Chemosphere

Removal of estrogenic activity of natural steroidal hormone estrone by ligninolytic enzymes from white rot fungi

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Abstract

Natural steroidal hormone estrone (E_1) was treated with the white rot fungus *Phanerochaete sordida* YK-624 under ligninolytic condition with low-nitrogen and high-carbon culture medium. E_1 decreased by 98% after 5 d of treatment and the activities of ligninolytic enzymes, manganese peroxidase (MnP) and laccase, were detected during treatment, which suggested that the disappearance of E_1 is related to ligninolytic enzymes produced extracellularly by white rot fungus. Therefore, E_1 was treated with MnP and laccase prepared from the culture of white rot fungi. HPLC analysis demonstrated that E_1 disappeared completely in the reaction mixture after 1 h of treatment with either MnP or laccase. Using the yeast two-hybrid assay system, it was also confirmed that both enzymatic treatments completely removed the estrogenic activity of E_1 after 2 h. These results strongly suggest that ligninolytic enzymes are effective in removing the estrogenic activity of E_1.

Keywords: Estrone; Estrogenic activity; Ligninolytic enzyme; Manganese peroxidase; Laccase; White rot fungus

1. Introduction

Effluents from sewage treatment plants (STPs) can discharge estrogenic contaminants into rivers at levels sufficient to induce vitellogenin biosynthesis in male fish (Jobling et al., 1998). To date, a multitude of chemicals have shown to be endocrine disrupters. Among these, natural and synthetic steroidal hormones (estrogens) are effective at the lower ng l^{-1} level, while most other chemicals having estrogenic effects are biologically active at the μg l^{-1} level (Purdom et al., 1994; Routledge et al., 1998; Metcalfe et al., 2001). Thus, considerable concern has been expressed that steroidal estrogens excreted into the environment by humans, domestic or farm animals, and other wildlife, in part via STPs, may be disruptive to the endocrine system. The natural estrogens 17β-estradiol (E_2) and estrone (E_1) have been detected ubiquitously in effluent from STPs and are thought to be responsible for most of the estrogenic effects observed in such effluent (Folmar et al., 1996; Harries et al., 1996, 1997; Desbrow et al., 1998).

It has been reported that E_2 is oxidized to E_1, which is further eliminated in aerobic batch experiments containing diluted slurries of activated sludge from an actual STP without any observed degradation products (Ternes et al., 1999). However, recent studies have claimed that the concentration of E_1 in final effluent at seven of the 25 STPs in Italy, Spain, and Canada is elevated above that in influent, and the removal efficiency of E_1 markedly lower than that of E_2 (Baronti et al., 2000; Carballa et al.,

2004; Servos et al., 2005). At least part of the increase in E_1 concentration in effluent is thus explained by the accumulation of E_1 resulting from biological oxidation of E_2 at STPs. Furthermore, it has been demonstrated that the amount of E_1 discharged from STPs into receiving waters is more than ten times that of E_2 (D'Ascenzo et al., 2003; Servos et al., 2005). Both in vitro (Routledge and Sumpter, 1997) and in vivo (Routledge et al., 1998) experiments have shown that the estrogenic potency of E_1 is half that of E_2. These observations led to the conclusion that, among natural estrogens, E_1 is by far the most important endocrine disrupter in the aquatic environment (D'Ascenzo et al., 2003).

Great interest is currently being expressed in lignin-degrading white rot fungi and their ligninolytic enzymes due to the recognized potential for degrading and detoxifying recalcitrant environmental pollutants such as dioxins (Bumpus et al., 1985), chlorophenols (Joshi and Gold, 1993), polycyclic aromatic hydrocarbons (Bezalel et al., 1996; Collins et al., 1996), and dyes (Ollikka et al., 1993; Nishida et al., 1999). Manganese peroxidase (MnP), laccase, and lignin peroxidase (LiP) produced extracellularly by white rot fungi have been shown to be involved in the degradation of lignin and these pollutants.

