

Hydroxylation of bisphenol A by hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under non-ligninolytic condition

著者	Wang Jianqiao, Yamamoto Ryoko, Yamamoto Yotaro, Tokumoto Toshinobu, Dong Jing, Thomas Peter, Hirai Hirofumi, Kawagishi Hirokazu
journal or publication title	Chemosphere
volume	93
number	7
page range	1419-1423
year	2013-10
出版者	Elsevier
権利	Copyright (C) 2013 Elsevier Ltd. Published by Elsevier Ltd. All rights reserved.
URL	http://hdl.handle.net/10297/7430

doi: 10.1016/j.chemosphere.2013.07.026

Technical Note

**Hydroxylation of bisphenol A by hyper lignin-degrading fungus *Phanerochaete sordida*
YK-624 under non-ligninolytic condition**

Jianqiao Wang^a, Ryoko Yamamoto^b, Yotaro Yamamoto^c, Toshinobu Tokumoto^a, Jing Dong^d,
Peter Thomas^d, Hirofumi Hirai^{c, e*}, Hirokazu Kawagishi^{a, c, e}

^a Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^b Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^c Graduate School of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^d Marine Science Institute, University of Texas at Austin, 750 Channel View Drive, Port Aransas, TX 78373, USA

^e Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

Key words: *Phanerochaete sordida* YK-624; Bisphenol A; Hydroxylation; Cytochrome P450

* Corresponding author: Hirofumi Hirai

Mailing address: Graduate School of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan.

Tel. & Fax: +81 54 238 4853

E-mail address: ahhirai@ipc.shizuoka.ac.jp

ABSTRACT

1 Bisphenol A (BPA) is one of the representative compounds of the endocrine disrupting
2 compounds group and the highest volume chemicals produced worldwide. As a result, BPA is
3 often detected in many soil and water environments. In this study, we demonstrated the
4 transformation of BPA from liquid cultures inoculated with hyper lignin-degrading fungus
5 *Phanerochaete sordida* YK-624. Under non-ligninolytic conditions, approximately 80% of
6 BPA was eliminated after 7 d of incubation. High-resolution electrospray ionization mass
7 spectra and nuclear magnetic resonance analyses of a metabolite isolated from the culture
8 supernatant suggested that BPA was metabolized to hydroxy-BPA,
9 4-(2-(4-hydroxyphenyl)propan-2-yl)benzene-1,2-diol, which has a much lower estrogenic
10 activity than BPA. In addition, we investigated the effect of the cytochrome P450 inhibitor
11 piperonyl butoxide (PB) on the hydroxylation of BPA, markedly lower transformation activity
12 of BPA was observed in cultures containing PB. These results suggest that cytochrome P450
13 plays an important role in the hydroxylation of BPA by *P. sordida* YK-624 under
14 non-ligninolytic conditions.

1. Introduction

15 The occurrence of endocrine disrupting compounds (EDCs) in the aquatic environment
16 has generated worldwide interest because these chemicals can cause feminization of fish as
17 well as interfere with the reproduction and development of other aquatic organisms (Purdom
18 et al., 1994; Harries et al., 1996; Larsson et al., 1999). One of the representative compounds
19 of the EDCs group is bisphenol A (2,2-bis(4-hydroxyphenyl)propane; BPA). BPA, a
20 monomer component of polycarbonate plastics, is used in many consumer products, including
21 lacquers applied as food-can linings and dental composite fillings and sealants (Brotons et al.,
22 1995; Olea et al., 1996). Many countries throughout the world have large production
23 capacities for BPA, especially Germany, the Netherlands, the USA and Japan. BPA is one of
24 the highest volume chemicals produced worldwide, with an estimated production of 5 Mt in
25 2010 (Ballesteros-Gómez et al., 2009). As a result, BPA has been detected often in many soil
26 and water environments (Kitada et al., 2008; Matsumura et al., 2009).

