목재공학 27(2) : 53~61
Mokchae Konghak 27(2) : 53~61, 1999

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

- Conditions of Manganese Peroxidase Production by Lignin-Degrading Fungus LSK-27 -

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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(III)[*1]

-리그닌分解菌 LSK-27에 의한 Manganese peroxidase 生産條件 -

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ABSTRACT

Effects of culture conditions and Mn(II) addition were investigated for production of extracellular manganese peroxidase by lignin-degrading fungus LSK-27. Nitrogen source was shown to more influence the production of extracellular manganese peroxidase by LSK-27 than carbon source. When peptone or yeast extract as nitrogen source was added, high MnP activity was obtained. Especially, nitrogen-sufficient culture condition was effective in MnP activity, showing significantly increase up to 1.0% peptone concentration, but carbon-sufficient was not. Mn(II) was shown to strongly induce the MnP production in culture fluids of LSK-27. Increase of MnP actiyity was obeserved up to addition of 100ppm Mn(II), and over this Mn(Ⅱ) concentration appeared to be inhibitory. The highest level of MnP activity was attained when Mn(II) was added after 2 day incubation.

Keywords : Lignin-degrading fungus LSK-27, manganese peroxidase, nitrogen and carbon source, peptone, Mn(II)

- 요 약 -

리그닌분해균 LSK-27 균주로부터 Manganese peroxidase (MnP) 생산을 위한 배지조건과 Mn(II) 첨가효과를 검토하였다. LSK-27균주에 의한 균체외 MnP 생산에는 탄소원보다 질소원의 영향이 크게 나타났다. 질소원으로는 peptone

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이나 yeast extract와 같은 복합 유기질소원이 효과적이었으며 특히 질소원의 농도가 높은 조건에서 MnP activity가 우수하였다. 질소원으로서 peptone 농도 1.0%까지는 뚜렷한 activity 증가를 가져왔으나 1.5%이상의 농도에서는 MnP activity 증가효과가 크게 나타나지 않았다. 반면에 탄소원의 농도에 의한 MnP activity 증가효과는 glucose농도 1.0%에서 3.0%까지 거의 비슷한 수준이었다. Mn(II)는 높은 MnP 유도효과를 나타냈으며 첨가농도 100ppm 까지 MnP activity의 증가효과를 보였고 그 이상의 농도에서는 MnP 생산이 억제되었다. Mn(II)는 배양 2일 후에 첨가하였을 때 MnP activity 증가가 가장 높게 나타났다.

INTRODUCTION

Ligninolytic enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase have been recently focused on fundamental researches because of their potential abilities to carry out biopulping and biobleaching and to degrade toxic pollutants. Of the ligninolytic enzymes, MnP was first discovered in *Phanerochate chrysosphorium* (Kuwahara *et al.*, 1984) and there are now many evidences that this enzyme plays a role in lignin depolymerization. The MnP specifically oxidizes Mn(Ⅱ) ions to Mn(Ⅲ) ions in the presence of H_2O_2 and appropriate Mn(Ⅲ)-chelating agents. The resulting Mn(Ⅲ) complexes can substantially oxidize broad spectra of phenolic and related compounds, including a variety of synthetic lignins (Johanson & Nyman, 1987; Warriishi *et al.*, 1989; Wariishi *et al.*, 1991), wood lignins (Datta *et al.*, 1991; Galliano *et al.*, 1991), and chloro lignin of pulp mill effluent (Lockner *et al.*, 1991; Michel *et al.*, 1991). However, this ligninolytic enzyme application in industrial processes on a large scale requires the understanding of physiology of lignin-degrading fungi and the use of efficient system for enzyme mass-production. Lignin-degrading fungi have shown the importance of nutritional factors in the appearance of ligninolytic activity (Kirk *et al.*, 1978). Many authors have investigated the role of environmental factors, such as carbon and nitrogen regulation, oxygen tension, and Mn(II) concentration promoting MnP production. In *P. chrysosphorium*, ligninolytic peroxidases are produced during the secondary metabolism and are synthesized in response to nitrogen, carbon or sulfur limitation (Jeffries *et al.*, 1981). In particular, manganese ion and nutrient nitrogen source have been shown to have strong regulating effects on MnP activity (Kirk & Tien, 1987; Gold & Alic, 1993), and those regulatory effects have been observed in other white-rot fungi as well (Bonnarme & Jeffries, 1990; Perez & Jeffries, 1992).

