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Biodegradation of Bisphenol A by Fungi

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Abstract

The biologic degradation of 2,2-*bis*(4-hydroxyphenyl)propane (bisphenol A [BPA]; **1**) was studied with 26 fungi. An initial BPA concentration of 40 ppm in an aqueous solution was degraded in the dark for 14 d. Among the 26 strains tested, 11 degraded BPA at 50% or more. Furthermore, four strains (*F. sporotrichioides* NFRI-1012, *F. moniliforme* 2-2, *A. terreus* MT-13, and *E. nidulans* MT-98) were more effective for degradation of BPA.

Index Entries: Bisphenol A; endocrine disrupter; fungi; biologic degradation; spores.

Introduction

2,2-*Bis*(4-hydroxyphenyl)propane (bisphenol A [BPA]; **1**) is generally used as a starting material for polymers including polycarbonates, epoxy resins, phenol resins, polyesters, and polyacrylates. This compound is commonly suspected to act as an endocrine disrupter *(1)*. Degradation of BPA has been extensively studied *(2–10)*, but such knowledge on fungi is limited. Some examples of the biologic degradation of BPA by bacteria *(2,3)* and basidiomycetes *(4)* have recently been reported. However, these biologic methods to eliminate the pollutant in an aqueous solution cannot completely decompose the total organic carbon. Hamada et al. *(11)* reported the phytoremediation of BPA by plant cultured cells of *Eucalyptus perriniana*. In a previous study, we found that BPA was degraded by cultured cells of *Caragana chamlagu* for 2 d in aqueous solutions, and two

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intermediates were then completely dissipated after 10 d *(12)*. We report here the total degradation of BPA in an aqueous solution by using some soil fungi in the biocatalytic reaction.

Materials and Methods

The 26 isolated fungi tested in the present study are given in Table 1. The fungi under the NFRI and IFO numbers were the stock cultures of the National Food Research Institute (Tsukuba, Japan) and the Institute for Fermentation (Osaka, Japan), respectively. *Aspergillus terreus* MT-13, *Aspergillus ustus* MT-3, *Aspergillus ustus* MT-23, and *Emericella nidulans* MT-78 were isolated from soil samples from Miyakojima, Okinawa, Japan. *Aspergillus* sp. MT-41, *Trichoderma viride* MT-6, and *Trichoderma viride* MT-40 were isolated from soil samples from Taramajima, Okinawa, Japan. *Fusarium moniliforme* 2-2 and *Trichoderma viride* PHF-2 were isolated from soil samples from Tsukuba, Japan. *Aspergillus sydowii* KF-17, *Fusarium* sp. KOM-191, and *Curvularia lunata* FUTA-N were isolated from rice grains. *Penicillium* sp. NIN-1 was isolated from garlic bulb.

BPA (GC grade >99%) was purchased from Tokyo Kasei (Tokyo, Japan). Czapek yeast-extract (CY) liquid medium or Czapek yeast-extract agar (CYA) medium was used for the growth of fungi. The CY medium contained per liter of distilled water: 3.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.01 g of $ZnSO_4 \cdot 7H_2O$, 0.005 g of $CuSO_4 \cdot 5H_2O$, 5.0 g of yeast extract, and 30.0 g of sucrose. For CYA medium, agar was added to CY medium at a concentration of 1.5%. Then, the media were sterilized at 125°C for 15 min. In a cube, the strains were grown on CY or CYA medium for 5 d at 25°C in the dark without BPA.

For the BPA-degrading microorganism, Czapek (CZ) liquid medium was used. The CZ medium contained per liter of distilled water: 3.0 g of $NaNO_3$, 1.0 g of K_2PO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $ZnSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, and 0.005 g of $CuSO_4 \cdot 5H_2O$. At this stage, the CZ liquid medium did not use sucrose and utilized the BPA as a sole source of carbon. After incubating in CYA or CY medium, the strains were inoculated in CZ medium (50 mL in 300-mL Erlenmeyer flasks) with 2 mg of BPA. In this step, the suspension medium was incubated by three methods to check the BPA degradation efficiency. In method A, the fungal spores that were precultivated in CYA medium were added directly by platinum loop, and then the suspension medium was incubated with standing at 25°C in the dark for degradation of BPA. In method B, the incubated fungal spores that were precultivated in CY medium were homogenized with distilled water (60 mL) to disperse the spores, and 2 mL (dry wt: 12 mg) of the suspension solution was added to the CZ medium. Then, the suspension medium was incubated with standing at 25°C in the dark similarly to method A. In method C, the fungal spores were added to the CZ medium in the same manner as in method B. To stimulate the spores, the suspension medium was incubated with shaking (120 rpm) at 30°C in the dark.

