Ann.Report,Inst.Forest Sci., Kangweon Nat'l.Univ.,Vol.5. 45~60 .1989.

Mechanisms of Lignin Biodegradation by Ligninase, Phanerochaete Chrysosporium Burds.[*1 *2]

Byung - Ho Hwang[*3]

Contents

Keywords : Lignin Biodegradation, Ligninase, Phanerochaete chrysosporium, Phenol oxidation, Aromatic ring cleavage, Side chain cleavage, Model compound.

Introduction

Lignin is a polymer composed of phenylpropane units interconnected by a number of stable carbon to carbon and carbon to ether linkages. Lignin is racemic and amorphous. It has highly resistant to biodegradation.

Hence, investigations on the biodegradation of the model compounds containing major

*1. Received January 10, 1989.
*2. This review article was presented at the 16th Pacific Congress in Seoul, 1987.
*3. Dept. of Forest, Products & Technology, College of Forestry, Kangweon National University, Chuncheon, 200-701 Korea.

substructures of lignin macromolocules have largely contributed to elucidating the degradation of lignin.

It is decomposed efficiently, however, by basidiomycetous white-rot fungus. The basidiomycete Phanerochaete chrysosporium Burds produces an extracellular lignin-degrading enzyme. It's enzyme discovery by Kent kirk's group and named" "Ligninase" or "Lignin peroxidase".[1)]

The ligninase (Lignin peroxidase) catalyzes a variety of seemingly different reactions in the presence of hydrogen peroxide, $C\alpha$-$C\beta$ cleavage of their aliphatic side chain of lignin models, hydroxylation of benzylic methylene groups, oxidation of benzylalcohols to the corresponding aldehyde or ketones, and phenol oxidation.

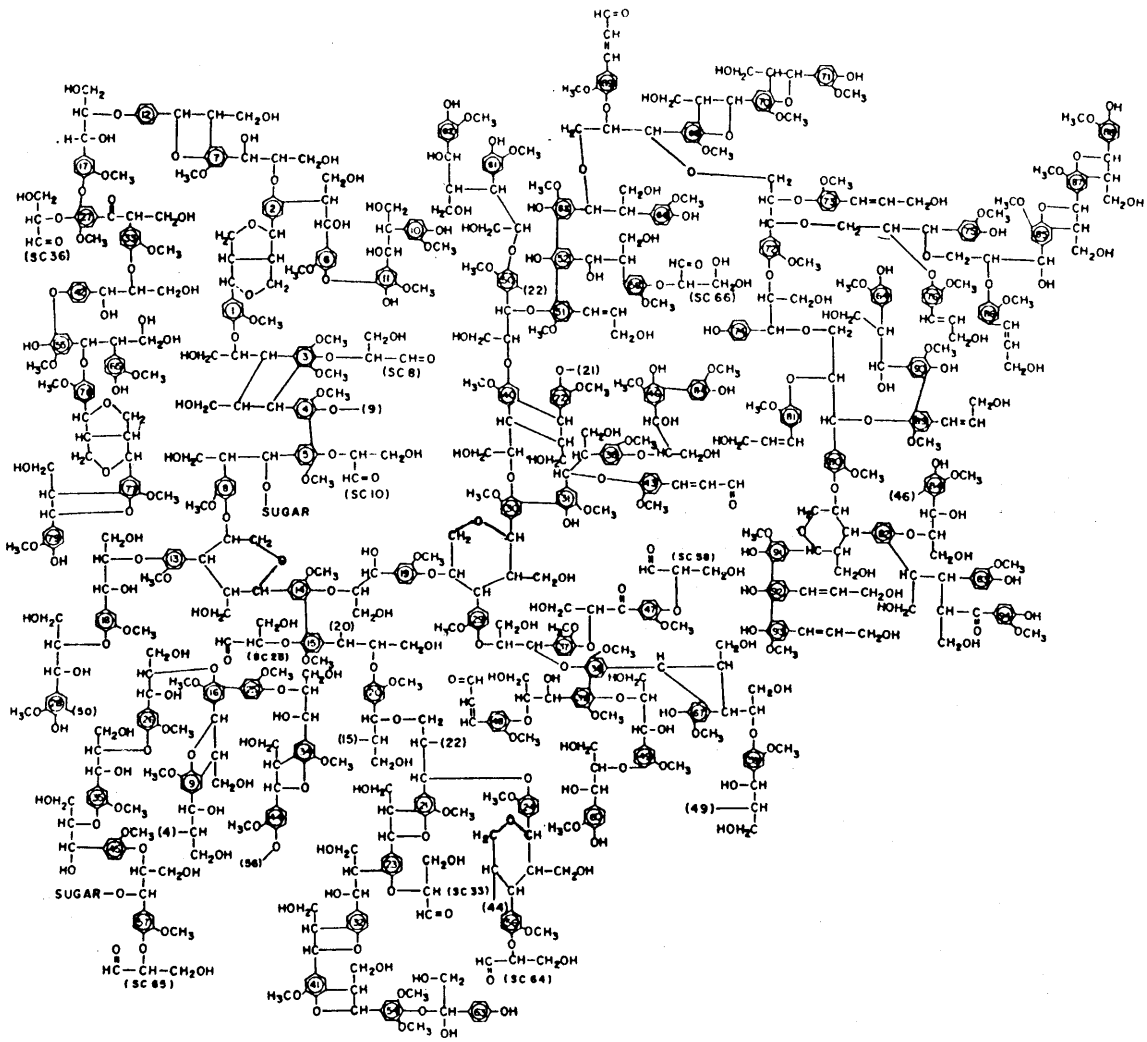


Fig. 1. Softwood lignin model designed by computerized evaluation (by courtesy of W. G. Glasser).

The most recently, the lignin model reported by Glasser and Glasser[2] was mainly based on the simulation of radical coupling reactions of the p-hydroxy cinnamyl alcohols, and was composed of 94 phenylpropane units (Fig. 1).

Lignin models used were arylglycerol-β-arylether (β-O-4), phenylcoumaran(β-5), diarylpropane(β-1), biphenyl(5-5), Aryl-Alkyl(β-6) and resional(β-β) compounds.

The general mechanisms for ligniase-catalyzed degradations of different model compounds are shown in Fig. 2 - Fig. 20.

