Copyright © 2007 by Humana Press Inc.
All rights of any nature whatsoever reserved.
0273-2289/1559-0291 (Online)/07/136/0039/$30.00

Degradation of Bisphenol A by *Bacillus pumilus* Isolated from Kimchi, a Traditionally Fermented Food

Hayato Yamanaka,* Kunihiko Moriyoshi, Takashi Ohmoto, Tatsuhiko Ohe, and Kiyofumi Sakai

Department of Environmental Technology, Osaka Municipal Technical Research Institute, 1-6-50, Morinomiya, Joto-ku, Osaka, 536-8553, Japan, E-mail: yamanaka@omtri.city.osaka.jp

Received December 16, 2005; Revised May 11, 2006; Accepted May 26, 2006

Abstract

Novel bisphenol A (BPA)–degrading bacterial strains, designated as BP-2CK, BP-21DK, and BP-22DK, were isolated from kimchi, a traditionally fermented food. These isolates were identified as *Bacillus pumilus* and efficiently degraded BPA in a medium supplemented with nutrients such as peptone, beef extract, and yeast extract. Strains BP-2CK, BP-21DK, and BP-22DK successfully degraded 25, 25, and 50 ppm of BPA, respectively, and all strains exhibited BPA-degrading activity in the presence of 10% NaCl. Accumulation of the metabolites including 4-hydroxyacetophenone, one of the intermediates produced by the other BPA-degrading bacteria, was not observed in BPA degradation by the isolated strains. These results indicate that the isolated food-derived bacteria are applicable for the construction of efficient and safer systems for the removal of BPA.

Index Entries: Bisphenol A; biodegradation; *Bacillus pumilus*; kimchi; endocrine–disrupting chemical.

Introduction

Bisphenol A (2,2-bis[4-hydroxyphenyl]propane, or BPA) is widely used as the starting material for the industrial production of polycarbonates, epoxy resins, and other specialty chemicals. They are frequently found in a wide range of products including consumer products such as plastic bottles and the linings of canned goods. Owing to its mass production and

*Author to whom all correspondence and reprint requests should be addressed.

widespread use, the possibility of environmental contamination of BPA has increased. Environmental releases are possible via permitted outfalls of industrial wastewater treatment systems or sewage treatment plants that receive BPA. Other possible sources of BPA are found in the environment, such as waste plastics in waste landfills and sewage sludge from wastewater treatment facilities.

Abiotic and biotic degradation of BPA were demonstrated in several studies *(1,2)*. The overall residence time for the removal of BPA from the environment was estimated within 7 d, and BPA has been considered to be not persistent in the environment *(3)*. However, BPA under certain conditions, such as in anoxic sediments *(4)* and in seawater *(5)*, can persist for an extended period time. Moreover, there are numerous monitoring data on BPA in Europe, the United States, and Japan, and certain levels of BPA were detected in many samples *(3)*. Yamamoto et al. *(6)* reported that the levels of BPA in hazardous waste landfill leachates ranged from 1.3 to 17,200 μg/L.

BPA was revealed to show an acute toxicity within the range of 1–10 mg/L toward algae, invertebrates, and fish *(7)*. The effects of BPA on human health have also been of concern, and BPA was found to have mutagenicity in human RSa cells within the range of 10^{-7}–10^{-5} *M (8)*. In addition, BPA has been suspected of being an endocrine-disrupting chemical *(9)*. Thus, BPA is an environmental contaminant of great concern, and the construction of efficient systems for its removal is necessary.

The application of BPA-degrading microorganisms is one possible approach to achieving this goal. There have been several reports on the microbial degradation of BPA. Lobos et al. *(10)* isolated a Gram-negative aerobic bacterium designated as strain MV-1 from sludge taken from a wastewater treatment plant at a plastics-manufacturing facility. Ike et al. *(11)* isolated *Pseudomonas paucimobilis* FJ-4 from sludge taken from a wastewater treatment plant at an epoxy resin–manufacturing facility. We have been interested in the environmental fate of BPA and screened BPA-degrading microorganisms. As a result, we isolated *Sphingomonas yanoikuyae* BP-7, a BPA-degrading bacterium, and *Pseudomonas fluorescens* BP-14, a symbiotic bacterium, from seawater *(12)*. Another BPA-degrading bacterium, *Sphingomonas yanoikuyae* BP-11R, was also isolated from river water *(13)*. These results suggest that BPA-degrading microorganisms are widely distributed in the natural environments and that isolation of novel BPA-degrading microorganisms is possible by expanding the range of screening.

In this article, we describe the isolation and characterization of novel BPA-degrading bacteria from kimchi, a traditionally fermented food. One advantage of using foods as the source of microorganisms is that it allows the isolation of nonpathogenic and nontoxicogenic microorganisms, and, therefore, construction of safer systems that can clean up the contaminants without damage to human health or the living environment can accomplished.