Table 1
Degradation of BPA Using Fungi[a]

Fungi	Run	Time (d)	Degradation (%)[b]
Aspergillus			
Aspergillus niger NFRI-1540	1	14	—[c]
Aspergillus oryzae NFRI-1571	2	14	26
Aspergillus sydowii KF-17	3	14	44
Aspergillus terreus MT-13	4	14	>99
Aspergillus ustus MT-3	5	14	34
Aspergillus ustus MT-23	6	14	27
Aspergillus sp. MT-41	7	14	46
Fusarium			
Fusarium graminearum NFRI-1280	8	14	78
Fusarium mornilforme 2-2	9	14	>99
Fusarium oxysporum NFRI-1011	10	14	—[c]
Fusarium sporotrichioides NFRI-1012	11	14	>99
Fusarium sp. KOM-191	12	14	95
Penicillium			
Penicillium chrysogenum NFRI-1241	13	14	—[c]
Penicillium citrinum NFRI-1019	14	14	39
Penicillium expansum NFRI-1021	15	14	83
Penicillium frequentans NFRI-1022	16	14	23
Penicillium hirstum IFO-32031	17	14	—[c]
Penicillium sp. NIN-1	18	14	59
Fungi imperfecti			
Curvularia lunata FUTA-N	19	14	41
Paecilomyces lilacinus IFO-31847	20	14	98
Trichoderma viride MT-6	21	14	41
Trichoderma viride MT-40	22	14	57
Trichoderma viride PHF-2	23	14	49
Ascomycetes			
Byssochlamys fuluva NFRI-1226	24	14	68
Emericella nidulans MT-78	25	14	>99
Zygomycetes			
Rhizopus stoonifer NFRI-1030	26	14	25

[a]Reaction conditions: BPA (2 mg), culture medium (50 mL), and fungal spores were incubated with standing at 25°C (method A).
[b]Degradation rate was determined by HPLC peak area.
[c]No growth.

After the indicated incubation period, the spores were removed by filtration, the filtrate was extracted with distilled ether, and the spores were extracted with MeOH. The amount of residual BPA was measured by high-performance liquid chromatography (HPLC) (LC-9A; Shimadzu, Japan) using a reverse-phase column Shim-pack CLC-ODS (6.0 × 150 mm) (Shimadzu). A fluorescence detector (SPD-6A; Shimadzu) was set with

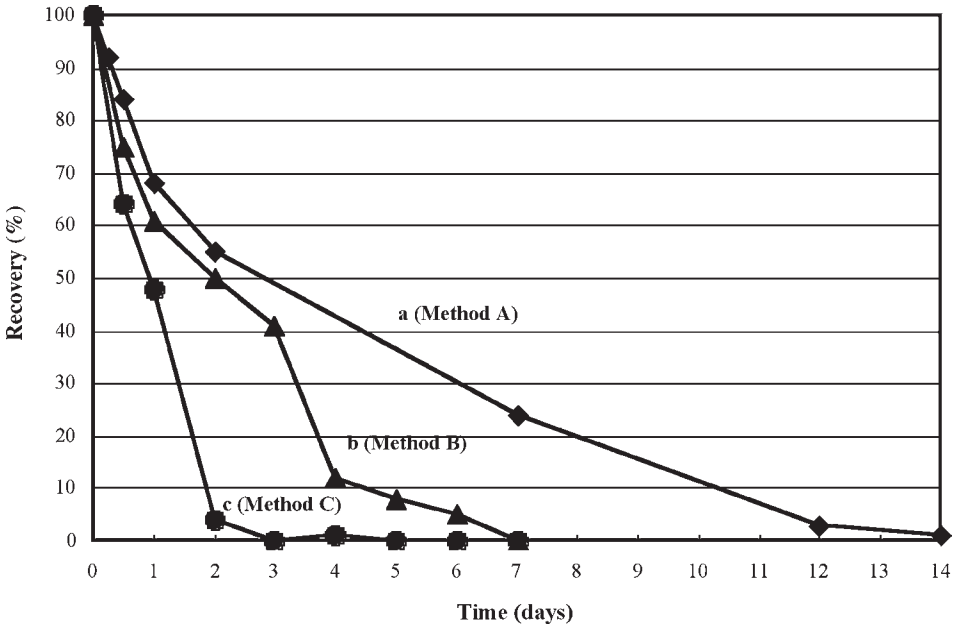

Fig. 1. Time-course reaction of *F. sporotrichioides* NFRI-1012.

excitation at 275 nm. The mobile phase consisted of methanol:water (70:30 [v/v]) and was pumped at a flow rate of 1 mL/min under isocratic conditions. The column temperature was 25°C, and the sample injection volume was 20 µL. The chemical structures of the reaction products were determined by gas chromatography-mass spectroscopy (GC-MS) using a JMS-GCmate II (JEOL, Japan). GC (6890 Series GC system; Agilent) using a DB-1 column (0.32 mm × 30 m) (Agilent) was programmed to raise the oven temperature from 50 to 250°C at 18°C/min, and MS was conducted at 70 eV (electron impact).