1. Cation radical

Recently, Kersten and Kirk[3] have demonstrated that the ligninase of P. chrysosporium generates cation radicals from methoxybenzenes. Evidence given Fig. 2 indicated that these are formed directly one-electron oxidation followed by a reverse disproportionation reaction. The detection of cation radicals in the enzymatic system from methoxybenzens permitted predictions of the products formed from ligninase-catalyzed oxidation. The mechanism of demethoxylation presumably involves one-electron of the methoxybenzene by the oxidized enzyme and subsequent addition of H_2O to the cation radical, followed by or simultaneous with methoxyl aromatic cation radicals has been noted in other systems. Fig. 2 shows that 1,4-dimethoxybenzene is converted to p-benzoquinone and methanol. The ligninase reaction is a new biological mechanism for ether clearage.

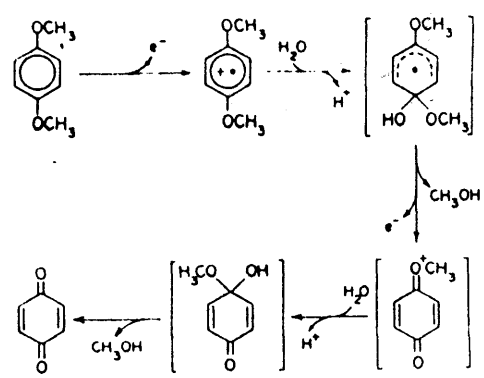


FIG. 2. **Hypothetical scheme showing two sequential one-electron oxidations of 1,4-dimethoxybenzene and addition of water with loss of methanol.**

2. Arylglycerol-β-Arylether Linkage

The arylglycerol-β-arylether bond (β-O-4 substructure) is the most abundant interphenylpropane linkage in lignin Higuchi group reported that P. chrysosporium and C. Versicolor degrade both phenolic and nonphenolic β-O-4 compounds. Fig. 3 shows that a phenolic β-O-4 model, arylglycerol-β-coniferyl ether(1) is converted by Phanerochaete

(pathway B.) to arylglycerol-β-guaiacylglycerol ether(2') which is converted to vanillic acid ether(5) via vanillin ether(4). The vanillic acid ether(5) is than degraded either to glycerol-2-vanillic acid ether(6) and dimethoxy-p-benzoquinone(7) by alkyl-phenyl cleavage or to vanillyl alcohol(9), probably via vanillin as primary product by oxidative Cα-Cβ clevage, as found for nonphenolic β-O-4 models. Both alkyl-phenyl and Cα-Cβ cleavage could occur via phenoxy radicals of the substrate mediated by lignin peroxidase.[4,5]

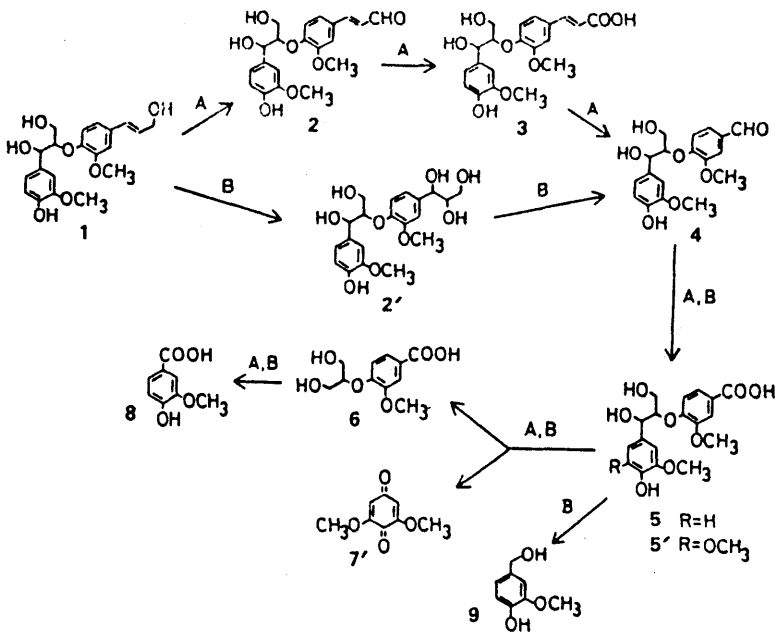


Fig. 3. Degradation pathways of guaiacylglycerol-β-coniferyl ether by *F. solani* M-13-1 and *Phanerochaete chrysosporium*. A. Pathway by *Fusarium*; B. pathway by *Phanerochaete*.

To elucidate the degradation mechanism is very importment for nonphenolic β-O-4 substructure by lignin degrading fungi. Higuchi's group[6] started degradation study in 1981 on β-O-4 model compounds (10) using P. chrysosporium. They identified arylglycerol(11), guaiacol(12), C_6 C_α (benzylalcohol) derivative(13) and guaiacoxy ethanol(14). Guaiacoxyethanol(14) was suggested to be formed by retroaldol reaction of γ-aldehydeic β-O-4 dimers shown in Fig. 4.

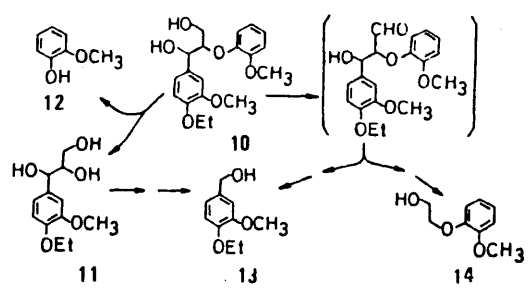


Fig. 4. Degradation pathways of nonphenolic arylglycerol-β-aryl ether models by *P. chrysosporium*.

So, to elucidate the mechanism of Cα- Cβ cleavage, Higuchi group[7] synthesized a trimer (15) composed of β-O-4 and α-O-γ substructure, i.e, the trimer model, and used it as substrate for ligninolytic culture of P. chrysosporium.

Fig. 5 shows the degradation of γ-benzyl ether of β-O-4 model(15) with phanerochaete. Benzyloxyethanol(16), a Cβ- Cγ fragment compound was first identified by Higuchi group, in addition to a C_6- Cα derivative(13) and arylglycerol derivative(17) as degradation products.

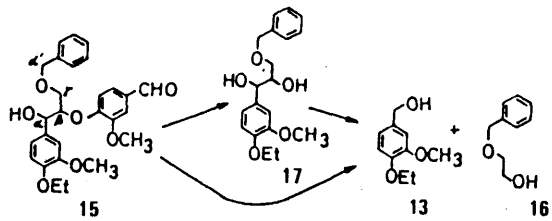


Fig. 5. Degradation pathways of a trimer composed of β-O-4 and α-O-γ substructure, γ-benzyl ether of β-O-4 substructure model by *P. chrysosporium*.