27 Lignin-degrading white-rot fungi, which have the unique ability to degrade lignin to the
28 level of CO₂ (Kirk and Farrell, 1987), and their ligninolytic enzymes have also attracted
29 interest for the biotransformation of contaminants because of their industrial potential for
30 degrading recalcitrant environmental pollutants, such as polychlorinated dibenzodioxin
31 (Kamei et al., 2005), lindene (Bumpus et al., 1985), chlorophenols (Joshi and Gold, 1993),
32 mycotoxin aflatoxin B₁ (Wang et al., 2011), and acetamiprid as a neonicotinoid insecticide
33 (Wang et al., 2012a).

34 The degradation of EDCs by ligninolytic enzymes has attracted the attention of many
35 researchers (Hirano et al., 2000; Suzuki et al., 2003; Saito et al., 2004; Tamagawa et al., 2006).
36 The degradation of BPA using fungi is also reported (Chai et al., 2005; Shin et al., 2007) but
37 the detail mechanisms are still unknown. More recently, we have reported that the removal of
38 BPA by lignin peroxidase from hyper lignin-degrading fungus *Phanerochaete sordida*
39 YK-624 (Wang et al., 2012b) and *P. sordida* YK-624 under ligninolytic condition (Wang et
40 al., 2013). In our previous reports, BPA dimers have been formed as metabolites by radical
41 couplings of BPA phenoxy radical generated by ligninolytic enzymes. Unfortunately, these
42 BPA dimers are possible to be decomposed to toxic BPA by various reactions. Therefore, the
43 transformation techniques of BPA without polymerization are necessary. In the present study,
44 we examined the removal of BPA by *P. sordida* YK-624 under non-ligninolytic condition
45 which hardly oxidize BPA to BPA phenoxy radical. We also detected the metabolite from
46 BPA and proposed the metabolic pathway of BPA by *P. sordida* YK-624.

2. Materials and methods

47 2.1. Fungi and chemicals

48 *P. sordida* YK-624 (ATCC 90872), which has been isolated from rotted wood, was
49 used in the present study (Hirai et al., 1994). The fungus was maintained on potato dextrose
50 agar (PDA) slants at 4 °C.

51 BPA was purchased from Alfa Aesar, A Johnson Matthey (Lancashire, United
52 Kingdom). All other chemicals were analytical pure grade and were used without further
53 purification.

54 2.2. Transformation of BPA

55 Potato dextrose broth (PDB) medium which is a non-lignolytic (no production of
56 lignolytic enzymes) condition was used for BPA transformation experiments. The fungus was
57 incubated on a PDA plate at 30 °C for 3 d, and then 10 mm-diameter disks were punched out
58 from the growing edge of mycelium. Two disks were each placed into a 100-mL Erlenmeyer
59 flask containing 10 mL of PDB medium. After statically incubating the flasks at 30 °C for 3 d,
60 100 µL of 100 mM BPA/dimethyl sulfoxide were added to the cultures (final concentration at
61 1 mM), and then further incubated for 0-7 d (each in triplicate). The internal standard material
62 was added to the culture, and then the culture was homogenized with 20 mL of acetone by
63 using a Polytron PT1200E (Kinematica, Canada). The homogenate was filtrated and then
64 evaporated to dryness. The residue was analyzed by HPLC for the quantification of BPA
65 under the following conditions: column, Wakosil-II 5C18HG (4.6×150 mm; Wako Pure
66 Chemical Industries, Japan); mobile phase, 10 to 100% MeOH gradient for 60 min; flow rate,
67 1 mL min⁻¹; and UV wavelength, 277 nm.

68 2.3. Metabolism experiment of BPA

69 Inoculated cultures of *P. sordida* YK-624 were prepared under the PDB medium. After
70 static incubation at 30 °C for 3 d, BPA (final concentration, 1 mM) was added to these
71 cultures. These cultures were further incubated for 7 d and then filtrated with membrane
72 filters (pore size; 0.2 µm). The resulting solution was extracted 3 times with equal amount of
73 ethyl acetate (EtOAc). The EtOAc extract was dried over anhydrous sodium sulfate and then
74 evaporated to dryness. The residue was cleaned on a thin-layer chromatography (TLC, glass
75 plates) to obtain 3 fractions. Each fraction was analyzed by TLC (aluminum sheets). Silica gel
76 plates (Merck F₂₅₄; Merck, Darmstadt, Germany) were used for TLC. The metabolite was
77 further separated by HPLC (column: CAPCELL PAK C18 AQ; 20×250 mm, Shiseido,