MnP is a heme peroxidase produced by white rot fungi and catalyzes the oxidation of Mn(II) to Mn(III) in the presence of H_2O_2. Malonate, oxalate, and α-hydroxy acids such as malate, lactate, and tartrate chelate the generated Mn(III) and

release Mn(III) from the manganese-binding site of MnP. The released Mn(III)-organic acid complex in turn oxidizes various phenolic compounds, including lignin (Glenn et al., 1986; Wariishi et al., 1989). Fungal laccase is a multicopper oxidase that catalyzes the single-electron oxidation of phenolic compounds by reducing molecular oxygen to water (Reinhammer, 1984). We recently demonstrated that MnP and laccase are effective in removing the estrogenic activities of E_2, ethinylestradiol, bisphenol A (BPA), nonylphenol (NP), and phytoestrogen genistein (Tsutsumi et al., 2001; Suzuki et al., 2003; Tamagawa et al., 2005) and in degrading methoxychlor (Hirai et al., 2004) and the antifouling compound Irgarol 1051 (Ogawa et al., 2004). These studies prompted investigation into the removal of estrogenic activity of E_1 by ligninolytic enzymes, which are able to degrade various aromatic compounds.

In this study, we examined the disappearance of E_1 by the white rot fungus *Phanerochaete sordida* YK-624 and investigated the enzymes related to its disappearance. Furthermore, we applied the related enzymes, MnP and laccase, to the treatment of E_1 and describe the removal of its estrogenic activity as measured by a yeast two-hybrid system (Nishikawa et al., 1999).

2. Materials and methods

2.1. Treatment of E_1 with white rot fungus

P. sordida YK-624 (ATCC 90872) was used in this study. The fungus was

maintained in potato dextrose agar (PDA; Difco Laboratories) slants. A new PDA plate was inoculated with fungus, which was then precultured for 5 d at 30ºC. Five disks punched from the grown edge of the precultured mycelium on the PDA plate were homogenized for 30 s with 50 ml of PMY medium (3.0% glucose, 1.0% peptone, 1.0% malt extract, 0.4% yeast extract), which was then put into a 500-ml Erlenmeyer flask with 150 ml of PMY medium and shaken at 150 rpm at 30ºC to give a mycelium suspension culture. After 3 d, 2.2 ml of this culture was homogenized once again for 30 s with 17.6 ml of Kirk medium (Tien and Kirk, 1988) and added to a 100-ml Erlenmeyer flask with 0.2 ml of 10^{-2} M E_1 (final concentration of 10^{-4} M E_1; Tokyo Chemical Industry, Tokyo, Japan). The flask was shaken at 150 rpm at 30ºC.

2.2. Enzyme assay and preparation

Laccase activity was determined by monitoring the oxidation of 2,6-dimethoxyphenol (DMP) at 470 nm and 30ºC. The reaction mixture contained 1 mM DMP and 50 mM malonate buffer (pH 4.5). MnP activity was determined in the same manner, except that the reaction mixture also contained 0.1 mM $MnSO_4$ and 0.2 mM H_2O_2. LiP activity was determined by monitoring the oxidation of veratryl alcohol (VA) at 310 nm and 30ºC. The reaction mixture contained 1 mM VA, 20 mM succinate buffer (pH 3.0), and 0.2 mM H_2O_2. One katal (kat) of enzyme activity was defined as the amount of enzyme producing 1 mol of quinone dimer (49.3 $mM^{-1}cm^{-1}$ at 470 nm)

or veratraldehyde (9.3 $mM^{-1}cm^{-1}$ at 310 nm) from DMP or VA, respectively, per s (Lee and Edlin, 1985; Wariishi et al., 1992).

Partially purified MnP and laccase were prepared from cultures of *P. sordida* YK-624 and *Trametes versicolor* IFO-6482, respectively, as described previously (Tsutsumi et al., 2001). Each partially purified ligninolytic enzyme preparation contained no other ligninolytic enzyme activities.

2.3. Treatment of E_1 with ligninolytic enzymes

For treatment with MnP, the reaction mixture consisted of 10^{-5} M E_1, partially purified MnP (10 nkat ml^{-1}), 50 mM malonate buffer (pH 4.5), $MnSO_4$ (0.1 mM), Tween 80 (0.1%), and glucose (25 mM) and glucose oxidase (3.33 nkat ml^{-1}; Wako, Osaka, Japan) to supply H_2O_2. The reaction was performed at 30ºC while stirring at 150 rpm. Laccase treatment was conducted in the same manner except that laccase (10 nkat ml^{-1}) was used instead of MnP, and $MnSO_4$, Tween 80, glucose, and glucose oxidase were omitted from the reaction mixture.