On the other hand, Gold's group[8] reported that on the degradation of 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether(10) with ligninolytic culture of P. chrysosporium. Enoki et al.[9,10] identified 4-ethoxy-3-methoxyphenylglycerol(11), 4-ethoxy-3-mothoxybenzylalcohol(13), guaiacol(12), glycerol-2-guaiacylether(14) and guaiacoxyethanol(18) by GC-MS, and concluded that nonphenolic β-O-4 model are converted via three pathway as shown Fig. 6.

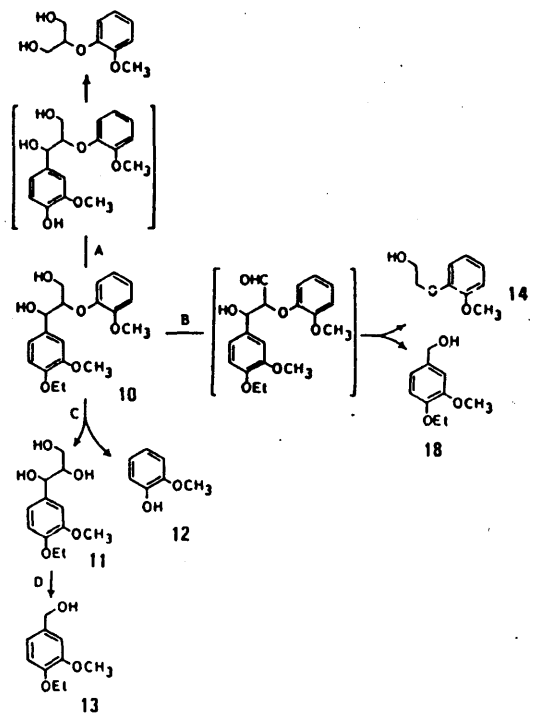


Fig. 6. Degradation pathways of nonphenolic arylglycerol-β-aryl ether models by *Phanerochaete chrysosporium*.

1) Alkyl-phenyl cleavage (pathway A), presumably preceeded by 4-O-dealkylation to give guaiacylglycerol-β-guaiacyl ether and catalyzed by PO_5,

2) Cα- Cβ cleavage (pathway B), presumably to involve Cγ-oxidation, which has not been established, followed by a reversed aldol reaction.

3) β-ether cleavage (pathway C), followed by Cα-Cβ cleavage in the phenylglycerol product (pathway D).

On the other hand, Nakatsubo[11] reported that on incubation of veratrylglycerol-β-coniferyl ether(19) with P. chrysosporium the substrate is converted to the glycerol ether(20), which is then converted to the vanillic acid ether(22) via the vanillin ether(21) as shown Fig. 7.

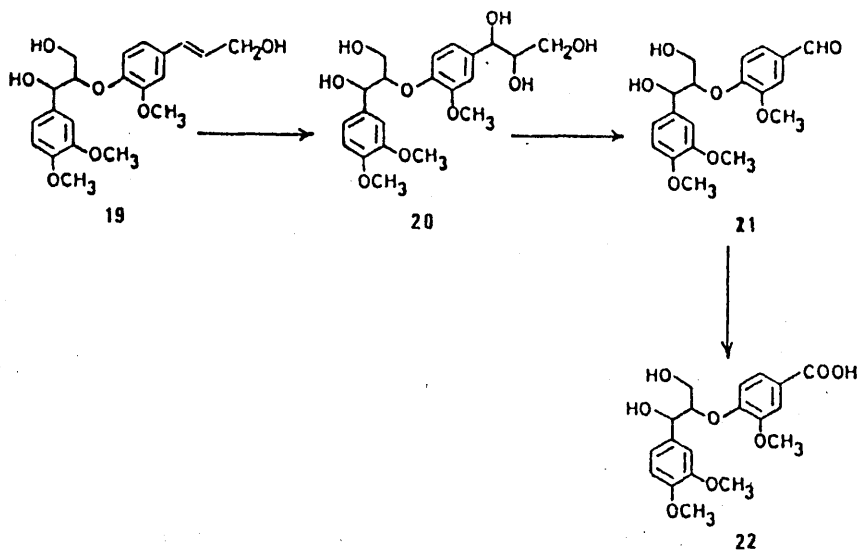


Fig. 7. Pathway for partial degradation of veratrylglycerol-β-coniferyl ether by *Phanerochaete chrysosporium*

Also, Tien and Kirk[12] have isolated and characterized an enzyme that directly cleavages the Cα-Cβ linkage of arylglycerol-β-arylether as shown Fig. 8. The enzyme has been purified from the culture filtrate of P. chrysosporium. The arylglycerol-β-aryl ether compound converted to veratryl aldehyde and guaiacol.

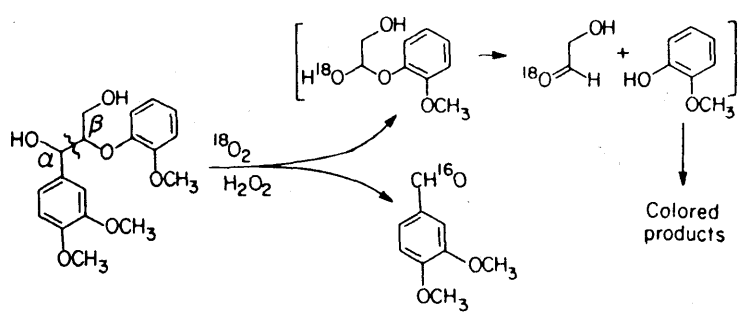


Fig. 8. Cleavage of a β-*O*-4 model compound by a ligninolytic oxygenase from *Phanerochaete chrysosporium* is analogous to cleavage of β-1 models (Fig.). Compounds in brackets are suspected products, based on work with related models. From Tien and Kirk (1984).

Recently, Kirk group[3,13] reported that ligninase functions by single-electron oxidation of the aromatic nuclei to unstable cation radicals, which decompose via various reactions to yield the observed products Schomemaker et al[14] have suggested a smilar mechanism.
In the case of β-O-4 substructures, generation of a cation radical in ring A is expected to lead mainly to Cα-Cβ cleavage, whereas smilar oxidation instead in ring B is expected to lead in part to O-C cleavage. Kirk group[15] reported that a mechanism for Cα-Cβ cleavage via a cation radical intermediate can be formulated as illustrated in Fig. 9(a).