78 Japan) using 10 to 100% MeOH gradient. The purified metabolite was analyzed by
79 HR-ESI-MS and NMR, including distortionless enhancement by polarization transfer,
80 correlated spectroscopy, heteronuclear multi quantum correlation, and heteronuclear multiple
81 bond correlation (HMBC) experiments, respectively. The HR-ESI-MS data were measured
82 using a JMS-T100LC mass spectrometer. ¹H-NMR spectra were recorded using a Jeol
83 Lambda-500 spectrometer at 500 MHz, while ¹³C-NMR spectra were recorded on the same
84 instrument at 125 MHz.

85 2.4. Cytochrome P450 inhibitor experiment

86 After preculturing *P. sordida* YK-624 for 3 d, BPA (final concentration at 1 mM) and
87 the cytochrome P450 inhibitor piperonyl butoxide (PB, final concentration, 0, 0.1 and 1 mM)
88 were added to cultures. The cultures were further incubated for 1-4 d, and each culture was
89 then subjected to HPLC for the quantification of BPA, as described above.

90 2.5. Competition assay using membrane estrogen receptor

91 Culture of HEK231 cells were stably transfected with membrane estrogen receptor
92 (GPR30) and estrogen receptor competition studies were carried out as described before
93 (Thomas and Dong, 2006). Briefly, human HEK293 cells stably transfected with human
94 GPR30 were cultured in dulbecco's modification of eagle's medium/Ham's F-12 medium with
95 5% fetal bovine serum and 100 µg mL⁻¹ of G418 in 150 mm diameter plates. The medium
96 was replaced every 1-2 d and the cells which were reached 80% confluence after 3 d were
97 used in the experiments.

98 Procedures for the preparation of plasma membrane fractions and assay of steroid
99 membrane receptors were followed with few modifications for measurement of [³H] estradiol
100 (E₂) binding to plasma membranes of HEK293 cells transfected with goldfish mPRα
101 (Tokumoto et al., 2007) except that presoaking with Tween 80 was omitted. As a
102 radio-labeled steroid [2, 4, 6, 7-³H]E₂ ([³H]E₂, ~89 Ci mmol⁻¹) was purchased from
103 PerkinElmer Life & Analytical Sciences (Waltham, MA). The competitive binding assay
104 tubes contained 4 nM [³H]E₂ and the competitors (concentration range: 1 nM to 10 µM).
105 After a 30 min incubation at 4 °C with the membrane fractions, the reaction was stopped by
106 filtration (Whatman GF/B filters), the filters were washed and bound radioactivity measured
107 by scintillation counting. The displacement of [³H]E₂ binding by the competitors was
108 expressed as a percentage of the maximum specific binding of E₂.

3. Results

109 *3.1. Transformation of BPA by P. sordida YK-624 under non-ligninolytic condition*

110 In the present study, PDB medium was used for the transformation experiment of BPA.
111 When *P. sordida* YK-624 was cultured in PDB medium, BPA was decreased by 51, 71, 83%
112 after 1, 4, and 7 d of incubation, respectively (Fig. 1a). These results suggested that *P. sordida*
113 YK-624 had the ability for the transformation of BPA under the non-ligninolytic conditions
114 such as PDB medium.

