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Preliminary Communication

Biodegradation of Bisphenol A by Cultured Cells of *Caragana chamlagu*

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The biological degradation of 2,2-bis(4-hydroxyphenol)propane (1; bisphenol A, BPA), a representative endocrine disruptor, was studied with plant-cultured cells of *Caragana chamlagu*. An initial BPA concentration of 425 μM in an aqueous solution was degraded by *C. chamlagu* at 25°C for 2 days in the dark, and two intermediates were then completely dissipated after 10 days.

Key words: bisphenol A; plant-cultured cells; *Caragana chamlagu*; biological degradation; endocrine disrupter

2,2-Bis(4-hydroxyphenyl)propane (bisphenol A or 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 disruptor.[1)] Some examples of the biological degradation of BPA by bacteria[2)] and basidiomycetes[3)] have recently been reported. However, these biological methods to eliminate the pollutant in an aqueous solution cannot completely decompose the total organic carbon.

There is growing interest in the ability of plant-cultured cells. One example is the convertion of foreign substances in the stereo- and regioselective control of organic synthesis.[4,5)] However, to our knowledge, there has been no study on the decomposition of BPA by means of plant-cultured cells. We have been investigating oxidation by using plant-cultured cells. In particular, the plant-cultured cells of *Caragana chamlagu* had high ability for oxidation.[6)] We report here the total degradation of BPA in an aqueous solution by using *C. chamlagu* in the biocatalytic reaction.

BPA (GC grade > 99%) was purchased from Tokyo Kasei (Tokyo, Japan), and BPA-d_{16} (98 atom%D) was purchased from Aldrich Chemical (Milwaukee, WI, U.S.A.). The Murashige and Skoog medium was purchased from Wako Pure Chemical Industries (Osaka, Japan), as were 2,4-dichlorophenoxyacetic acid (Ti grade > 98%) and sucrose.

The callus tissue induced from the stem of *Caragana chamlagu* (Leguminosae) that was used in our previous study[6)] was also used in this investigation, this callus tissue of *C. chamlagu* having been maintained for approximately 6 years. The callus tissue of *C. chamlagu* was transferred to a freshly prepared Murashige-Skoog medium[7)] (containing 1 ppm of 2,4-dichlorophenoxyacetic acid as auxin, 3% sucrose, and 0.8% agar), and then grown at 25°C in the dark.

The cultured cells of *C. chamlagu* (10 g) were transplanted to the MS medium (200 ml) containing 1 ppm of 2,4-dichlorophenoxyacetic acid, and the suspension cells were then incubated while shaking (110 rpm) at 25°C in the dark for 5 days. BPA (**1**; 20 mg, 425 μM) was then added to the suspension cells which were further incubated. After the indicated incubation period, the cultured cells were removed by filtration, and the filtrate was extracted with distilled ether. The amount of residual BPA was measured by gas chromatography-mass spectrometry (GC-MS: GCMS-QP5050, Shimadzu, Tokyo, Japan). GC was programmed to raise the oven temperature from 50°C to 250°C at 18°C/min, and MS was conducted at 70 eV (EI) or 300 eV (CI). The reaction products were isolated by column chromatography on silica gel 60 (Merck, Darmstadt, Germany), using *n*-hexane-Et_2O (3:1) as the eluent. The chemical structures of the reaction products were determined by GC-MS, IR spectra (Jasco FT-IR 230 spectrometer, Japan), and ^{1}H and ^{13}C-NMR spectra which were measured with a Jeol GSX 400 spectrometer (Nihon Denshi, Tokyo, Japan) in deu-