2.4. Analyses of E_1 treated with ligninolytic enzymes

Residual E_1 concentrations in the enzymatic reaction mixtures were determined by high-performance liquid chromatography (HPLC) analysis. HPLC analyses were carried out with a Wakosil-II 5C18HG (Wako) column using an isocratic elution with

50% acetonitrile aqueous containing 1% acetic acid at a flow rate of 0.8 ml min^{-1} with detection at 285 nm.

2.5. Estrogenic activity of E_1 treated with ligninolytic enzymes

Estrogenic activity of E_1 before and after enzymatic treatment was evaluated by an *in vitro* screening test for chemicals with hormonal activities that used the yeast two-hybrid estrogenic assay system, as developed by Nishikawa et al. (Nishikawa et al., 1999). Yeast Y190 transformed with the pGBT9-estrogen receptor ligand-binding domain (pGBT9-ER LBD) and pGAD424-coactivator was provided by Nishihara (Osaka University). In the yeast, the GAL4 DNA binding domain-ER LBD and the GAL4 activation domain-coactivator fusion proteins were expressed by these expression plasmids. Y190 harbors a GAL4 binding site upstream of the *lacZ* gene, and thus β-galactosidase activity corresponds to the strength of interaction between estrogen receptor and coactivator. The protein-protein interaction between estrogen receptor and coactivator was strictly dependent on the presence of estrogen (Nishikawa et al., 1999).

In the assay system, 2.5 μl of test sample was added to 50 μl of yeast culture and 200 μl of Sabouraud's dextrose medium (Tsutsumi et al., 2001). The test sample for the assay system was prepared as follows; each reaction mixture (200 μl) was lyophilized and dissolved in dimethyl sulfoxide (20 μl), thus ensuring that the

concentration of E_1 before enzymatic treatment corresponded to 10^{-6} M in the assay system. Relative estrogenic activity (%) was defined as the percentage of β-galactosidase activity of enzyme-treated E_1 as compared to that of untreated E_1. In the experiment on dose response curve for estrogenic activity described in Fig. 1, 2.5 μl of various concentrations of E_1, E_2, NP, and BPA dissolved in dimethyl sulfoxide were used instead of reaction mixtures to evaluate their estrogenic activities.

3. Results and Discussion

3.1. Treatment of E_1 with white rot fungus

Ligninolytic activity of white rot fungi appears as a secondary metabolic event, and nutritional nitrogen or carbon limitation (or both) allows extensive degradation of lignin (Keyser et al., 1978; Kirk et al., 1978). Thus, the treatment of E_1 with *P. sordida* YK-624 was investigated under ligninolytic condition with low-nitrogen and high-carbon culture medium (0.02% ammonium tartrate, 1% glucose) (Tien and Kirk, 1988). Figure 2 shows the decrease in E_1 observed during treatment with *P. sordida* YK-624. No decrease in E_1 was observed during the first two days of treatment, but marked decreases were noted after 2 d of treatment and E_1 concentration decreased by 98% after 5 d of treatment.

It has been suggested that MnP, LiP, and laccase produced extracellularly by white rot fungi are involved in the oxidative breakdown of lignin (Dodson et al., 1987;

Gold et al., 1984; Tien and Kirk, 1983). Therefore, these enzyme activities were determined during treatment of E_1 with *P. sordida* YK-624 (Fig. 2). MnP and laccase activities were detected after 2 d of treatment, but LiP activity was not detected throughout the treatment. The profiles of MnP and laccase productions and E_1 decrease during treatment were very similar. These results strongly suggest that MnP and laccase are involved in the disappearance of E_1 by *P. sordida* YK-624.

3.2. Treatment of E_1 with ligninolytic enzymes

We applied two enzymatic treatments, MnP and laccase, in order to remove the estrogenic activity of E_1. As shown in Fig. 3, HPLC detection of residual E_1 on an octadecylated silica column (285 nm) during these two enzymatic treatments revealed that E_1 completely disappeared in the reaction mixture after 1 h of treatment. This indicates that two enzymatic treatments effectively decrease E_1. However, the greatest focus concerning the biodegradation of endocrine-disrupting (estrogenic) chemicals should be on the removal of estrogenic activity. We therefore attempted to assay the estrogenic activity of the E_1 reaction mixtures during enzymatic treatment using the yeast two-hybrid estrogenic assay system. This system is newly developed and is based on the ligand-dependent interaction between the nuclear hormone receptor and its coactivator. The method is rapid and has been confirmed to be reliable for measuring estrogenic activity (Nishikawa et al., 1999).