115 *3.2. Identification of the metabolite from BPA*

116 To determine the metabolite(s) produced during the degradation of BPA, 7-d cultures of
117 *P. sordida* YK-624 in PDB medium was supplemented with 0.5 mM BPA were subjected to
118 TLC and HPLC. From this analysis, one potential BPA metabolite was detected (data not
119 shown). The purified metabolite was then subjected to high-resolution electrospray ionization
120 mass spectrometry analysis, which yielded a molecular ion at m/z 243.1015 [M-H]⁻
121 (calculated for C₁₅H₁₅O₃, 243.1021), indicating that the molecular formula of this compound
122 was C₁₅H₁₆O₃. This formula suggested that the metabolite might be a hydroxylation form of
123 BPA. The structure of the purified metabolite was further characterized by NMR analyses.
124 Table 1 lists the chemical-shift assignment data for the metabolite of BPA. HMBC (Fig. 2)
125 (H-3/C-2', H-5/C-2') confirmed that the metabolite was
126 4-(2-(4-hydroxyphenyl)propan-2-yl)benzene-1,2-diol, which hydroxylated at the
127 *ortho*-position of BPA.

128 *3.3. Effect of cytochrome P450 inhibitor*

129 The effect of the cytochrome P450 inhibitor on the transformation of BPA by *P. sordida*
130 YK-624 was investigated using PB. The transformation rate of BPA after the addition of 0.1
131 and 1 mM PB into cultures of *P. sordida* YK-624 is shown in Fig. 1b. In contrast to the
132 transformation rate of BPA in cultures without PB, markedly lower transformation activity of
133 BPA was observed in cultures containing PB. In the presence of 0.1 and 1 mM PB, 63 and 38%
134 of BPA were eliminated after 4 d of incubation, respectively, compared to 70% in cultures
135 lacking PB.

136 *3.4. Steroid binding assay of BPA and the metabolite*

137 To evaluate the estrogenic activity of BPA metabolite, we used the steroid binding assay
138 in the present study. BPA showed significant competition with IC₅₀ of 1.8 μM. Just a few
139 affinities for binding to the membrane estrogen receptor were detected in hydroxy-BPA. These

140 results demonstrated that hydroxylation of BPA by *P. sordida* YK-624 causes the 67% loss of
141 estrogenic activity of BPA (Fig. 3). In other words hydroxylation of BPA by *P. sordida*
142 YK-624 is sufficient for diminish possible environmental estrogenic activity of BPA.

4. Discussion

143 Biotransformation of BPA has also been reported by many researchers. More recently,
144 we reported the removal of BPA by *P. sordida* YK-624 under ligninolytic condition (Wang et
145 al., 2013). BPA dimers are formed by radical couplings at extracellular region without the
146 incorporation into the cell of *P. sordida* YK-624. However, BPA dimers may be returned to
147 BPA by various reactions. BPA glucuronide is the major metabolite of BPA in rat hepatocytes
148 (Nakagawa and Tayama, 2000; Pritchett et al., 2002). In plants, cells of *Eucalyptus*
149 *perriniana* and *Portulaca oleracea* cv. metabolize BPA to its hydroxyl products (Hamada et
150 al., 2002; Watanabe et al., 2012). In fungi, BPA is metabolized to
151 BPA-*O*- β -D-glucopyranoside by *Aspergillus fumigatus* (Yim et al., 2003). In the present study,
152 we detected a hydroxy-BPA as a metabolite of BPA.

153 Watanabe et al. (2012) suggested polyphenol oxidases were likely to contribute to BPA
154 hydroxylation by portulaca. However, *P. sordida* YK-624 produces lignin peroxidases and
155 manganese peroxidase as ligninolytic enzymes but not laccase. The functional diversity of
156 cytochrome P450s in white-rot fungi has been studied because cytochrome P450s play an
157 important role on degrading a wide variety of recalcitrant aromatic compounds (Hiratsuka et
158 al. 2001; Xiao et al., 2011; Wang et al., 2012a). Recently, Hata et al. (2010) suggested that
159 hydroxylation catalyzed by cytochrome P450 in *P. sordida* YK-624 might be involved in the
160 elimination and detoxification of diclofenac and mefenamic acid. Our present study showed
161 that the transformation of BPA was efficiently inhibited by the addition of PB (Fig. 1b), which
162 is a common inhibitor of cytochrome P450 and is often used for demonstrating whether a
163 reaction is catalyzed by cytochrome P450 enzymes (Mori and Kondo, 2002; Mori et al., 2003;
164 Kamei et al., 2005). Since hydroxylation of BPA was inhibited by PB, we propose that
165 cytochrome P450s are involved in the hydroxylation of BPA by *P. sordida* YK-624 under
166 non-ligninolytic condition. We have described for the first time the hydroxylation of BPA by a
167 white-rot fungus.

