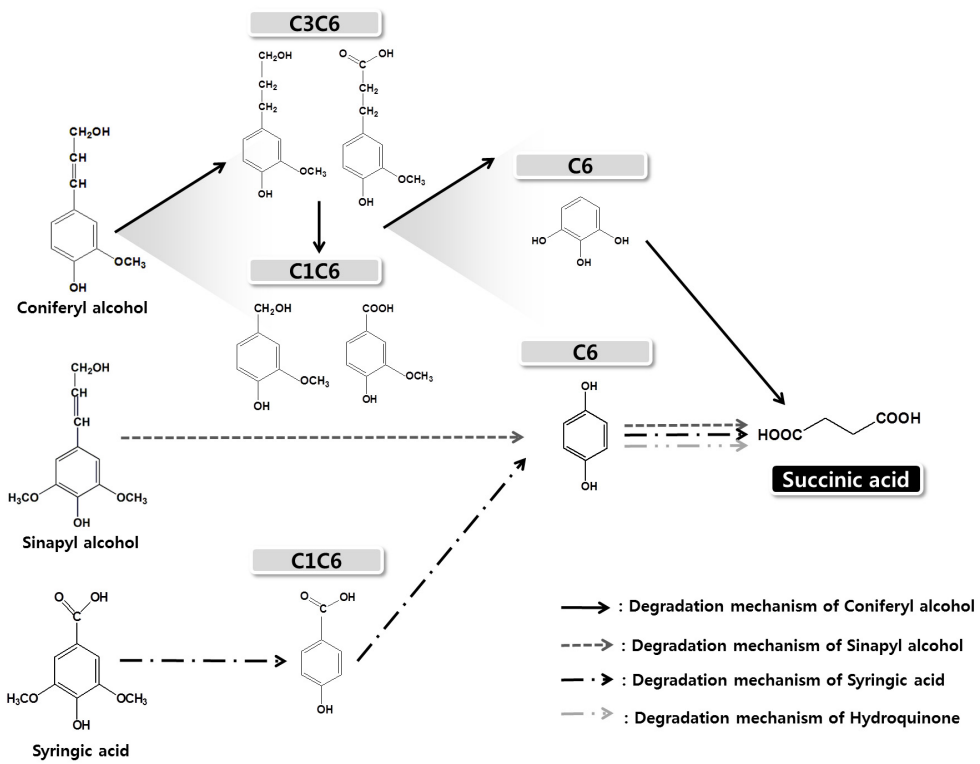

Fig. 6. Degradation mechanism of lignin derived-aromatic compounds by P. chrysosrporioum in the presence of reducing agent.

osproium modified the coniferyl alcohol (C3C6 type) to 3-vanilpropanol, 3-(4-hydroxy-3-methoxy phenyl) propanoic acid (oxidized and reduced C3C6 type), vanillyl alcohol, vanillic acid (C1C6 type) and trihydroxybenzene (C6 type). This fungus degraded sinapyl alcohol (C3C6 type) and syringic acid (C1C6 type) to hydroquinone (C6 type). Finally, main degradation product of aromatic compounds by *P. chrysosproium* was succinic acid. Compared with free-ascorbic acid experiment in our previous study, various degraded products of monolignols and syringic acid were detected. Accordingly, addition of ascorbic acid added as reducing agent caused preferentially the oxidation of substrates, blocking the formation of oxidative radicals from monomers. These observations established the accurate formation mechanism of succinic acid from aromatic compounds by *P. chrysosporium.* Furthermore, it suggested that catabolic system of *P. chrysosporium* has excellent ability to degrade aromatic compounds to valuable acid compounds.

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REFERENCES

Adav, S.S., Ravindran, A., Sze, S.K. 2012. Quantitative proteomic analysis of lignocellulolytic enzymes by *Phanerochaete chrysosporium* on different lignocellulosic biomass. *Journal of proteomics* 75(5): 1493～1504.

Ander, P., Eriksson, K.-E. 1985. Methanol formation during lignin degradation by *Phanerochaete chrysosporium*. *Applied microbiology and biotechnology* 21(1-2): 96～102.