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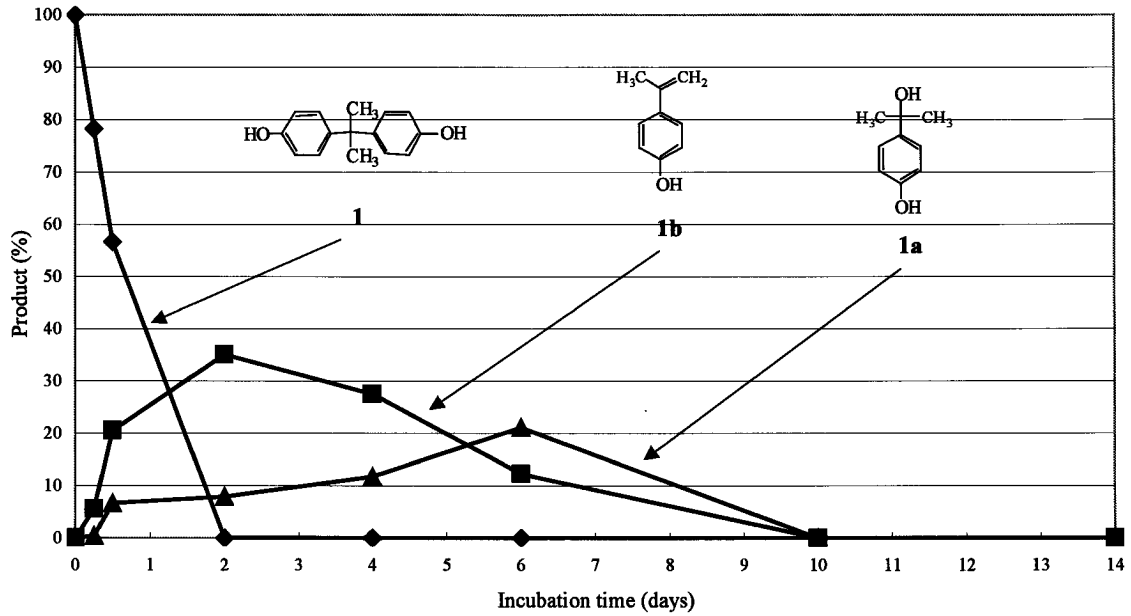
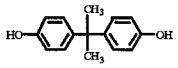
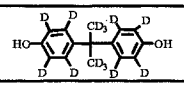
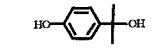
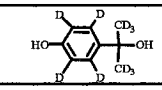
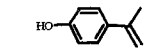
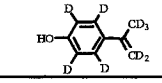


Fig. 1. Biocatalyic Degradation of Bisphenol A by *C. chamlagu*.

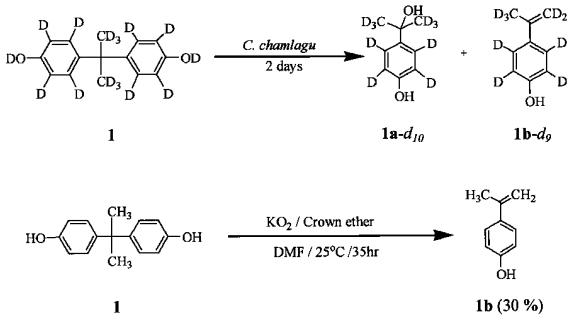
Table 1. Main Fragment Ion (m/z) of the Reaction Compounds by a GC-MS Analysis (EI)

Compound	Retention time/min	Fragment Ion m/z (% abundance)	Molecular weight (m/z)	Structure
BPA	13.2	228 (21), 213 (100), 119 (48), 91 (32)	228	
BPA-d_{14}	13.2	242 (22), 224 (100), 125 (42), 97 (25)	242	
1a	8.5	152 (16), 137 (100), 119 (6), 91 (6)	152	
1a-d_{10}	8.5	162 (16), 144 (100), 125 (12), 97 (10)	162	
1b	7.9	134 (100), 119 (89), 91 (53)	134	
1b-d_9	7.9	143 (100), 125 (91), 97 (43)	143	

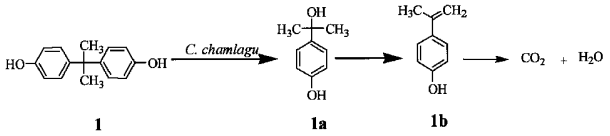
teriochloroform solutions with tetramethylsilane as an internal standard.