In previous studies, the screened lignin-degrading fungi LSK-27 had high extarcellular MnP activity in glucose peptone broth, as compared with other screened lignin-degrading fungi (Jung *et al.*, 1995). In order to exploit capabilities of this MnP for biotechnological applications, its structual and functional properties need to be studied. Therefore, in the present study, the growing conditions of lignin-degrding fungus LSK-27 were investigated to optimize the MnP production for purification of the MnP.

MATERIALS AND METHODS

Microorganism

The fungus used for MnP production was lignin-degrading fungus LSK-27, which has been reported previously (Jung *et al.*, 1995). This fungus was maintained at 4℃ on potato-dextrose agar slant, from which it was transferred to glucose-peptone plate (glucose 30g, peptone 10g, KH_2PO_4 1.4g, $MgSO_4$ 0.5g, $CuSO_4$ 20mg, and thiamin HCl 2mg in one liter of

disstilled water). It was incubated at 28℃ for 5 days before use as the inoculum in experiments. The experiments were inoculated with two agar plugs of 5 mm diameter as described previously (Jung *et al.*, 1996).

Culture conditions for MnP production

To examine the production of MnP in different nitrogen concentration based media, lignin-degrading fungus LSK-27 was incubated in four culture media of the following composition (wt/v) : GP (3% glucose, 1% peptone), GY (3% glucose, 1% yeast extract), GA (3% glucose, 1% ammonium tartrate), and Kirk's medium (Kirk & Tien, 1988). Mn(Ⅱ) was added as $MnSO_4$. All Mn(Ⅱ) concentrations given in parts per million (miligrams per liter) are for the free ion and not the salt. The basal level of Mn(Ⅱ) used was 11.15 ppm, as given by Kirk and Tien (1988). The pH was adjusted to 5.0 after addition of salts (KH_2PO_4 1.4g, $MgSO_4$ 0.5g, $CuSO_4$ 20mg, thiamin HCl 2mg per liter) with exception of Kirk's medium.

Carbon-limited culture was performed with 0.1% glucose as carbon source, and nitrogen-limited culture was prepared with 0.05% peptone or yeast extract as nitrogen source.

The effects of different concentrations of carbon, nitrogen, and Mn(Ⅱ) on MnP production were studied in glucose-peptone medium. The glucose concentrations were 0.1, 1.0, 2.0, and 3.0%, and the nitrogen concentrations were 0.05, 0.5, 1.0, and 1.5%. The Mn(Ⅱ) concentration was varied to give 1, 40, 100, 200, and 500ppm. And to evaluate the effect of Mn(Ⅱ) addition time, Mn(Ⅱ) of 40 ppm was added after growth of 0, 1, 2, 3, and 4 days in glucose-peptone broth. LSK-27 isolate for MnP production was incubated in stationary cultures.

MnP activity assay

MnP activity was measured spectrophotometrically (465nm) at 30℃ according to Paszczynski *et al.* (1985) with guaiacol as a substrate. The reaction mixture contained 0.1M sodium tartrate buffer (pH 5.0), 0.1mM guaiacol, 0.1mM $MnSO_4$, and 0.1mM H_2O_2. The reaction was initiated by the addition of H_2O_2. Enzyme boiled for 5 minutes was used in the control.