The estrogenic activity of E_1, expressed as β-galactosidase activity, is compared with those of BPA, NP, and E_2, which are known endocrine disrupters (Fig. 1). The activity of E_1 (10^{-8} M) was almost the same as those of BPA (10^{-4} M), NP (10^{-5} M), and E_2 (~5×10^{-9} M). This finding is consistent with a previous report in which the relative estrogenicities of E_1, BPA, and NP were found to be 0.6, 5×10^{-5} and 4×10^{-4}, respectively, as compared with E_2 (Nakamuro et al., 2002).

Figure 4 shows that MnP and laccase reduced the estrogenic activity of E_1 by 99% and 97% after a 1-h treatment, respectively, and completely removed the activity after 2 h of treatment. On the other hand, HPLC detection of the residual E_1 during enzymatic treatment revealed that E_1 completely disappeared in the reaction mixture after a 1-h treatment with either MnP or laccase (Fig. 3); the residual concentration of E_1 was below the HPLC detection limit (10^{-8} M). Figure 1 shows that E_1 exhibits estrogenic activity at very low concentrations. Thus, the fact that 1-3% of the estrogenic activity of E_1 remained after a 1-h treatment (Fig. 4) may be due to residual traces of E_1. In HPLC analyses, we detected traces of unknown compounds, presumably degradation products, in the reaction mixtures after 1 h of treatment with either MnP or laccase. However, their peak area was much less than the amount of E_1 consumed, even if differences in absorption coefficients are considered. In a previous report, we demonstrated that treatment of BPA and NP with MnP and laccase resulted in oligomeric reaction products through the formation of phenoxy radicals of these

chemicals followed by radical coupling (Tsutsumi et al., 2001). Thus, the removal of estrogenic activity of E_1 may be due to polymerization brought about by enzymatic oxidation, because E_1 has a *para*-substituted phenol structure, as do BPA and NP.

Very little is known about the disappearance of E_1, and no data exist on the microbial removal of the estrogenic activity of E_1. Therefore, this is the first report to show that the ligninolytic enzymes MnP and laccase from white rot fungus can effectively decrease E_1 and remove its estrogenic activity.

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References

Baronti, C., Curini, R., D'Ascenzo, G., Corcia, A. D., Gentili, A. and Samperi, R., 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environ. Sci. Technol. 34, 5059-5066.

Bezalel, L., Hadar, Y., Fu, P. P., Freeman, J. P. and Cerniglia, C., 1996. Initial oxidation products in the metabolism of pyrene, anthracene, fluorine, and dibenzothiophene by the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiol. 62, 2554-2559.

Bumpus, J. A., Tien, M., Wright, D. and Aust, S. D., 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science 228, 1434-1436.

Carballa, M., Omil, F., Lema, J. M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M. and Ternes, T., 2004. Behavior of pharmaceuticals, conmetics and hormones in a sewage treatment plant. Water Res. 38, 2918-2926.

Collins, P. J., Kotterman, M. J., Field, J. A. and Dobson, A. W., 1996. Oxidation of anthracene and benzo(a)pyrene by laccases from *Trametes versicolor*. Appl. Environ. Microbiol. 62, 4563-4567.

D'Ascenzo, G., Di Corcia, D., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M. and Samperi, R., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302, 199-209.

Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and Waldock, M., 1998.

Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. Environ. Sci. Technol. 3, 1549-1558.

Dodson, P. J., Evans, C. S., Harvey, P. J. and Palmer, J. M., 1987. Production and properties of an extracellular peroxidase from *Coriolus versicolor* which catalyzes cleavage in a lignin model compound. FEMS Microbiol. Lett. 42, 17-22.

Folmar, L. C., Denslow, N. D., Rao, V., Chow, M., Crain, D. A., Enbrom, J., Marcino, J. and Guillette, L. J., 1996. Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near major metropolitan sewage treatment plant. Environ. Health Perspect. 104, 1096-1101.

Glenn, J. K., Akileswaran, L. and Gold, M. H., 1986. Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. Arch. Biochem. Biophys. 251, 688-696.