Ander, P., Eriksson, K.-E., Yu, H.-s. 1983. Vanillic acid metabolism by *Sporotrichum pulverulentum*: evidence for demethoxylation before ring-cleavage. *Archives of microbiology* 136(1): 1～6.

Ander, P., Eriksson, M.E., Eriksson, K.E. 1985. Methanol production from lignin-related substances by *Phanerochaete chrysosporium*. *Physiologia Plantarum* 65(3): 317～321.

Beckham, G. T., Johnson, C. W., Karp, E. M., Salvachua, D., & Vardon, D. R. 2016. Opportunities and challenges in biological lignin valorization. *Current opinion in biotechnology* 42: 40-53.

Enoki, A., Goldsby, G.P., Gold, M.H. 1981. β-Ether cleavage of the lignin model compound 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether and derivatives by *Phanerochaete chrysosporium. Archives of Microbiology* 129(2): 141～145.

Glenn, J.K., Morgan, M.A., Mayfield, M.B., Kuwahara, M., Gold, M.H. 1983. An extracellular H_2O_2-requiring enzyme preparation involved in lignin biodegradation by the white rot basidiomycete *Phanerochaete chrysosporium*. *Biochemical and Biophysical Research Communications* 114(3): 1077～1083.

Gunsch, C.K., Cheng, Q., Kinney, K.A., Szaniszlo, P.J., Whitman, C.P. 2005. Identification of a homogentisate-1, 2-dioxygenase gene in the fungus *Exophiala lecanii-corni*: analysis and implications. *Applied microbiology and biotechnology* 68(3): 405～411.

Harayama, S., Kok, M., Neidle, E. 1992. Functional and evolutionary relationships among diverse

oxygenases. *Annual Reviews in Microbiology* 46(1): 565～601.

Higuchi, T. 1990. Lignin biochemistry: biosynthesis and biodegradation. *Wood Science and Technology* 24(1): 23～63.

Hirosue, S., Tazaki, M., Hiratsuka, N., Yanai, S., Kabumoto, H., Shinkyo, R., Arisawa, A., Sakaki, T., Tsunekawa, H., Johdo, O. 2011. Insight into functional diversity of cytochrome P450 in the white-rot basidiomycete *Phanerochaete chrysosporium*: involvement of versatile monooxygenase. *Biochemical and biophysical research communications* 407(1): 118～123.

Hofrichter, M. 2002. Review: lignin conversion by manganese peroxidase (MnP). *Enzyme and Microbial technology* 30(4): 454～466.

Hong, C.Y. 2016. Biomodification of Lignin Compounds by *Abortiporus biennis* and *Phanerochaete chrysosporium* and Investigation of Related Enzymes by Transcriptomic Analysis. Ph.D. Thesis, Seoul National University, Seoul, Korea.

Hong, C.Y., Park, S.Y., Kim, S.H., Lee, S.Y., Ryu, S. H., Choi, I.G. 2016. Biomodification of ethanol organolsolv lignin by *Abortiporus biennis* and its structural change by addition of reducing agent. *Journal of Korean Wood Science and Technology* 44(1): 124～134.

Kelly, S.L., Lamb, D.C., Jackson, C.J., Warrilow, A.G., Kelly, D.E. 2003. The biodiversity of microbial cytochromes P450. *Advances in microbial physiology* 47: 131～186.

Kersten, P.J., Tien, M., Kalyanaraman, B., Kirk, T.K. 1985. The ligninase of *Phanerochaete chrysosporium* generates cation radicals from methoxybenzenes. *Journal of Biological Chemistry* 260(5): 2609～2612.

Keyser, P., Kirk, T., Zeikus, J. 1978. Ligninolytic enzyme system of *Phanaerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. *Journal of bacteriology* 135(3): 790～797.

Kinne, M., Poraj-Kobielska, M., Ralph, S.A., Ullrich, R., Hofrichter, M., Hammel, K.E. 2009. Oxidative cleavage of diverse ethers by an extracellular fungal peroxygenase. *Journal of biological chemistry* 284(43): 29343～29349.

Kirk, T.K., Farrell, R.L. 1987. Enzymatic "combustion": the microbial degradation of lignin. *Annual Reviews in Microbiology* 41(1): 465～501.