168 Due to the high production volumes, the toxicity of BPA has been intensively studied
169 since the 1970s. Estrogenic activity of BPA was first reported in 1993 (Krishnan et al., 1993).
170 We used the steroid binding assay to evaluate the estrogenic activity of BPA and BPA
171 metabolite in the present study. BPA showed significant competition with IC₅₀ of 1.8 μ M. Just

172 a few affinities for binding to the membrane estrogen receptor were detected in hydroxy-BPA
173 (Fig. 3). Since the metabolite hydroxy-BPA has lower estrogenic toxicity than BPA,
174 diminishing environmental estrogenic activity of BPA is possible using *P. sordida* YK-624.

175 The findings of the present study confirm that *P. sordida* YK-624 can remove the
176 estrogenic activity of BPA by converting to hydroxy-BPA. In the next study, we will
177 investigate whether hydroxy-BPA could be further metabolized by *P. sordida* YK-624 for
178 complete removal of estrogenic activity.

179

180 **5. Acknowledgement**

181 This work was partially supported by Grants-in-Aid for Scientific Research (No.
182 21780296) from the Ministry of Education, Culture, Sports, Science and Technology of
183 Japan.

184 **6. References**

184 Ballesteros-Gómez, A., Rubio, S., Pérez-Bendito, D., 2009. Analytical methods for the
185 determination of bisphenol A in food. *J. Chromatogr. A* 1216, 449-469.

186 Brotons, J.A., Olea-Serrano, M.F., Villalobos, M., Pedraza, V., Olea, N., 1995.
187 Xenoestrogens released from lacquer coatings in food cans. *Environ. Health Persp.* 103,
188 608-612.

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

191 Chai, W., Handa, Y., Suzuki, M., Saito, M., Kato, N., Horiuchi, C.A., 2005.
192 Biodegradation of bisphenol A by fungi. *Appl. Biochem. Biotechnol.* 120, 175-182.

193 Hamada, H., Tomi, R., Asada, Y., Furuya, T., 2002. Phytoremediation of bisphenol A by
194 cultured suspension cells of *Eucalyptus perriniana*-regioselective hydroxylation and
195 glycosylation. *Tetrahedron Lett.* 43, 4087-4089.

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

199 Hata, T., Kawai, S., Okamura, H., Nishida, T., 2010. Removal of diclofenac and
200 mefenamic acid by the white rot fungus *Phanerochaete sordida* YK-624 and identification of
201 their metabolites after fungal transformation. *Biodegradation* 21, 681-689.

202 Hirai, H., Kondo, R., Sakai, K., 1994. Screening of lignin-degrading fungi and their
203 ligninolytic enzyme activities during biological bleaching of kraft pulp. *Mokuzai Gakkaishi*
204 40, 980-986.

205 Hirano, T., Honda, Y., Watanabe, T., Kuwahara, M., 2000. Degradation of bisphenol A by
206 the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot
207 basidiomycete, *Pleurotus ostreatus*. *Biosci. Biotechnol. Biochem.* 64, 1958-1962.

208 Hiratsuka, N., Wariishi, H., Tanaka, H., 2001. Degradation of diphenyl ether herbicides
209 by the lignin-degrading basidiomycete *Coriolus versicolor*. *Appl. Microbiol. Biotechnol.* 57,
210 563-571.

211 Joshi, D.K., Gold, M.H., 1993. Degradation of 2,4,5-trichlorophenol by the
212 lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* 59,
213 1779-1785.

214 Kamei, I., Sahara, H., Kondo, R., 2005. Phylogenetical approach to isolation of white-rot
215 fungi capable of degrading polychlorinated dibenzo-*p*-dioxin. *Appl. Microbiol. Biotechnol.*
216 69, 358-366.

217 Kirk, T.K., Farrell, R.L., 1987. Enzymatic combustion: the microbial degradation of
218 lignin. *Annu. Rev. Microbiol.* 41, 465-505.