RESULTS AND DISCUSSION

Influence of nitrogen source on MnP proction

Lignin-degrading fungus LSK-27 had higher extarcellular MnP activity in glucose peptone medium, as compared with other lignin-degrading fungi screened in my laboratory (Jung *et al.*, 1995). Thus to investigate the influence of nitrogen sources on MnP production, LSK-27 was incubated for 10 days in various nitrogen sources. As shown in Fig. 1, MnP production by LSK-27 was apparantly higher in peptone and yeast extract media than in Kirk's and ammonium tartrate media, with and without Mn(Ⅱ). Without Mn(Ⅱ), MnP was reached to maximal activity after 5 to 7 days incubation in peptone and yeast extract media, after which time it slowly decreased. On the other hand, MnP activity was shown to very low level in Kirk's and ammonium tartrate media. Kimura *et al.* (1990) found that wild-type *Bjerkandera adusta* produced LiP only in an organic nitrogen-rich medium. Also Erwin *et al.* (1993) indicated that organic nitrogen-sufficient media induced LiP production and stimulated MnP production in *Bjerkandera sp.* strain BOS55, and these ligninolytic enzymes production could also be induced by peptone, soybean protein, and yeast extract, but not by NH_4^+. In accordance with their results, MnP of LSK-27 was exhibited to be induced by organic nitrogen such as peptone or yeast extract and not by the vitamins or by the minerals in Kirk's medium.

There are many literatures that Mn(Ⅱ) has a potent inducing effect on the expression of MnP in many white rot fungi (Bonnarme & Jeffries,

1990; Brown *et al.*, 1990; Woude *et al.*, 1993). Also the results of this study demonstrated that Mn(II) has a strong inducing effect on the appearance and the MnP activity in the extracellular fluids of LSK-27. When Mn(II) of 11.15 ppm was added to these culture media, MnP activities in peptone and yeast extract media were first detectable on 2 days incubation, and significantly increased throughout the course of incubation. Also the inducing effect of MnP activity by Mn(II) addition were obtained in Kirk's and ammonium tartrate media, but the increase of MnP activity in these media was not high. The decline in MnP activity during secondary metabolism was reported to be due to degradation of MnP proteins by a protease induced under starvation conditions (Dass *et al.*, 1996). With Mn(II), MnP activity showed to increase after 5 to 7 days incubation, as compared with the media without Mn(II). It is not known by these results whether the increase of MnP in final stage incubation is due to the restriction of specific proteases production by Mn(II) or the inducing effect of MnP. However, it was demonstrated that at least Mn(II) could play a role in MnP production and regulation of LSK-27.

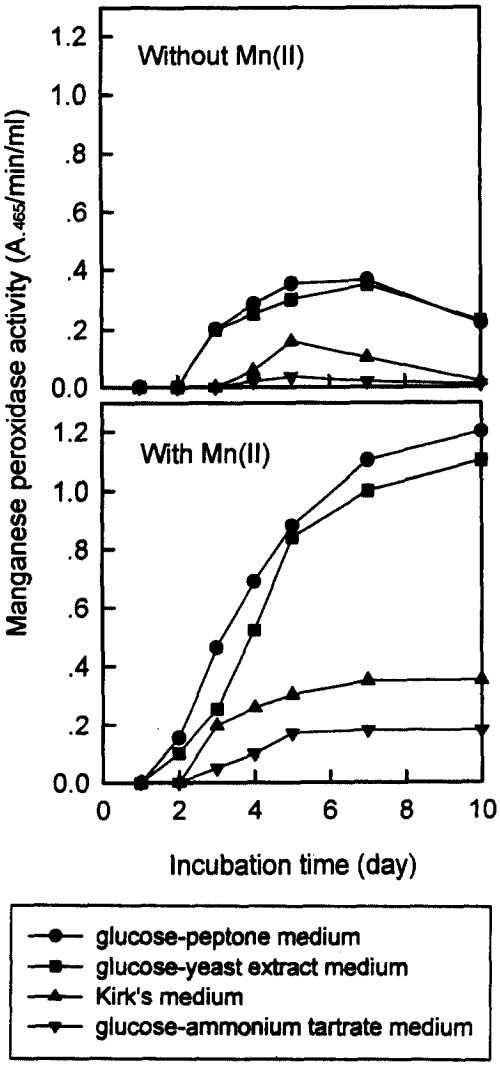

Fig. 1. Manganese peroxidase activity in various culture conditions.