Kirk, T.K., Nakatsubo, F. 1983. Chemical mechanism of an important cleavage reaction in the fungal degradation of lignin. *Biochimica et Biophysica Acta (BBA)-General Subjects* 756(3): 376～384.

Lipscomb, J.D. 2008. Mechanism of extradiol aromatic ring-cleaving dioxygenases. *Current opinion in structural biology* 18(6): 644～649.

Lopretti, M., Cabella, D., Morais, J., Rodrigues, A. 1998. Demethoxylation of lignin-model compounds with enzyme extracts from *Gloeophilum trabeum*. *Process biochemistry* 33(6): 657～661.

Martinez, D., Larrondo, L.F., Putnam, N., Gelpke, M.D.S., Huang, K., Chapman, J., Helfenbein, K.G., Ramaiya, P., Detter, J.C., Larimer, F. 2004. Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nature biotechnology* 22(6): 695～700.

Matsuzaki, F., Shimizu, M., Wariishi, H. 2008. Proteomic and metabolomic analyses of the white-rot fungus *Phanerochaete chrysosporium* exposed to exogenous benzoic acid. *Journal of proteome research* 7(6): 2342～2350.

Miyauchi, K., Adachi, Y., Nagata, Y., Takagi, M. 1999. Cloning and sequencing of a novel meta-cleavage dioxygenase gene whose product is involved in degradation of γ-hexachlorocyclohexane in

Sphingomonas paucimobilis. Journal of bacteriology 181(21): 6712～6719.

Munir, E., Yoon, J.-J., Tokimatsu, T., Hattori, T., Shimada, M. 2001a. New role for glyoxylate cycle enzymes in wood-rotting basidiomycetes in relation to biosynthesis of oxalic acid. *Journal of wood science* 47(5): 368～373.

Quideau, S., Ralph, J. 1992. Facile large-scale synthesis of coniferyl, sinapyl, and p-coumaryl alcohol. *Journal of agricultural and food chemistry* 40(7): 1108～1110.

Ravalason, H., Jan, G., Mollé, D., Pasco, M., Coutinho, P.M., Lapierre, C., Pollet, B., Bertaud, F., Petit-Conil, M., Grisel, S. 2008. Secretome analysis of *Phanerochaete chrysosporium* strain CIRM-BRFM41 grown on softwood. *Applied microbiology and biotechnology* 80(4): 719～733.

Shimizu, M., Yuda, N., Nakamura, T., Tanaka, H., Wariishi, H. 2005. Metabolic regulation at the tricarboxylic acid and glyoxylate cycles of the lignin-degrading basidiomycete *Phanerochaete chrysosporium* against exogenous addition of vanillin. *Proteomics* 5(15): 3919～3931.

Tien, M., Kirk, T.K. 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. *Science(Washington)* 221(4611): 661～662.

Umezawa, T., Nakatsubo, F., Higuchi, T. 1982. Lignin degradation by *Phanerochaete chrysosporium*: Metabolism of a phenolic phenylcoumaran substructure model compound. *Archives of microbiology* 131(2): 124～128.

Warrilow, A., Ugochukwu, C., Lamb, D., Kelly, D., Kelly, S. 2008. Expression and characterization of CYP51, the ancient sterol 14-demethylase activity for cytochromes P450 (CYP), in the white-rot fungus *Phanerochaete chrysosporium. Lipids* 43(12): 1143～1153.

Weinstein, D.A., Krisnangkura, K., Mayfield, M.B., Gold, M.H. 1980. Metabolism of radiolabeled β-guaiacyl ether-linked lignin dimeric compounds by *Phanerochaete chrysosporium*. *Applied and environmental microbiology* 39(3): 535～540.

Wong, L.-L. 1998. Cytochrome P450 monooxygenases. *Current opinion in chemical biology* 2(2): 263～268.

Yadav, J.S., Soellner, M.B., Loper, J.C., Mishra, P.K. 2003. Tandem cytochrome P450 monooxygenase genes and splice variants in the white rot fungus *Phanerochaete chrysosporium*: cloning, sequence analysis, and regulation of differential expression. *Fungal genetics and biology* 38(1): 10～21.