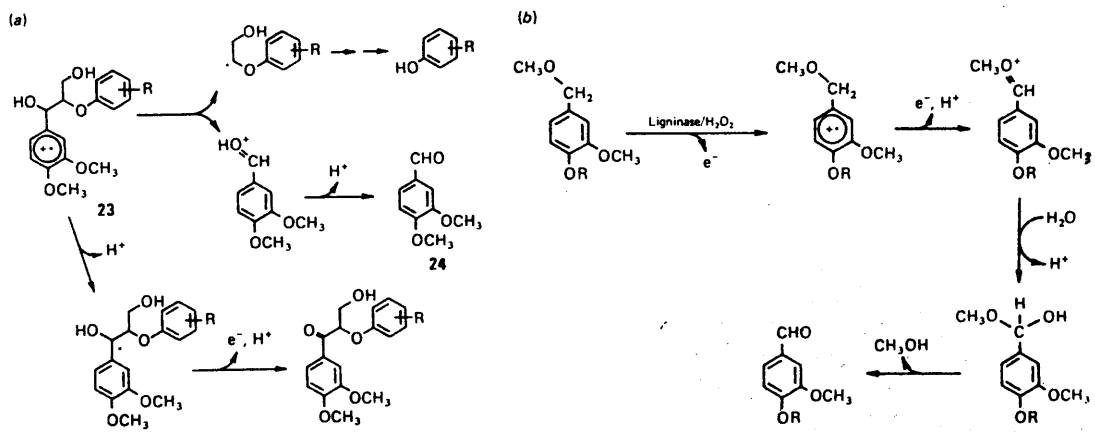
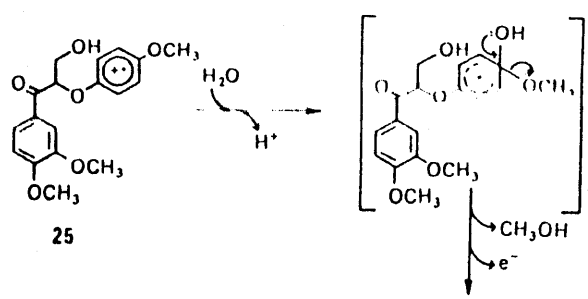


Fig. 9. (*a*) Possible mechanisms for $C_{(\alpha)}$–$C_{(\beta)}$ cleavage, $C_{(\alpha)}$ oxidation and (*b*) $C_{(\alpha)}$-methoxy-group demethoxylation via cation radical intermediates

Also, a mechanism of Cα-oxidation via cation radical intermediate is illustrated in this Fig. 9(a).
Fig. 10 illustrates a possible mechanism[15] for formation of products 26, p-benzoquinone and methanol on ligninase oxidation of model compound 25. Formation of a small amount of methanol from model 25 compound reveals that ligninase probably is responsible for at least some of the methoxy group loss that accompanies lignin degradation by P. chrysosporium. (Chen and Chang[16] Ander and Eriksson).[17]
The role of ligninase in the demethoxylation of lignin is not get known, and other enzymes might well be involved. Crawford[18] has reported aryldemethoxylase activity in the culture filtrate of P. chrysospoirum.



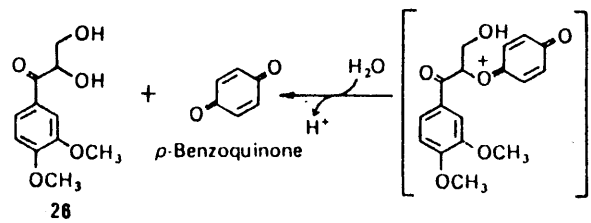


Fig. 10. Possible mechanism for formation of product (26), *p*-benzoquinone and methanol from model compound (25) via initial formation of a cation radical in ring B

The carbonyl group render the aromatic nuclei resistant to oxidation by ligninase, it seems evident that other enzymes are involved in further degradation of the Cα-carbonyl-bearing structures. P. chrysosporium enzyme apparently are not able to reduce such Cα-carbonyl groups.

In addition to cleaving the Cα-Cβ bonds, the fungas appears to be able to cleave the Cβ-Cγ bonds in side chains also.

An example of the Cβ-Cγ cleavage is shown in Fig. 11 for a β-O-4 substructure(Chen and Chang[19]). The side chain of the A ring is subsequently cleaved via Cα-Cβ cleavage to form a benzoic acid derivative, whereas the α-hydroxy group of the side chain of the B ring is oxidized to an α-carbonyl group.

The resulting intermediate presumably is converted to a substructure of the type(27) via a reverse aldol type reaction and subsquent release of this substructure from the lignin macromolecule would give compound 30.

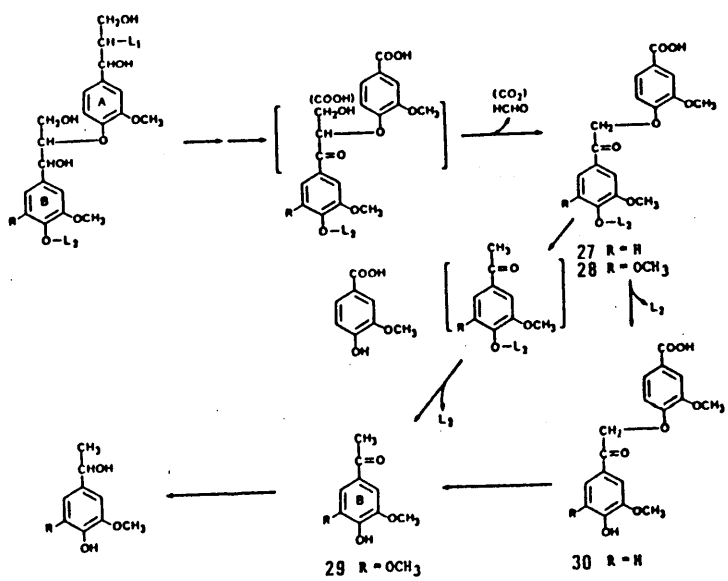


Fig. 11 Possible pathways for fungal degradation of nonphenolic β-O-4 substructures involving cleavages of Cα-Cβand Cβ-Cαbonds in side chains: L_1, L_2 = lignin molety.

Whereas structure 27 and 28 are detected by ^{13}C-NMR in polymeric degraded lignins, compound 29 and acetosyringone 30 are identified among the low molocular weight degradation lignin.

3. Phenylcoumaran (β-5) Linkage

Phenylcoumaran substructure accounts for 6% and 9-12% of the intermonomer linkage in birch and spruce wood lignins, respectively.

Recently, in ligninolytic cultures of P. chrysosporium Nakatsubo et al[20] reported that the cinuamyl alcohol side chain of 4-O-methyldehydrodiconiferyl alcohol(31) is first converted to the corresponding glycerol structure(32), which is then converted (by loss of the two terminal carbon atoms, B and C) to a Cα-aldehyde derivative(33).