Effect of carbon and nitrogen concentration on MnP production

The ligninolytic machinery in white rot fungi is highly regulated by nutrients. In particular, Mn(II) and nitrogen have been shown to have strong regulating effects (Harvey *et al.*, 1986; Gold & Alic, 1993). In various culture media for MnP production, nitrogen sources positively affected the levels of MnP activity, and the highest levels of MnP activity were obtained in peptone medium, followed in yeast extract. Peptone and yeast extract are undefined sources of organic nitrogen. It is mainly composed of peptides of different sizes, and it has been suggested that some of them could induce secondary-metabolism events such as the secretion of ligninolytic peroxidases because they are similar to peptides released during mycelium autolysis at the end of growth phase (Erwin *et al.*, 1993). To examine the effect of carbon and nitrogen limitation on MnP production, LSK-27 was incubated for 7 days under culture conditions of 0.1% glucose as carbon limitation and 0.05% peptone or yeast extract as nitrogen limitation, with 11.15 ppm Mn(II).

As shown in Table 1, when LSK-27 was

Table 1. Effect of carbon and nitrogen limitation on MnP production

Medium	M.W.(g/35ml)	MnP activity(A_{465}/min/ml)
GP	0.2532	0.8800
GLP	0.2055	0.4506
GLY	0.2158	0.4032
GPL	0.1595	0.1021

M.W:mycelium weight, GP:glucose-peptone medium, GLP:glucose limited-peptone medium, GLY:glucose limited-yeast extract medium, GPL:glucose-peptone limited medium.

incubated under conditions of carbon limitation, its mycelium weights were almost similar level with that in glucose-peptone medium, and MnP activities were detected to some extent. Whereas in condition of nitrogen limitation, MnP activity was almost not detected, and mycelial growth was a little low level in comparison with the other two media.

Effects of various concentrations of glucose and nitrogen on MnP activity were shown in Fig. 2, and 3. In high nitrogen condition (1% peptone), MnP activities were almost similiar level in various glucose concentrations with exception of 0.1% glucose as carbon-limited condition. On the other hand, in high carbon condition (2% glucose), MnP activity was increased according to the increase of peptone concentration, showing significant increase up to 1.0% peptone concentration. But over 1.5 % peptone concentration had not influence on the increase of MnP avtivity. In general, nitrogen limitation has often been used to induce ligninolytic activity, and the highest MnP levels are produced in nitrogen-limiting conditions during secondary metabolism. This is true for most of the white rot fungi that have been

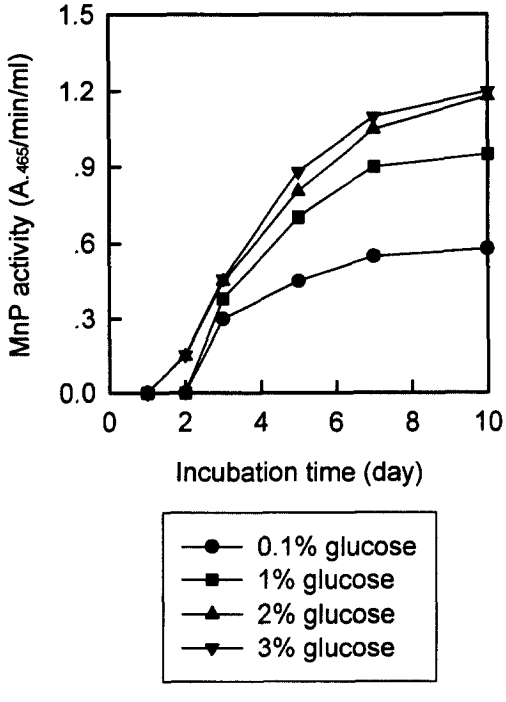

Fig. 2. Effect of glucose concentration on maganese peroxidase activity.