Gold, M. H., Kuwahara, M., Chiu, A. A. and Glenn, J. K., 1984. Purification and characterization of extracellular H_2O_2-requiring diaryl propane oxygenase from the white rot basidiomycete, *Phanerochaete chrysosporium*. Arch. Biochem. Biophys. 234, 353-362.

Harries, J. E., Sheahan, D. A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E. J., Rycroft, R., Sumpter, J. P. and Tylor, T., 1996. A survey of estrogenic activity in United Kingdom inland waters. Environ. Toxicol. Chem. 15, 1993-2002.

Harries, J. E., Sheahan, D. A., Jobling, S., Matthiessen, P., Neall, P., Sumpter, J. P.,

Tylor, T. and Zaman, N., 1997. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. Environ. Toxicol. Chem. 16, 534-542.

Hirai, H., Nakanishi, S. and Nishida, T., 2004. Oxidative dechlorination of methoxychlor by ligninolytic enzymes from white-rot fungi. Chemosphere 55, 641-645.

Jobling, S., Noran, M., Tyler, C. R., Brighty, G. and Sumpter, J. P., 1998. Widespread sexual disruption in wild fish. Environ. Sci. Technol. 32, 2498-2506.

Joshi, D. K. and Gold, M. H., 1993. Degradation of 2, 4, 5-trichlorophenol by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 59, 1779-1785.

Keyser, P., Kirk, T. K. and Zeikus, J. G., 1978. Ligninolytic enzymes system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. J. Bacteriol. 135, 790-797.

Kirk, T. K., Schultz, E., Connors, W. J., Lorenz, L. F. and Zeikus, J. G., 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol. 117, 277-285.

Lee, S. W. and Edlin, G., 1985. Expression of tetracycline resistance in pBR322 derivatives reduces the reproductive fitness of plasmid-containing *Esherichia coli*. Gene 39, 173-180.

Metcalfe, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E. and Potter, T., 2001. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese Medaka (*Oryzias latipes*). Environ. Toxicol. Chem. 20, 297-308.

Nakamuro, K., Ueno, H., Okuno, T., Sakazaki, H., Kawai, H., Kamei, T. and Ugawa, M., 2002. Contribution of endocrine-disrupting chemicals to estrogenicity of environmental water. J. Jpn. Soc. Water Environ. 25, 355-360 (in Japanese).

Nishida, T., Tsutsumi, Y., Kemi, M., Haneda, T. and Okamura, H., 1999. Decolorization of anthraquinone dyes by white-rot fungi and its related enzymes. J. Jpn. Soc. Water Environ. 22, 465-471 (in Japanese).

Nishikawa, J., Saito, K., Goto, J., Dakeyama, F., Matsuo, M. and Nishihara, T., 1999. New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. Toxicol. Appl. Pharmacol. 154, 76-83.

Ogawa, N., Okamura, H., Hirai, H. and Nishida, T., 2004. Degradation of the antifouling compound Irgarol 1051 by manganese peroxidase from the white rot fungus *Phanerochaete chrysosporium*. Chemosphere 55, 487-491.

Ollikka, P., Alhonmäki, K., Leppänen, V-M., Glumoff, T., Raijola, T. and Suominen, I., 1993. Decolorization of azo, triphenyl methane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete* c*hrysosporium*. Appl. Environ. Microbiol. 59, 4010-4016.

Purdom, C. E., Hardiman, P. A., Bye, V. J., Eno, N. C., Tyler, C. R. and Sumpter, J. P., 1994. Estyrogenic effects of effluents from sewage treatment works. Chem. Ecol. 8, 275-285.

Reinhammer, B., 1984. Laccase. In: Lontie, R. (Ed.), Copper Proteins and Copper Enzymes, Vol. 3. CRC Press, Boca Raton, FL, pp. 1-35.

Routledge, E. J. and Sumpter, J. P., 1997. Structural features of alkylphenolic chemicals associated with estrogenic activity. J. Biol. Chem. 272, 3280-3288.

Routledge, E. J., Sheahan, D., Desbrow, C., Sumpter, J. P. and Waldock, M., 1998. Identification of estrogenic chemicals in STW effluent. 2. in vivo responses in trout and roach. Environ. Sci. Technol. 32, 1559-1565.