The coumaran ring is then converted to a coumarone(34) which is metabolized slowly to a C_6-C_1 acid compound as shown in Fig. 12.

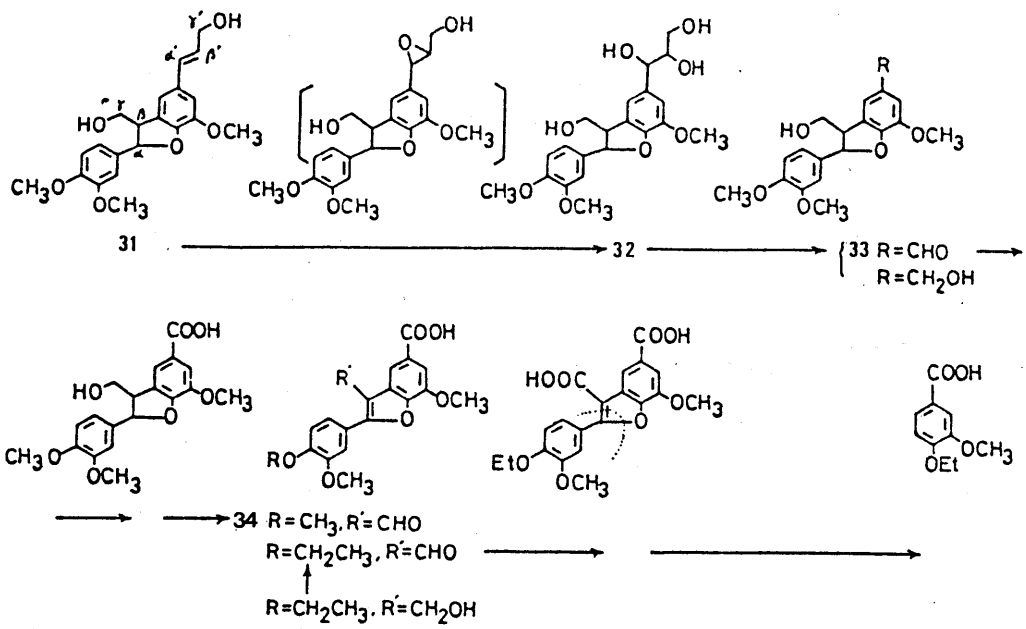


Fig. 12. Degradation pathway of 4-*O*-methyldehydrodiconiferyl alcohol by *Phanerochaete chrysosporium*

Umezawa et al[21] also reported that further experiments using phenylcoumaran-α-aldehyde with syringyl ring(35) showed the formation of phenylcoumarone(36) and a-hydroxyphenyl-coumaran(37) which was found to be degraded to 2,6-dimethoxy-p-benzoquinone(38) as shown in Fig. 13.

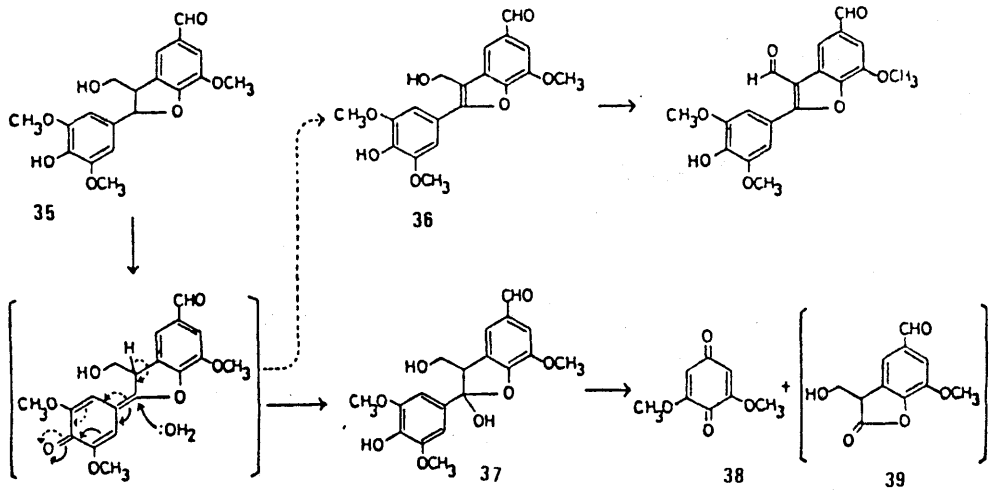


Fig. 13. Degradation pathway of dehydrodiconiferyl alcohol derivative by *Phanerochaete chrysosporium*

It seems that α-hydroxyphenylcoumaran(34) is an intermediate to give rise 2,6-dimethoxy-p-benzoquinone(38) and the compound(39) in degradation of phenolic phenylcoumaran.

4. Diarylpropane (β-1) Linkage

Diarylpropane-1,3-diol is one of the major substructure of lignin (5 - 15% in lignin). Fig. 14 shows the degradation pathway of 1,2-diarylpropane-1,3-diol(40) by ligninolytic culture of P. chrysosporium. (Higuchi group[22,23]).

The compound(40) was degraded to syringylglycerol(43), α-hydroxyacetosyringone(45), syringaldehyde(44) and syringyl alcohol(46), but 2,6-dimethoxy-p-benzoquinone(7') was scarecely detected. This suggests that the degradation of phenolic β-1 models with P. chrysosporium proceeds mainly via Cα- Cβ cleavage.

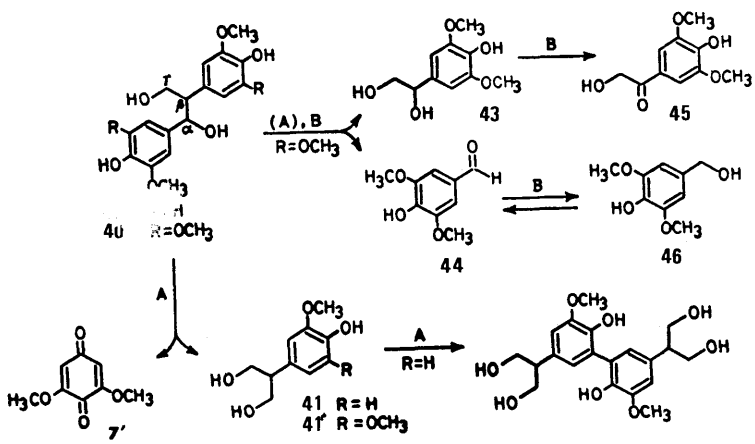


Fig.14. Degradation pathways of 1,2-diarylpropane-1,3-diols by *F. solani* M-13-1 and *P. chrysosporium*. A, Pathway by *Fusarium*; B, pathway by *Phanerochaete*.