Results and Discussion

The activity of the selected fungi in degrading BPA in vivo was investigated. The CZ medium (50 mL) spiked with BPA (2 mg, 40 ppm) was prepared to test the degradation of BPA by 26 strains of soil fungi. Table 1 presents the results of the degradation experiments. Among the 26 strains tested, 22 showed a good growth for this initial concentration of BPA. The values in Table 1 are expressed as a percentage of total degradation of BPA. After 14 d of reaction, these strains were grown to about 60 mg (dry wt). The 22 strains that had good growth had the ability to degrade BPA, and 11 strains degraded BPA at 50% or higher. The highest degradation (>99%) was found in *Aspergillus*, with *A. terreus* MT-13; *Fusarium*, with *F. moniliforme* 2-2 and *F. sporotrichioides* NFRI-1012; and *Ascomycetes*, with *Emericella nidulans* MT-78. It can be seen that *Fusarium* was the most efficient group among the six groups in Table 1.

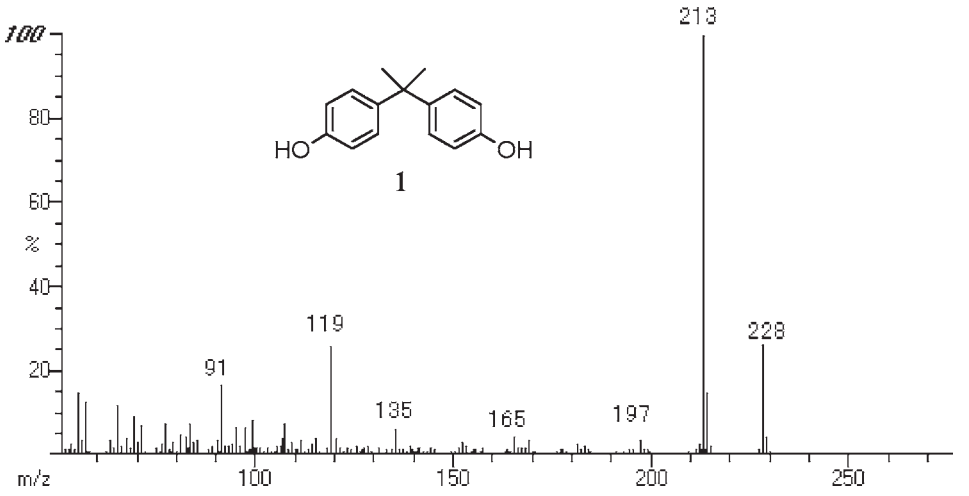

Fig. 2. Spectrum of BPA analyzed by GC-MS.

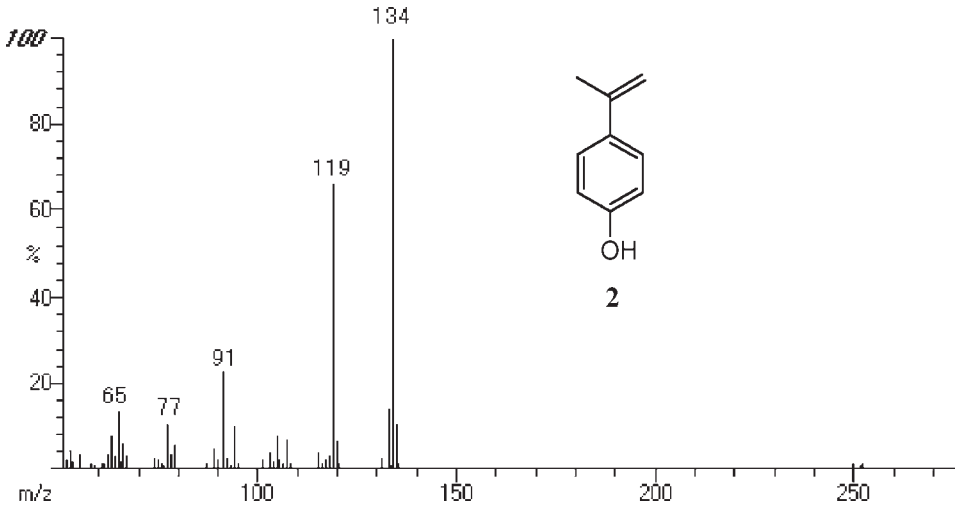

Fig. 3. Degradation product of BPA analyzed by GC-MS.