219 Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L., Feldman, D., 1993. Bisphenol-A: an
220 estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology*
221 132, 2279-2286.

222 Kitada, Y., Kawahata, H., Suzuki, A., Oomori, T., 2008. Distribution of pesticides and
223 bisphenol A in sediments collected from rivers adjacent to coral reefs. *Chemosphere* 71,
224 2082-2090.

225 Larsson, D.G.J., Adolfsson-Erici, M., Parkkonen, J., Pettersson, M., Berg, A.H., Olsson,
226 P., Forlin, L., 1999. Ethinylloestradiol - an undesired fish contraceptive? *Aquat. Toxicol.* 45,
227 91-97.

228 Matsumura, Y., Hosokawa, C., Sasaki-Mori, M., Akahira, A., Fukunaga, K., Ikeuchi, T.,
229 Oshiman, K., Tsuchido, T., 2009. Isolation and characterization of novel
230 bisphenol-A--degrading bacteria from soils. *Biocontrol Sci.* 14, 161-169.

231 Mori, T., Kondo, R., 2002. Oxidation of chlorinated dibenzo-*p*-dioxin and dibenzofuran
232 by white-rot fungus, *Phlebia lindtneri*. *FEMS Microbiol. Lett.* 216, 223-227.

233 Mori, T., Kitano, S., Kondo, R., 2003. Biodegradation of chloronaphthalenes and
234 polycyclic aromatic hydrocarbons by the white-rot fungus *Phlebia lindtneri*. *Appl. Microbiol.*
235 *Biotechnol.* 61, 380-383.

236 Nakagawa, Y., Tayama, S., 2000. Metabolism and cytotoxicity of bisphenol A and other
237 bisphenols in isolated rat hepatocytes. *Arch. Toxicol.* 74, 99-105.

238 Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza,

239 V., Soto, A.M., Sonnenschein, C., 1996. Estrogenicity of resin-based composites and sealants
240 used in dentistry. *Environ. Health Persp.* 104, 298-305.

241 Pritchett, J.J., Kuester, R.K., Sipes, I.G., 2002. Metabolism of bisphenol a in primary
242 cultured hepatocytes from mice, rats, and humans. *Drug Metab. Dispos.* 30, 1180-1185.

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

245 Saito, T., Kato, K., Yokogawa, Y., Nishida, M., Yamashita, N., 2004. Detoxification of
246 bisphenol A and nonylphenol by purified extracellular laccase from a fungus isolated from
247 soil. *J. Biosci. Bioeng.* 98, 64-66.

248 Shin, E.H., Choi, H.T. Song, H.G., 2007. Biodegradation of endocrine-disrupting
249 bisphenol A by white rot fungus *Irpex lacteus*. *J. Microbiol. Biotechnol.* 17, 1147-1151.

250 Suzuki, K., Hirai, H., Murata, H., Nishida, T., 2003. Removal of estrogenic activities of
251 17 β -estradiol and ethinylestradiol by ligninolytic enzymes from white rot fungi. *Water Res.*
252 37, 1972-1975.

253 Tamagawa, Y., Yamaki, R., Hirai, H., Kawai, S., Nishida, T., 2006. Removal of
254 estrogenic activity of natural steroidal hormone estrone by ligninolytic enzymes from white
255 rot fungi. *Chemosphere* 65, 97-101.

256 Thomas, P., Dong, J., 2006. Binding and activation of the seven-transmembrane estrogen
257 receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine
258 disruption. *J. Steroid. Biochem.* 102, 175-179.

259 Tokumoto, T., Tokumoto, M., Thomas P., 2007. Interactions of diethylstilbestrol (DES)
260 and DES analogs with membrane progestin receptor-alpha and the correlation with their
261 nongenomic progestin activities. *Endocrinology* 148, 3459-3467.

262 Wang, J., Hirai, H., Kawagishi, H., 2012a. Biotransformation of acetamiprid by the
263 white-rot fungus *Phanerochaete sordida* YK-624. *Appl. Microbiol. Biotechnol.* 93, 831-835.