The Cα-Cβ cleavage reaction in nonphenolic β-1 models with which the enzyme(ligninase) was first detected by Kirk group[24].

Fig. 15 shows the cleavage pathway of nonphenolic 1,2-diarylpropane-1,3-diol(47) by ligninase of P. chrysosporium.

The compound(47) was degraded to 5-methoxyveratrylaldehyde(48), veratrylaldehyde(49) and w-methylhydroxy veratrylaldehyde derivative(50).

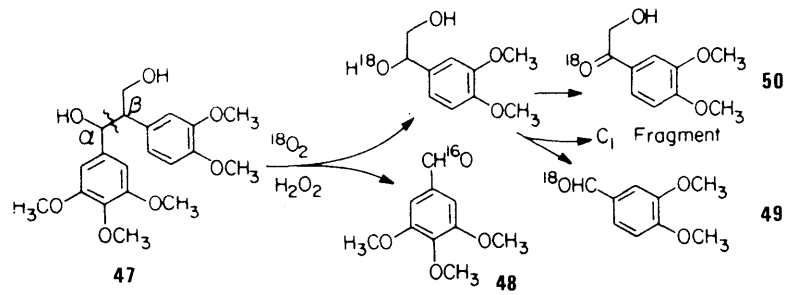


Fig.15. Cleavage of a β-1 model compound by a ligninolytic oxygenase from *Phanerochaete chrysosporium* involves C_α—C_β cleavage.

In addition to the Cα-Cβ cleavage the purified enzyme catalyzes the oxidation of benzylalcohols to aldehyde and ketones.

5. Resinol (β-β) Linkage

Degradation of β-β' linked lignin substructure models was investigated with ligninolytic cultures of a white-rot basidiomycete, Phanerochaete chrysosporium[22,24].

Fig. 16(pathway B) shows the degradation pathway of syringaresinol derivatives by P. chrysosporium. Syringaresinol(51), a major substructure of hardwood lignin and its monomethyl ether(52) are converted to the Cα-oxidized compounds(53, 54), 3-O-demethylated compound(59), γ-lactons(54, 56), methoxy-p-benzoquinone(7') and lactol(58).

γ-Lacton was converted to 3,4,5-trimethoxybenzonic acid(57) via Cα-Cβ clevage. The formation of these degradation products indicates that the resinols were degraded via oxidative alkyl-phenyl cleavage and Cα-Cβ cleavage, both probably mediated by lignin peroxidase. d,l-Pinoresinol monomethyl ether is converted analogous degradation products. The Cα-oxidized compounds(53, 54) occur in two chemical structures, hemiketal-keto alcohol, due to the ring-chain tautomerism.

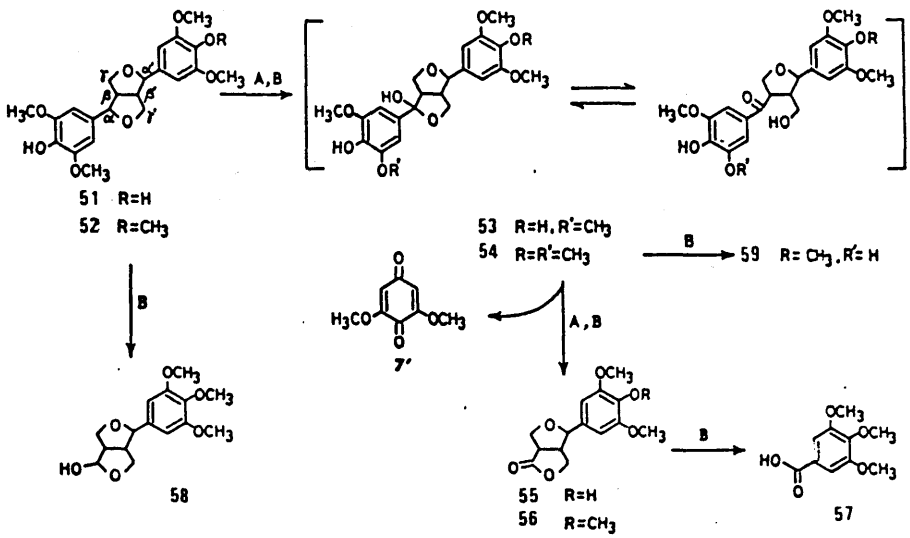


Fig. 16. Degradation pathways of syringaresinol derivatives by *F. solani* M-13-1 and *P. chrysosporium*. A, Pathway by *Fusarium*; B, pathway by *Phanerochaete*.

6. Biphenyl (5 - 5) Linkage

Biphenyl structure is an important linkage unit in natural lignin marcromolecule and resistant to microbial attack.

Higuchi group[25] found that pinoresinol monoethyl ether(60), a β-β' linked lignin substructure model, was largly dimerized at free otho-position of the phenolic hydroxyl group, probably by the action of fungal phenol-oxidizing enzymes. (Fig. 17)

The biphenyl dimer(61) was rather stable, but slowly degraded to give 6-oxo-2-(4-ethoxy-3-methoxyphenyl)-3,7-dioxabicyclo[3,3,0] octane(62) in 5 days.

About 75% of compound 61 was recovered.

This biphenyl dimer 61 was degraded mainly via alkyl-aryl($C\alpha$-C_1) cleavage, after oxidation of the benzylic position($C\alpha$).

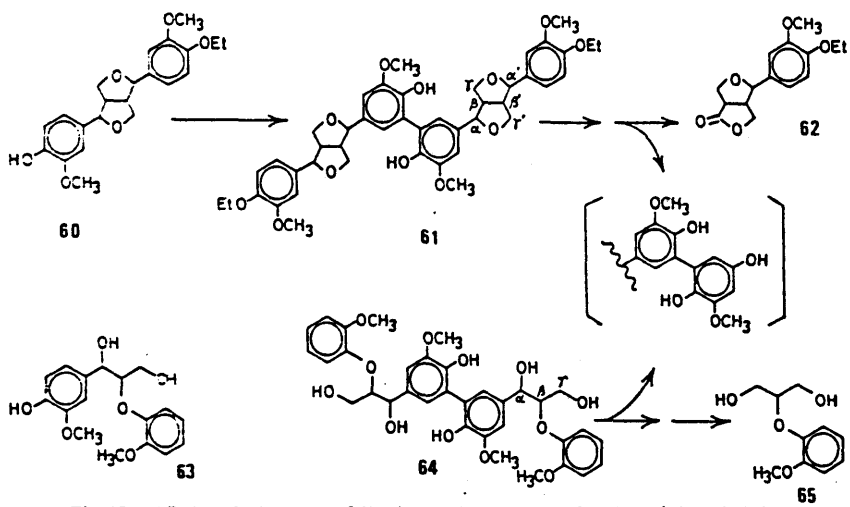


Fig. 17. Alkyl-aryl cleavage of lignin model compounds with biphenyl linkage by *Phanerochaete chrysosporium*.