To research the biodegradation conditions, the time-course degradation of BPA by *F. sporotrichioides* NFRI-1012 was investigated. In Fig. 1, which presents the results of the time-course experiments, curve **a** shows the results by method A. It can be seen that BPA disappeared after 14 d of incubation. Curve **b** shows that BPA disappeared after 7 d by method B. Curve **c** shows that the incubation method of BPA degradation was the most rapid (5 d) by method C.

In *Fusarium*, *F. solani* was used for the biodegradation of dichlorodiphenyltrichloroethane (DDT) *(13)*. In addition, Yue et al. *(14)* reported that *F. moniliforme* could be used to biodegrade 2-benzoxazolinone and

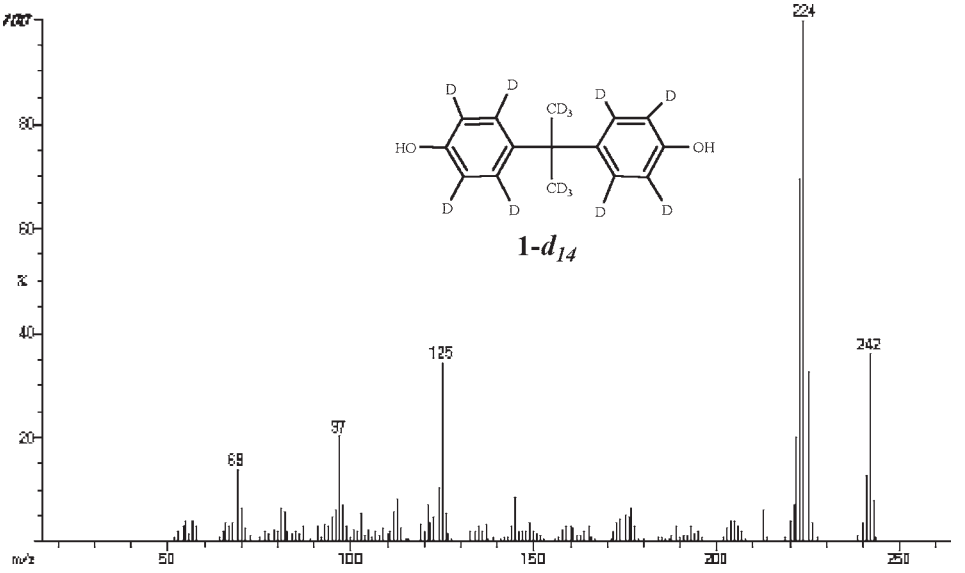

Fig. 4. Spectrum of BPA-d_{14} analyzed by GC-MS.

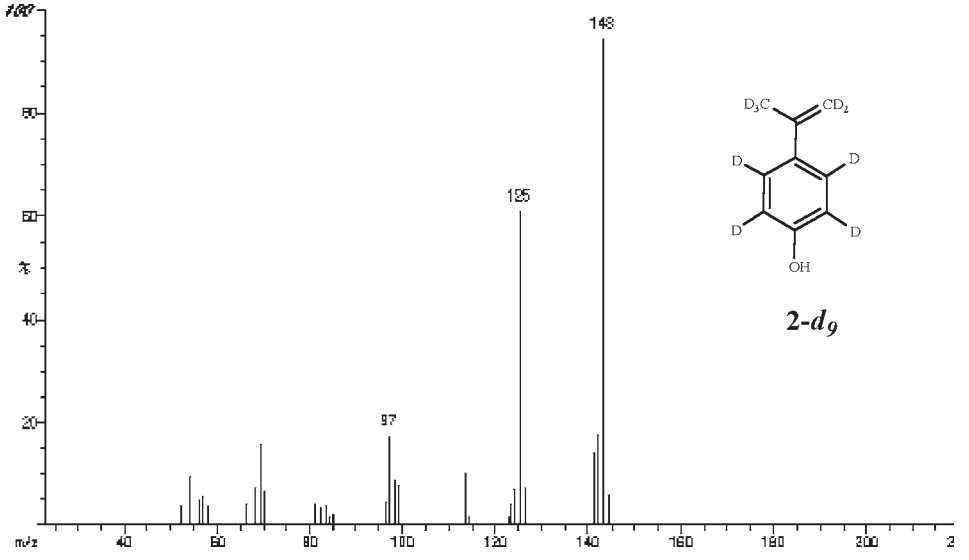

Fig. 5. Degradation product of BPA-d_{14} analyzed by GC-MS.