264 Wang, J., Majima, N., Hirai, H., Kawagishi, H., 2012b. Effective removal of
265 endocrine-disrupting compounds by lignin peroxidase from the white-rot fungus
266 *Phanerochaete sordida* YK-624. *Curr. Microbiol.* 64, 300-303.

267 Wang, J., Ogata, M., Hirai, H., Kawagishi, H., 2011. Detoxification of aflatoxin B₁ by
268 manganese peroxidase from the white-rot fungus *Phanerochaete sordida* YK-624. *FEMS*
269 *Microbiol. Lett.* 314, 164-169.

270 Wang, J., Yamamoto, Y., Hirai, H., Kawagishi, H., 2013. Dimerization of bisphenol A by
271 hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under ligninolytic condition.
272 *Curr. Microbiol.* 66, 544-547.

273 Watanabe, I., Harada, K., Matsui, T., Miyasaka, H., Okuhata, H., Tanaka, S., Nakayama,
274 H., Kato, K., Bamba, T., Hirata, K., 2012. Characterization of bisphenol A metabolites
275 produced by *Portulaca oleracea* cv. by liquid chromatography coupled with tandem mass
276 spectrometry. Biosci. Biotechnol. Biochem. 76, 1015-1017.

277 Xiao, P., Mori, T., Kamei, I., Kondo, R., 2011. A novel metabolic pathway for
278 biodegradation of DDT by the white rot fungi, *Phlebia lindtneri* and *Phlebia brevispora*.
279 Biodegradation 22, 859-867.

280 Yim, S.H., Kim, H.J., Lee, I.S., 2003. Microbial metabolism of the environmental
281 estrogen bisphenol A. Arch. Pharm. Res. 26, 805-808.

282

283 Fig. 1 Time course for BPA transformation by *P. sordida* YK-624 under non-ligninolytic
284 condition (a) and effect of cytochrome P450 inhibitor PB on the transformation of BPA by *P.*
285 *sordida* YK-624 (b). ● without PB, ▲ 0.1 mM PB, ■ 1 mM PB. Values are the means \pm
286 SD of triplicate samples.

287

288 Fig. 2 COSY and HMBC correlations for the identified BPA metabolite.

289

290 Fig. 3 Competition by BPA and hydroxy-BPA for binding to the membrane estrogen
291 receptor. Samples were incubated with 4 nM [³H]E2 and 10 μ M competitor. Competition rate
292 (%) is represented as relative value of the displacement of [³H]E2 binding by BPA set as
293 100 %.