On the other hand, another type of biphenyl model 64 prepared from the β-ether dimer 63 was more actively degraded by the P. chrysosporium and disappeared completely in 5 days.

Only one product was isolated and identified as glycorol-2-guaiacyl ether 65. This results indicated that compound 60 was also degraded via alkyl-aryl cleavage, followed by reduction of glyceradehyde-2-guaiacyl ether as speculative intermediate to give compound 65.

7. Alkyl-aryl (β-6) Linkage

Lignin peroxidase catalyzing Cα-Cβ cleavage is an oxygenase, incorporating an oxygen atom into the β-carbon and requiring an α-hydroxyl for cleavage reaction to proceed, as shown in Fig. 18. (chen and Chang).[26]

The cleavage results in the formation of benzaldehyde derivatives that are either further oxidized to benzoic acid derivatives or reduced to benzyl alcohol derivatives.

The Cα-Cβ cleavage probably also occurs in many different lignin substructures as long as there is a hydroxyl group at the α-carbon.

Judging from the many aromatic dicarboxylic acids identified, the Cα- Cβ cleavage probably also occurs condensed structures such as β-5 and β-6 substructures.

Chen and Chang[26] reported that the β-6 dimeric compound was degraded to 2-carbonyl-5-hydroxy-4-methoxy benzoylformic acid(66), m-hemipimic acid(67), 4-methoxy-m-hemipimic acid(68) and vanillic acid(69).

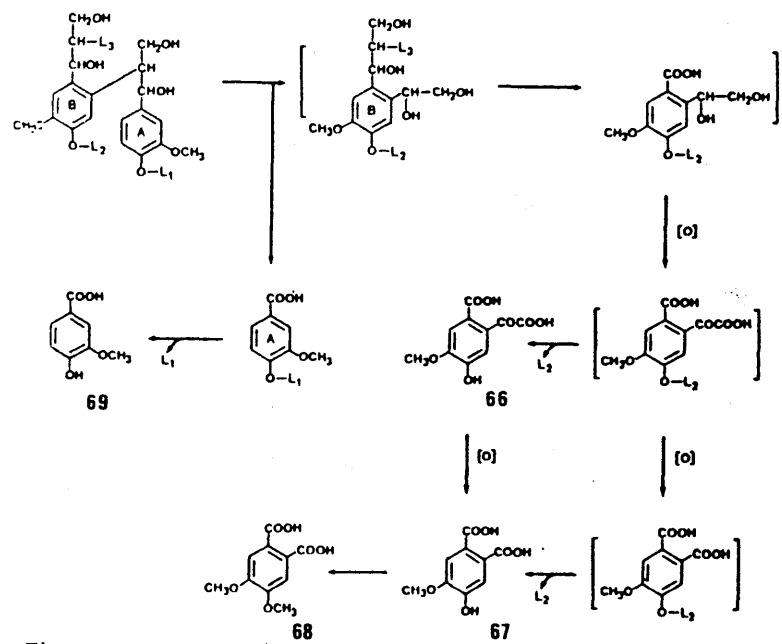


Fig. 18 Possible pathways for fungal degradation of nonphenolic β-O-4 and β-6 substructures involving cleavage of $C_\alpha - C_\beta$ bonds in side chains; L_1, L_2, L_3 = lignin moiety.

8. Conclusive remarks

The oxidation patterns revealed by the degradation products of main dilignols such as guaiacylglycerol-β-coniferyl alcohol ether, dehydrodiconiferyl alcohol, syringaresinol ether, 1,2-diguaiacylpropane-1,3-diol, biphenyl compound and β-6 compound by P. chrysosporium. Higuchi group[27] reported that ally alcohol side chains of dilignols were degraded in a different way by this fungi (Fig. 19).

1) Cinnamyl alcohol groups are oxidazed to the corresponding cinnamic acids via cinnamaldehyde by Fusarium but are converted to glycerol group by Phenerochaete.
2) Both cinnamic acid and glycerol side chains thus formed are converted to the C_6-C_1 aldehyde groups and these to the C_6-C_1 acid groups.

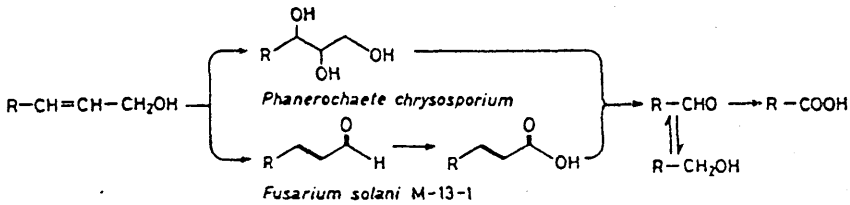


Fig. 19. Oxidative degradation of allyl alcohol side chains of dilignols by *Fusarium solani* M-13-1 and *Phanerochaete chrysosporium*

Fig. 20 shows the degradation products identified so far from β-O-4 substructure model compounds by culture of P. chrysosporium and lignin peroxide. (Higuchi group[23]). These are yielded by Cα- Cβ cleavage, O-4 cleavage, alkyl-phenyl cleavage, Cα-oxidation, oxidation of β-hydroxy group and subsequent reaction with hydroxyl groups, and aromatic ring cleavage derived from the oxidation of either A ring or B ring by lignin peroxide.