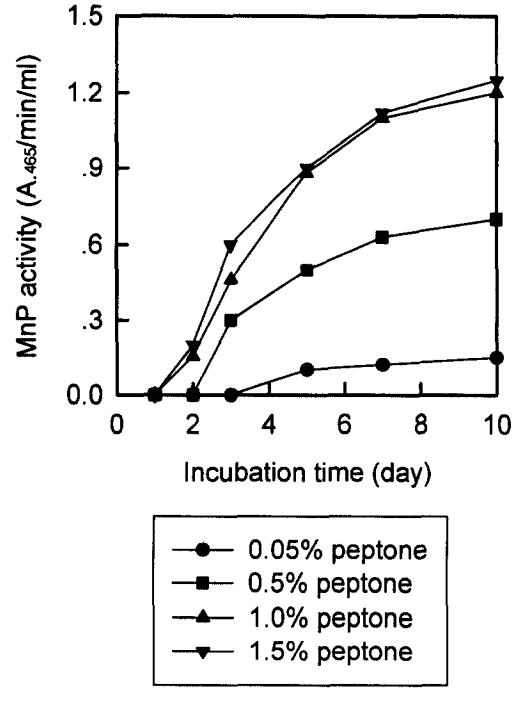

Fig. 3. Effect of peptone concentration on maganese peroxidase activity.

studied, including *P. chrysosphorium* (Keyser *et al.*, 1978; Jeffries *et al.*, 1981), *Phlebia radiata* (Niku-Paavola *et al.*, 1988), *Phlebia brevispora* (Ruttimann *et al.*, 1992), and *Coriolus versicolor* (Jonson *et al.*, 1987). In contrast, *Cereporiopsis subvermispora* produces higher levels of MnP and laccase when grown under nitrogen- and carbon-sufficient conditions (Ruttimann *et al.*, 1992), and *Dichomitus squalens* degrades lignin and expresses its lignin-degrading enzymes under nitrogen-sufficient as well as nitrogen-limiting conditions (Perie & Gold, 1991). Also, lignin biodegradation can be sustained as long as carbohydrate is present, but it is very diffcult to maximize extracellular enzyme production while restricting available nitrogen (Bonnarme & Jeffries, 1990). From our results it can be concluded that excess glucose did not stimulate MnP production, whereas peptone suppliments did. However, the importance of nitrogen stimulation in nature is not clear, because the nitrogen content of most wood is very low. Some conditions in which the fungi could have access to high levels of nitrogen could occur naturally. Larsen *et al.* (1978) suggested that some N_2-fixing bacteria are associated with major decay fungi in wood. Then, because this LSK-27 is wild type lignin-degrading fungus screened from decayed chip in soil, it was thought to be produced MnP in responce to nitrogen-suffcient conditions.

Effect of concentration and addition time of Mn(II) on MnP activity

The presence of Mn(Ⅱ) in cultures controls the expression of MnP and the extent of lignin degradation by white-rot fungi (Perez & Jeffries, 1992). However, ligninolytic enzyme production and lignin degradation can be inhibited by high concentration of Mn(Ⅱ), even for MnP activity. Bonnarme and Jeffries (1990) showed that Mn (Ⅱ) had an inductive effect on MnP activity and repressive effect on LiP activity when a high Mn(II) concentration was used, and when Mn

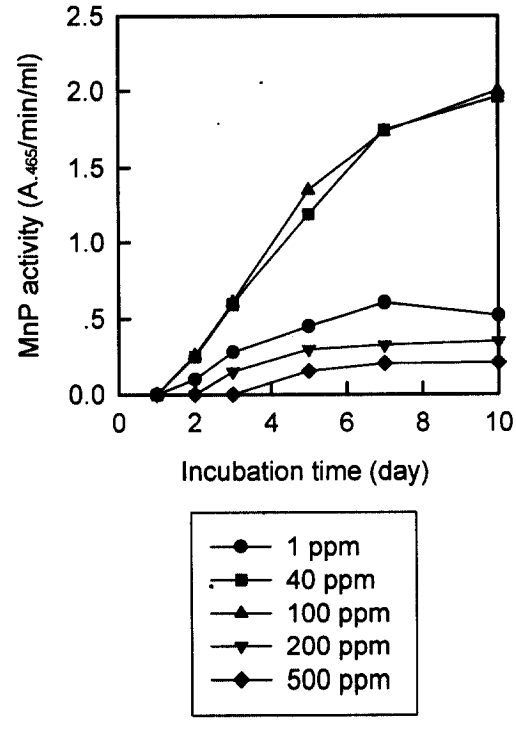

Fig. 4. Effect of Mn(Ⅱ) concentration on maganese peroxidase activity.