294

295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338

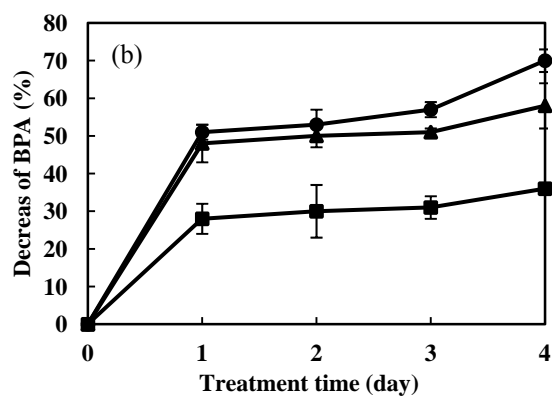
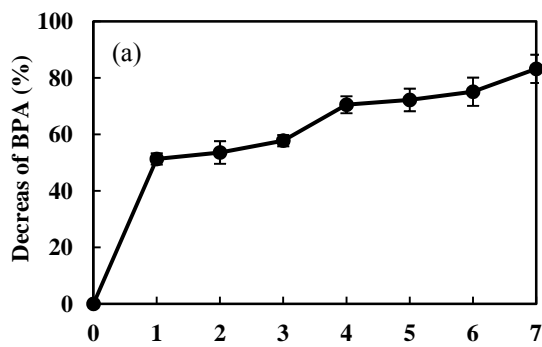
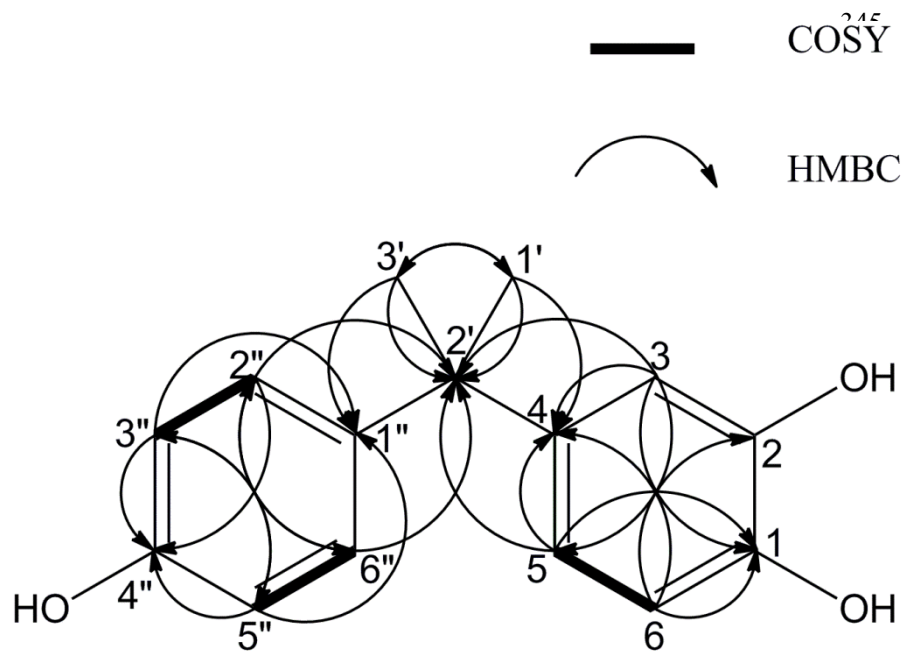


Fig. 1

339
340
341
342
343
344

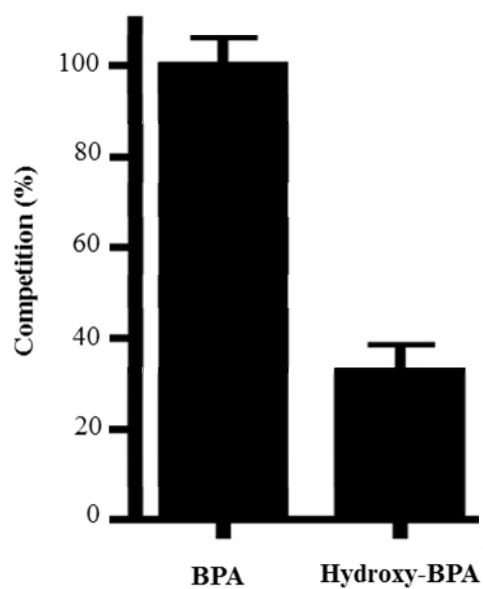


361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382

4-(2-(4-hydroxyphenyl)propan-2-yl)benzene-1,2-diol

Fig. 2

383
384
385
386
387
388
389
390
391
392
393
394
395
396



397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412

Fig. 3

413
414
415
416
417
418
419
420
421
422
423

Table 1 ^1H - and ^{13}C -NMR data for BPA metabolite (in CD_3OD)

Position	^1H	^{13}C
	δ_{H} (mult, J in Hz)	d
1	-	142.3
2	-	142.9
3	6.61 (d, 2.2)	114.1
4	-	141.9
5	6.56 (dd, 7.9, 2.2)	117.3
6	6.64 (d, 7.9)	113.9
1''	-	143.9
2''	7.02 (d, 9.0)	127.2
3''	6.65 (d, 8.5)	113.9
4''	-	154.3
5''	6.65 (d, 8.5)	113.9
6''	7.02 (d, 9.0)	127.2
1'-CH ₃	1.54 (s)	30.1
2'	-	41.0
3'-CH ₃	1.54 (s)	30.0