The activity of *C. chamlagu* in degrading BPA *in vivo* was investigated. First, the degradation of BPA by the suspension cells of *C. chamlagu* was examined. The results of time-course experiments are shown in Fig. 1. It can be seen from Fig. 1 that BPA disappeared after 2 days of incubation, and then intermediates **1a** and **1b** disappeared after 10 days. Compound **1b** was isolated by column chromatography and followed by GC-MS, IR, and NMR analysis. The spectral data of **1b** are as follow: IR ν_{max} (KBr) cm^{-1}: 3405; NMR δ_H ($CDCl_3$): 2.12 (3H, s), 4.98 (1H, s), 5.27 (1H, s), 6.79 (2H, d, $J=8.8$ Hz) and 7.36 (2H, d, $J=8.8$ Hz); NMR δ_C ($CDCl_3$): 29.2, 110.1, 115.8, 127.4, 142.8, 155.9; EIMS m/z: 134 (100) $[M]^+$, 119 (89) $[M\text{-}CH_3]^+$, 91 (53), 77 (27), 65 (42) and 51 (47); CIMS m/z: 135 (100) $[M+H]^+$. The spectra of **1b** show that the structure is in agreement with that of 4-isopropenylphenol. **1a** was deduced to be 4-(2-propanol)phenol by its GC-MS spectrum. In order to clarify that their origin was not in the cells, the biodegradation of BPA-d_{16} by *C. chamlagu* was carried out. The results of GC-MS spectra show that the deuterides of **1a** and **1b** (**1a**-d_{10} and **1b**-d_9) were obtained as intermediates, proving that **1a** and **1b** were intermediates of the biodegradation of BPA.

The results of the time-course experiments indicate that oxidative cleavage of the C-C bond had occurred. In order to identify the pathway for the bio-



Scheme 1. Verification Experiments of Pathway for BPA Biodegradation.



Scheme 2. Pathway for BPA Biodegradation by Cultured Cells of *C. chamlagu*.

degradation, we investigated the oxidation of the superoxide anion radical [O_2^-] with BPA (**1**). A typical procedure was the reaction of potassium superoxide (20.0 mmol), 18-crown 6-ether (10.0 mmol), BPA (**1**, 2.0 mmol) and DMF (60 ml) at 25°C for 35 hours. The resulting mixture was extracted with distilled ether and gave 4-isopropenylphenol (**1b**, 30%).

In the biological degradation of BPA with the bacterium or basidiomycete, the reaction pathway can be explained by the work of the lignin-degrading enzyme.[3] However, it is not believed that the degradation pathway of BPA using plant-cultured cells is similar to the reaction of the lignin-degrading enzyme. On the basis of the foregoing results, it is possible that the biological degradation of BPA proceeded by oxidation of the superoxide anion radical [O_2^-] with dioxygenases, which is similar to the reaction of BPA by KO_2. This is the first time that a BPA concentration of 425 μM in an aqueous solution has been completely dissipated by plant-cultured cells of *C. chamlagu*.

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References

1) Alexander, H. C., Dill, D. C., Smith, L. W., Guiney, P. D., and Dorn, P., Bisphenol A: Acute aquatic toxicity. *Environmental Toxicol. Chem.*, **7**, 19–26 (1988).
2) Spivack, J., Leib, T. K., and Lobus, J. H., Novel pathway for bacterial metabolism of bisphenol A. *J. Biol. Chem.*, **269**, 7323–7329 (1994).
3) Hirano, T., Honda, Y., Watanabe, T., and Kuwahara, M., Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot basidiomycete, *Pleurotus ostreatus*. *Biosci. Biotechnol. Biochem.*, **64**, 1958–1962 (2000).
4) Suga, T., and Hirata, T., Review article number 55, biotransformation of exogenous substrates by plant cell cultures. *Phytochemisty*, **29**, 2393–2406 (1990).
5) Chai, W., Hamada, H., Suhara, J., and Horiuchi, C. A., Biotransformation of (+)- and (−)-camphorquinones by plant cultured cells. *Phytochemistry*, **57**, 669–673 (2001).
6) Sakamaki, H., Kitanaka, S., Chai, W., Hayasida, Y., Takagi, Y., and Horiuchi, C. A., Biotransformation of thujopsene by *Caragana chamlagu*. *J. Nat. Prod.*, **64**, 630–631 (2001).
7) Murashige, T., and Skoog, F., A resised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.*, **15**, 473–497 (1962).