6-methoxybenzoxazolinone. These findings indicate that oxidative cleavage was promoted by *Fusarium* strains. In our previous work, we reported biodegradation of BPA by plant cultured cells *(12)*. We found that 4-isopropenylphenol and 4-(2-propanol)phenol were obtained as intermediates of degradation products. Furthermore, we suggested that the

biologic degradation of BPA proceeded by oxidation of the superoxide anion radical with dioxygenases, which is similar to the degradation of BPA by TiO_2 photocatalyst *(5)*.

To investigate the degradation pathway of BPA (Fig. 2) by the fungi tested, the products of BPA degradation rate >99% were analyzed by GC-MS. From the spectrum of GC-MS, compound **2** showed the spectral data (Fig. 3) as follows: electron impact mass spectrometry *m/z*: 134 (100) $[M]^+$, 119 (66) $[M\text{-}CH_3]^+$, 91 (24), 77 (10), and 65 (14). The spectra of **2** are in agreement with those of 4-isopropenylphenol. It is considered that the intermediate of the biologic degradation of BPA by strains is 4-isopropenylphenol. To clarify that its origin was not in the cells, the biodegradation of BPA-d_{14} (Fig. 4) was carried out. The results of the GC-MS spectra (Fig. 5) show that the deuteride of 4-isopropenylphenol (**2**-d_9) was obtained as intermediate, proving that **2** was intermediate of the biodegradation of BPA. On the basis of the foregoing results, it is possible that the biologic degradation of BPA using selected fungi proceeds by oxidative cleavage of the C-C bond.

Conclusion

For investigating the activity of BPA biodegradation of fungi, 26 selected fungi were tested. It was found that among these, 11 fungi could degrade BPA (40 mg/L) in liquid CZ medium >50%. This is the first time that a BPA in an aqueous solution has been dissipated by the 11 efficient strains. In particular, four strains (*F. sporotrichioides* NFRI-1012, *F. moniliforme* 2-2, *A. terreus* MT-13, and *E. nidulans* MT-78) were more effective for the degradation of BPA.

Acknowledgments

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References

1. Alexander, H. C., Dill, D. C., Smith, L. W., Guiney, P. D., and Dorn, P. (1988), *Environ. Toxicol. Chem.* **7,** 19–26.
2. Lobos, J. H., Leib, T. K., and Su, T.-M. (1992), *Appl. Environ. Microbiol.* **58,** 1823–1831.
3. Spivack, J., Leib, T. K., and Lobus, J. H. (1994), *J. Biol. Chem.* **269(10),** 7323–7329.
4. Hirano, T., Honda, Y., Watanabe, T., and Kuwahara, M. (2000), *Biosci. Biotechnol. Biochem.* **64,** 1958–1962.
5. Ohko, Y., Ando, I., Niwa, C., Tatsuma, T., Yamamura, T., Nakashima, T., Kubota, Y., and Fujishima, A. (2001), *Environ. Sci. Technol.* **35,** 2365–2368.
6. Ike, M., Chen, M.-Y., Jin, C.-S., and Fujita, M. (2002), *Environ. Toxicol.* **17,** 457–461.
7. Yoshida, M., Ono, H., Mori, Y., Chuda, Y., and Mori, M. (2002), *J. Agric. Food Chem.* **50,** 4377–4381.
8. Schmidt, B. and Schuphan, I. (2002), *Chemosphere* **49,** 51–59.
9. Kang, J.-H. and Kondo, F. (2002), *Arch. Environ. Contam. Toxicol.* **43,** 265–269.

10. Sajiki, J. and Yonekubo, J. (2003), *Chemosphere* **51,** 55–62.
11. Hamada, H., Tomi, R., Asada, Y., and Furuya, T. (2002), *Tetrahedron Lett.* **43(22),** 4087–4089.
12. Chai, W., Sakamaki, H., Kitanaka, S., Saitoh, M., and Horiuchi, C. A. (2003), *Biosci. Biotechnol. Biochem.* **67(1),** 218–220.
13. Mitra, J., Mukherjee, P. K., Kale, S. P., and Murthy, N. B. K. (2001), *Biodegradation* **12,** 235–245.
14. Yue, Q., Bacon, C. W., and Richardson M. D. (1998), *Phytochemistry* **48(3),** 451–454.