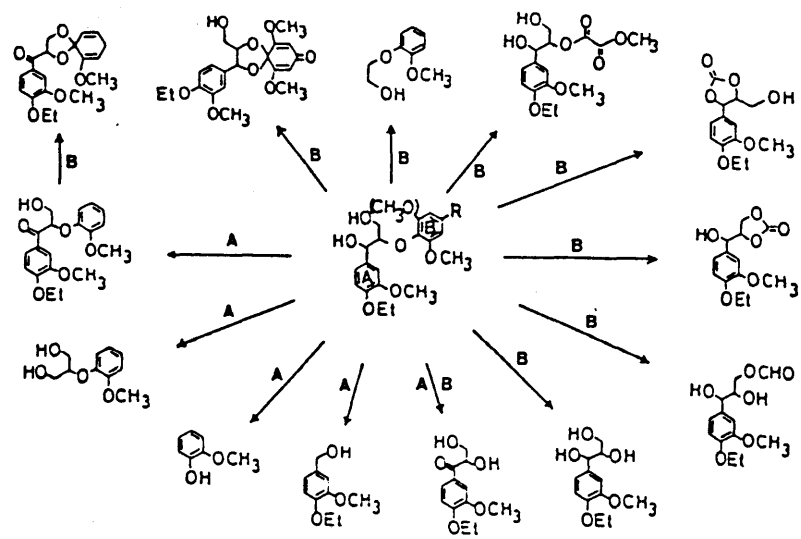


Fig. 20. Degradation products identified from β-O-4 substructure model compounds by the culture of *P. chrysosporium* and its lignin peroxidase. A. Degradation pathway started from aromatic ring A; B. degradation pathway started from aromatic ring B.

Acknowledgments

The author wishes to express his sincerest thanks to Prof. Dr. T. Kent Kirk, Forest Products Laboratory, USDA - Forest Service, Madison, Wisconsin, and Prof. Dr. Takayoshi Higuchi, Research Section of Lignin Chemistry, Wood Research Institute, Kyoto University, for his kindest discussions and suggestions.

요 약

리그닌 생분해의 분해경로 및 매카니즘에 관한 연구가 최근 Kirk와 Higuchi 등에 의하여 활발히 연구되고 있다. 특히, Phanerochaete chrysosporium이 생산해내는 Ligninase를 이용하여 매우 가치있는 연구 결과를 얻고 있다.

본 총설에서는 Kirk와 Higuchi의 허가를 얻어서 그들의 논문을 중심으로 리그닌의 중요한 결합 양식 별로 즉, β-O-4, β-5, β-1, β-6, 5-5등의 결합 모델 화합물들의 분해경로 및 매카니즘에 관하여 조사 정리하였다.

References

1. Tien, M., and T. K. Kirk, 1984, Proc. Nat'l Acad. Scie. USA 81, 2280-2284.
2. Glasser, W. G., and H. R. Glasser, 1981, Pap. Puu. 63, 71-83.
3. Kersten, P. J., Tien, M., Kalyanaraman, B., and T. K. Kirk, 1985, The Ligninase of Phanerochaete Chrysosporium Generates Cation Radicals from Methoxybenzenes, Bio. Chem. 260(5), 2609-2612.
4. Higuchi, T., 1985, Biosynthesis and Biodegradation of Wood Components, Acad. Press. New York, 558-561.
5. Higuchi, T., 1981, Biodegradation of Lignin, Wood Res. 67, 47-58.
6. Higuchi, T., 1983, Biosynthesis and Microbial Degradation of Lignin, The New Frontiers in Plant Biochemistry, 23-46.
7. Umezawa, T., and T. Higucui, 1985, Arylglycerol Formation in Degradation of β-O-4 Lignin Substructures with and without α-O-γ Bond by Phanerochaete Chrysosporium, Wood Res. 71, 25-31.
8. Enoki, A., Goldsby, G. P., and M. H. Gold, 198, Arch. Microbiol., 125, 227.
9. Enoki, A., Goldsby, G. P., Krisnangkura, K., and M. H. Gold, 1981, FEMS Microbiol. Lett. 10, 373-377.

10. Enoki, A., Goldsby, G. P., and M. H. Gold, 1981, Arch. Microbiol. 139, 141-145.
11. Nakatsubo, F., Reid, I. D., and T. K. Kirk, 1981, Biochem. Biophys. Res. Commun. 102, 484-491.
12. Kirk, T. K., 1984, In "Biochemistry of Microbial Degradation" (D.T. Gibson, ed.), Dekker, New York, 399-437.
13. Hammel, K. E., Tein, M., Kalyanaraman, B., and T. K. Kirk, 1985, Biol. Chem., 260, 8348-8353.
14. Schoemaker, H. E., Harvey, P. J., Bowen, R. M. and J. M. Palmer, 1985, FEBS Lett. 183, 7-12.
15. Kirk, T. K., Tein, M., Kersten, P. J., D. Mozuch and B. Kalyanaraman, 1986, Ligninase of Phanerochaete Chrysosporium, Biochem., 236, 279-287.
16. Chen, C.-L., and H.-M. Chang, 1985, In "Biosynthesis and Biodegradation of Wood Components" (Higuchi, T., ed.), Acad. Press, New York, 535-556.
17. Ander, P., and K.-E. Eriksson, 1985, Appl. Microbiol. Biotechnol. 21, 96-102.
18. Crawford, R.L. 1981, In "Lignin Biodegradation and Transformation" (R.L. Crawford), John Wiley and Sons, New York, 92-94.
19. Chen, C.-L., and H.-M. Chang, 1985, IN "Biosynthesis and Biodegradation of Wood Components" (Higuchi, T., ed.) Acad. Press, New York, 546-548.
20. Nakatsubo, F., Kirk, T.K., Shimada, M., and T. Higuchi, 1981, Metabolism of a Phenylcoumaran Substructure Lignin Model Compound in Ligninolytic Cultures of Phanerochaete Chrysosporium, Arch. Micro. 128, 416-420.
21. Umezawa, T., F. Nakatsubo, T., Higuchi, T., and T.K. Kirk, 1980, Abstracts 25th Sym. Lignin Chemistry (Japan), 29.
22. Kayama, Y., and T. Higuchi, 1984, Metabolism of 1,2-Disyringylpropane-1,3-diol by Phanerochaete Chrysosporium, Mokkuzai Gakkaishi, 30(3), 237-239.
23. Higuchi, T. 1986, Catabolic Pathways and Role of Ligninase for the Degradation of Lignin Substructure Models by White-Rot Fungi, Wood Res. 73, 58-81.
24. Kayama, T., F. Nakatsubo, and T. Higuchi, 1982, Arch. Microbiol. 131, 124.
25. _____, T. Higuchi, 1984, Degradation of Lignin Substructure Models with Biphenyl Linkage by Phanerochaete Chrysosporium Burds, Wood Res. 70, 25-28.
26. Chen, C.-L., Chang, H.-M. and T.K. Kirk, 1982, Holzforschung, 36, 3-9.
27. Higuchi, T. 1981, Biodegradation of Lignin, Wood Res. 67, 47-58.