(Ⅱ) was low, mineralization of DHP proceeded rapidly, whereas when Mn(Ⅱ) was high, mineralization of DHP was slower. To evalualte the effect of Mn(Ⅱ) concentration on MnP activity, LSK-27 was incubated in glucose-peptone medium containing various concentration of Mn(Ⅱ). As shown in Fig. 4, MnP activity was increased steadily with increasing Mn(Ⅱ) concentration and reached to maximum point at about 100ppm. Over this Mn(Ⅱ) concentration, it appeared to be inhibitory. Also low Mn(Ⅱ) concentration of 1 ppm showed to stimulate the production of MnP to some extent in comparison with the absence of Mn(Ⅱ). These results are consistent with work by Bonnarme and Jeffries (1990), in which they reported that addition of Mn(Ⅱ) up to 40 ppm stimulated the MnP activity, but 199 ppm Mn(Ⅱ) appeared to be slightly inhibitory in *P. chrysosphorium*. In high Mn(Ⅱ) concentration (>40 ppm), the mycelial mat became dark-brown

at later stage of incubation, probably reflecting MnO_2 formation (Boyle *et al.*, 1992). Therefore, these results showed that at least Mn(II) provides direct means of control over the production of MnP by LSK-27.

Fig. 5 showed the increasing rate of MnP activity versus control [without Mn(II)] with the addition time of Mn(II). The addition time of Mn(II) had a different effect on the appearence of MnP activity. The highest level of MnP activity were attained when Mn(II) was added after 2 days incubation. And when Mn(II) was added after 3 and 4 days, MnP activity appeared shortly and increased abruptly thereafter. However, on the whole MnP activities according to various addition time of Mn(II) were almost similar levels with exception of addition after 4 day incubation.

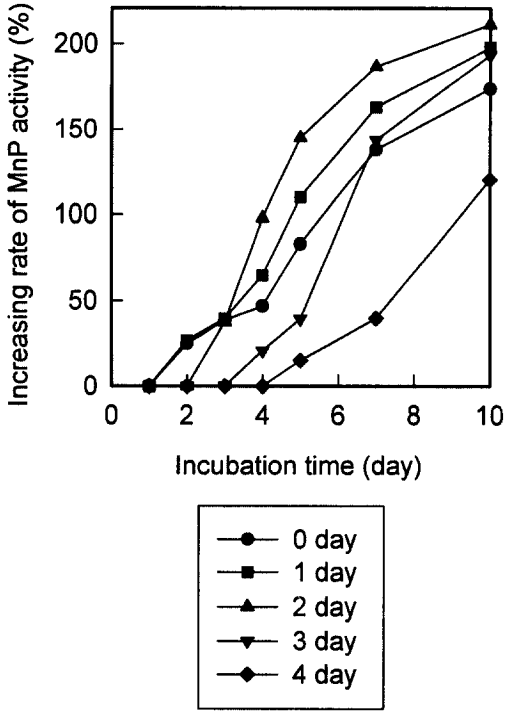

Fig. 5. Effect of Mn(II) addition time on maganese peroxidase activity.

CONCLUSION

Lignin-degrading fungus LSK-27 which has not been identified were studied under various culture conditions for production of MnP. In various culture media for MnP production, nitrogen sources positively affected the levels of MnP activity, and the highest levels of MnP activity were obtained in peptone medium, followed in yeast extract. In contrast, carbon sources and trace elements did not affect directly. When LSK-27 was incubated under condition of carbon limitation, its mycelium weight and MnP activity were similar level with that in glucose-peptone medium, and MnP activities were detected to some extent. Whereas in condition of nitrogen limitation, MnP activity was almost not detected, and mycellial growth was a little low level. In high nitrogen condition (1% peptone), MnP activities were almost similar level in various glucose concentrations with exception of 0.1% glucose as carbon-limited condition. On the other hand, in high carbon condition (2% glucose), MnP activity increased with peptone concentration, showing significant increase up to concentration of 1.0% peptone. But over 1.5 % peptone concentration had not influence on the increase of MnP avtivity.

Mn(II) has a strong inducing effect on the appearance and the activity of MnP in the extracellular fluids of LSK-27. MnP activity was increased steadily with increasing Mn(II) concentration and reached to maximum point at about 100ppm. Over this Mn(II) concentration, it appeared to be inhibitory. The highest level of MnP activity were attained when Mn(II) was added after growth of 2 day.