Servos, M. R., Bennie, D. T., Burnison, B. K., Jurkovic, A., McInnis, R., Neheli, T., Schnell, A., Seto, P., Smyth, S. A. and Ternes, T. A., 2005. Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. Sci. Total Environ. 336, 155-170.

Suzuki, K., Hirai, H., Murata, H. and Nishida, T., 2003. Removal of estrogenic activities of 17β-estradiol and ethinylestradiol by ligninolytic enzymes from white rot fungi. Water Res. 37, 1972-1975.

Tamagawa, Y., Hirai, H., Kawai, S. and Nishida, T., 2005. Removal of estrogenic activity of endocrine-disrupting genistein by ligninolytic enzymes from white rot fungi. FEMS Microbiol. Lett. 244, 93-98.

Ternes, T. A., Kreckel, P. and Mueller, J., 1999. Behaviour and occurrence of estrogens in municipal sewage treatment plants-II. Aerobic batch experiments with activated sludge. Sci. Total Environ. 225, 91-99.

Tien, M. and Kirk, T. K., 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosprium* Burd. Science 221, 661-663.

Tien, M. and Kirk, T. K., 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. Methods Enzymol. 161B, 238-249.

Tsutsumi, Y., Haneda, T. and Nishida, T., 2001. Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading basidiomycetes. Chemosphere 42, 271-276.

Wariishi, H., Dunford, H. B., MacDonald, I. D. and Gold, M. H., 1989. Manganese peroxidase from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J. Biol. Chem. 264, 3336-3340.

Wariishi, H., Valli, K. and Gold, M. H., 1992. Manganese (II) oxidation by manganese peroxidase from basidiomycete *Phanerochaete chrysosporium*, kinetic mechanism and role of chelators. J. Biol. Chem. 267, 23688-23695.

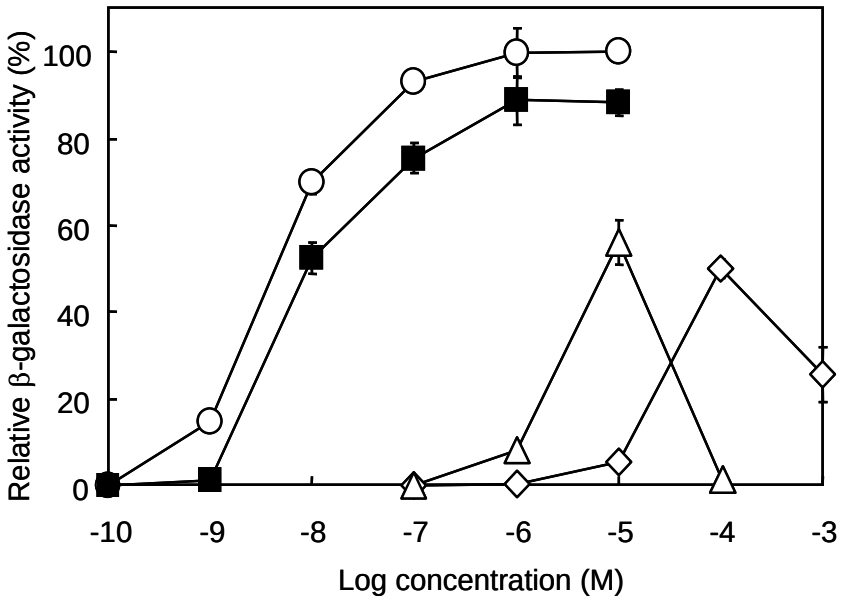

Fig. 1. Dose response curve for estrogenic activity as measured by yeast two-hybrid assay. Activity of E_2 at 10^{-5}M is 100% standard of relative activity. Indicated for each point are the mean and standard deviation of five experiments for E_1, E_2, NP, and BPA. (■), E_1; (○), E_2; (△), NP; (◇), BPA.

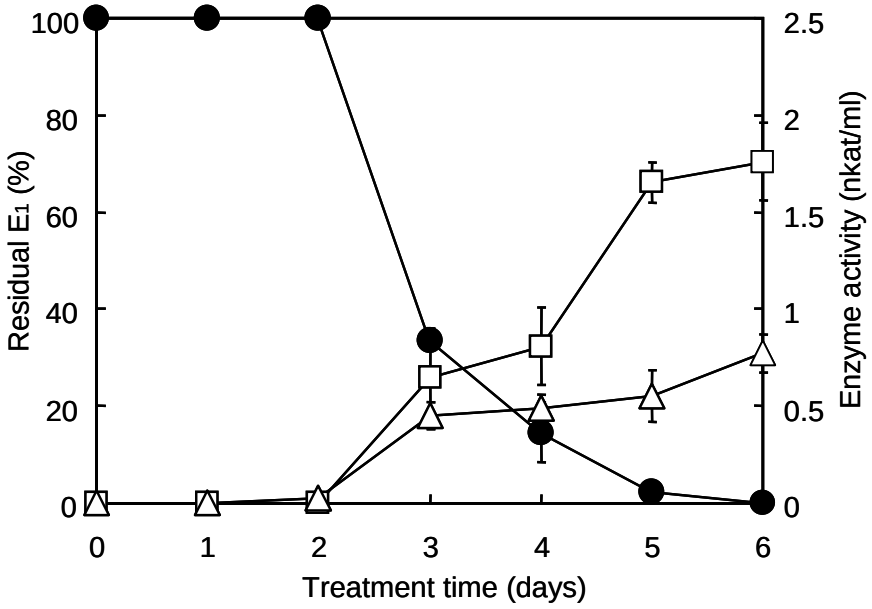

Fig. 2. Treatment of E_1 with *P. sordida* YK-624. Indicated for each point are the mean and standard deviation of triplicate cultures. (●), Residual E_1; (□), MnP activity; (△), laccase activity.

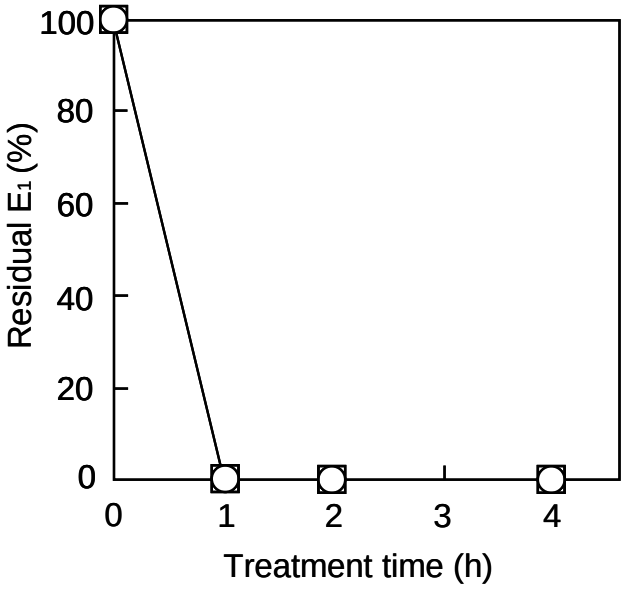

Fig. 3. Decrease in E_1 concentration by enzymatic treatment with MnP or laccase. (○), MnP; (□), laccase.

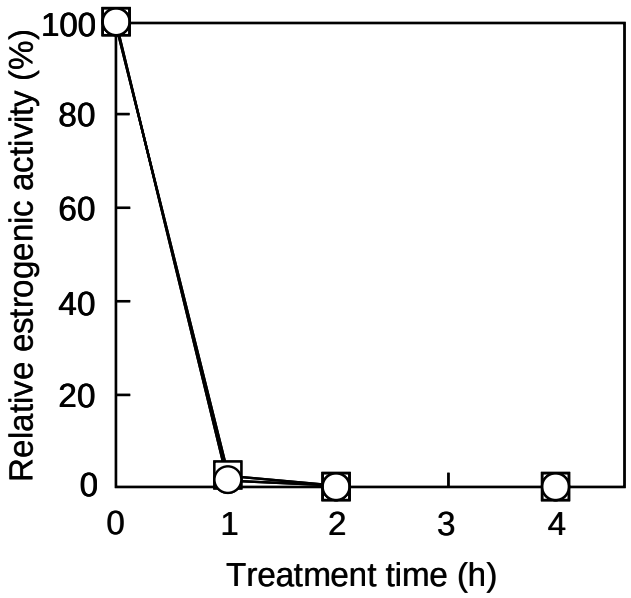

Fig. 4. Removal of estrogenic activity of E_1 by enzymatic treatment with MnP or laccase. (○), MnP; (□), laccase.