REFERENCES

1. Bonnarme, T. and T. W. Jeffries. 1990. Mn(II) regulation of lignin peroxidases and manganese-

dependent peroxidases from lignin-degrading white rot fungi. Appl. Environ. Microbiol. 56: 210-217.

2. Boyle, C. D., B. R. Kropp, and I. D. Reid. 1992. Solubilization and mineralization of lignin by white rot fungi. Appl. Environ. Microbiol. 58: 3217-3224.
3. Brown, J. A., J. K. Glenn, and M. H. Gold. 1990. Manganese regulates expression of manganese peroxidases by *Phanerochate chrysosphorium.* J. Bacteriol. 173:4101-4106.
4. Dass, B. S., C. G. Dosoretz, C. A. Reddy, and H. E. Grethlein. 1996. Extracellular proteases produced by the wood-degrading fungus *Phanerochate chrysosphorium* under ligninolytic and non-ligninolytic conditions. Arch. Microbiol. 163: 254-258.
5. Datta, K. A., A. Bettermann, and T. K. Kirk. 1991. Identification of specific manganese peroxidase among ligninolytic enzymes secreted by *Phanerochate chrysosphorium* during wood decay. Appl. Environ. Microbiol. 57: 1453-1460.
6. Erwin E. J. K., ED de Jong, and J. A. Field. 1993. Stimulation of ligninolytic peroxidase activity by nitrogen nutrients in the white rot fungus *Bjerkandera sp* strain BOS55. Appl. Environ. Microbiol. 59: 4031-4036.
7. Galliano, H., G. A. Gas, J. L. Seris, and A. M. Boudet. 1991. Lignin degradation by *Rigidoporous lignosus* involves synergistic action of two oxidizing enzymes: Mn peroxidase and laccase. Enzyme Microb. Technol. 13: 478-482.
8. Gold, M. H.. and M. Alic. 1993. Molecular biology of the lignin degrading basidiomycete *Phanerochate chrysosphorium.* Microbiol. Rev. 57: 605-622.
9. Harvey, P. J., H. E. Schoenmaker, and I. M. Palmer. 1986. Veratryl alcohols a mediater and the role of radical cations in lignin biodegradation by *Phanerochate chrysosphorium.* FEBS Lett. 195:242-246.
10. Jeffries, T. W., S. Choi and T. K. Kirk. (1981) Nutritional regulation of lignin degradation by *Phanerochate chrysosphorium.* Appl. Environ. Microbiol. 42:290-296.
11. Johannson, T., and P. O. Nyman. 1987. A manganese-dependent extracellular peroxidase in the white-rot fungus *Trametes versicolor.* Acta Chem. Scand. B41:762-765.
12. Jonson, L., T. Johansson, K. Sjostrom, and P. O. Nyman. 1987. Purification of ligninase isozymes from the white rot fungus *Trametes versicolor.* Acta. Chem. Scand. B41:766-769.
13. Jung, H. C., S. K. Park, B. S. Kim, and C. Y. Park. 1995. Screening and evaluating of wood-rotting fungi for lignin degradation and ligninolytic enzyme production (I). Screening of high active lignin-degrading fungi. Mokchae Konghak. 23(4): 108-116.
14. Jung, H. C., S. K. Park, B. S. Kim, and C. Y. Park. 1996. Screening and evaluating of wood-rotting fungi for lignin degradation and ligninolytic enzyme production (II). Laccase production by lignin-degrading fungi. Mokchae Konghak. 24(4): 74:81.
15. Keyser, P., T. K. kirk, and J. G. Zeikus. 1978. Ligninolytic enzyme system of *Phanerochate chrysosphorium* : synthesized in the absence of lignin in responce to nitrogen starvation. J. Bacteriol. 135: 790-797.
16. Kimura, Y., Y. Asada, and M. Kuwahara. 1990. Screening of basidiomycetes for lignin peroxidase genes using a DNA probe. Appl. Microbial. Biotechnol. 32: 436-442.
17. Kirk, T. K., E. Schultz, W. J. Connors, L. F. Lorenz and J. G. Zeikus. 1978. Influence of culture parameters on lignin metabolism by *Phanerochate. chrysosphorium.* Arch. Microbiol. 117: 277-285.
18. Kirk, T. K and M. Tien. 1988. Lignin peroxidase of *Phanerochate chrysosphorium.* Methods in Enzym. 161: 239-249.
19. Kirk, T. K. and R. L. Farrel. 1987. Enzymatic combustion the microbial degradation of lignin. Ann. Rev. Microbiol. 41: 465-505.
20. Kirk, T. K., S. Croan, M. Tien, K. E. Murtagh, and R. L. Farrel. 1985. Production of multiple ligninase by *Phanerochate chrysosphorium* : Effect of selected growth conditions and use of a

mutant strain. Enzyme Microbiol. Technol. 8: 27-32.

21. Kuwahara, M., J. K. Glenn, M. A. Morgan, and M. H. Gold. 1984. Separation and characterization of two extracellular H_2O_2-dependent oxidases from ligninolytic cultures of *Phanerochate chrysosphorium*. FEBS Lett. 169: 247-250.
22. Larsen, M. J., M. F. Jurgensen, and A. E. Harvey. 1978. N_2 fixation associated with wood decayed by some common fungi in western Montana. Can. J. For. Res. 8:341-345.
23. Lockner, R., E. Srebotnik, and K. Messner. 1991. Oxidative degradation of high molecular weight chloro lignin by manganese peroxidase of *Phanerochate chrysosphorium*. Biochem. Biophys. Res. Commun. 178: 14288-14293.
24. Michel, F. C., Jr. S. B. Dass, E. A. Grolke, and C. A. Reddy. 1991. Role of manganese peroxidases and lignin peroxidases of *Phanerochate chrysosphorium* in the decolorization of kraft bleach plant effluent. Appl. Environ. Microbiol. 57:2368-2375.
25. Niku-Paavola, M. L., E. Karhunen, P. Salola, and V. Raunio. 1988. Ligninolytic enzymes of the white rot fungus *Phlebia radiata*. Biochem. J. 254: 877-884.
26. Paszczynski, A., R. L. Crawford, and V. B. Huynh. 1988. Manganese peroxidase of *Phanerochate chrysosphorium* : purification. Methods in Enzym. 161: 266:271.
27. Paszcynski, A., V. B. Huynh, and R. Crawford. 1985. Enzymic activities of an extracellular, manganese-dependent peroxidase from *Phanerochate chrysosphorium*. Fems Microbiol. Lett. 29: 37-41.
29. Perez, J. and T. W. Jeffries. 1992. Roles of manganese and organic acid chelators in regulating lignin degradation and biosynthesis of peroxidases by *Phanerochate chrysosphorium*. Appl. Environ. Microbiol. 58: 2402-2409.
28. Perie, F. H., and M. H. Gold. 1991. Manganese regulation of manganese peroxidase expression and lignin degradation by the white rot fungus *Dichomitus squalens*. Appl. Environ. Microbiol. 57: 2240-2245.
30. Ruttimen, C., E. Schwember, L. Salas, D. Cullen, and R. Vicuna. 1992. Ligninolytic enzymes of the white rot basidiomycetes *Phlebia brevispora* and *Ceriporiopsis subvermispora*. Biotechnol. Appl. Biochem. 16: 64-76.
31. Van der Woude, M. W., K. Boominathan, and C. A. Reddy. 1993. Nitrogen regulation of lignin peroxidase and manganese-dependent peroxidase production is independent of carbon and manganese regulation in *Phanerochate chrysosphorium*. Arch. Microbiol. 160:1-4.
32. Wariishi, H., H. B. Dunford, I. D. Macdonald, and M. H. Gold. 1989. Manganese peroxidase from the lignin-degrading basidiomycete *Phanerochate chrysosphorium*. Transient state kinetics and reaction mechanism. J. Biol. Chem. 264: 3335-3340.
33. Wariishi, H., K. Valli, and M. H. Gold. 1991. In vitro depolymerization of lignin by manganese peroxidase of *Phanerochate chrysosphorium*. Biochem. Biophys. Res. Commun. 176: 269-275.