Materials and Methods

Chemicals

Various kinds of kimchi were purchased at local markets in Osaka, Japan. BPA was purchased from Nacalai Tesque (Kyoto, Japan). Beef extract was the product of Difco (Detroit, MI). Casein peptone and yeast extract were the products of Nihon Pharmaceutical (Tokyo, Japan). All other chemicals were also obtained from commercial sources.

Isolation of BPA-Degrading Microorganisms

In the screening experiments, BPA-NB agar plates containing 0.2 g/L of BPA, 10 g/L of peptone, 5 g/L of beef extract, 5 g/L of NaCl, and 15 g/L of agar (pH 7.0) were used. BPA concentration in the BPA-NB agar plates was above saturation at room temperature, and the plates were turbid, because BPA was precipitated after they solidified.

Each kind of kimchi was homogenized, diluted with saline solution, and spread on BPA-NB agar plates. Culture plates were incubated at 30°C, and colonies showing clear zones around them were picked up as the candidates of BPA-degrading microorganisms. Then each isolate was purified by serial single-colony isolation on BPA-NB agar plates.

The pure cultures obtained were tested for BPA-degrading activity. BPA-PBY medium was composed of 0.01 g/L of BPA, 5 g/L of peptone, 2.5 g/L of beef extract, 1 g/L of yeast extract, and 5 g/L of NaCl (pH 7.0). Each isolate was inoculated into 5 mL of BPA-PBY medium in a test tube and cultivated at 27°C for 7 d. Then BPA in the culture supernatant was quantified using high-performance liquid chromatography (HPLC). The residual cells were extracted with acetone, and the residue after evaporation was dissolved in acetonitrile and also subjected to HPLC analysis to estimate the amount of BPA in the cells.

Isolated BPA-degrading microorganisms were also examined for their ability to degrade 4-hydroxyacetophenone (4-HAP). Each isolate was inoculated into 5 mL of HAP-PBY medium in a test tube containing the same components as BPA-PBY medium except that 0.01 g/L of 4-HAP was added instead of BPA. Cultivation was carried out at 27°C for 7 d, and 4-HAP in the culture supernatant was quantified with HPLC.

Identification of the isolates having activity was conducted by NCIMB Japan (Shizuoka, Japan).

Degradation of BPA by BPA-Degrading Bacteria

BPA-PBYS medium, composed of 0.01 g/L of BPA, 5 g/L of peptone, 2.5 g/L of beef extract, 1 g/L of yeast extract, 50 g/L of NaCl, 1 g/L of NH_4NO_3, 0.5 g/L of KH_2PO_4, 1 g/L of K_2HPO_4, 0.3 g/L of KCl, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.2 g/L of $CaCl_2 \cdot 2H_2O$, and 0.01 g/L of $FeCl_2$ (pH 7.0), was used for the degradation experiments. Each strain was inoculated into 100 mL of BPA-PBYS medium in a 500-mL flask and precultivated at 27°C

for 20 h. Cells were collected by centrifugation, washed with sterilized water, and resuspended in 100 mL of fresh BPA-PBYS medium with the initial OD_{660} adjusted to 0.2. Then cultivation was carried out at 27°C and samples (1 mL each) were withdrawn at various time points. When the effects of the concentrations of culture components were examined, the composition of BPA-PBYS medium was modified as indicated in Table 2 and Figs. 2–4. All experiments were repeated at least two times to confirm reproducibility, and representative results are presented in Table 2 and Figs. 1–4.

Cultivation of BPA-Degrading Bacteria in a Mineral Salt Medium

Mineral salt (MS) medium was composed of 5 g/L of NaCl, 1 g/L of NH_4NO_3, 0.5 g/L of KH_2PO_4, 1 g/L of K_2HPO_4, 0.3 g/L of KCl, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.2 g/L of $CaCl_2 \cdot 2H_2O$, and 0.01 g/L of $FeCl_2$ (pH 7.0). To MS medium, 0.01 g/L of BPA or 10 g/L of glucose was added. Each strain was inoculated into 5 mL of the medium in a test tube and incubated at 27°C. Cell growth was observed after 14 d.

Resting Cell AsÔ/ys

For resting cell assays, 50 m*M* potassium phosphate buffer (pH 7.0) and MS medium were used. To these solutions, 0.01 g/L of BPA was added. Precultivation of the strains and preparation of cells were carried out using the same procedure described in a previous section. Then the cells obtained were suspended into 10 mL of the assay solution with the initial OD_{660} adjusted to 5. Incubation was carried out at 27°C for 7 d, and BPA in the assay solution was quantified using HPLC.

Evaluation of Salt Tolerance of BPA-Degrading Bacteria

PBY medium, composed of 5 g/L of peptone, 2.5 g/L of beef extract, 1 g/L of yeast extract, and various amounts of NaCl (pH 7.0), was used for the investigation of salt tolerance. The concentration of NaCl was varied from 0 to 20% at intervals of 1%. Each strain was inoculated into 5 mL of PBY medium in a test tube and incubated at 27°C. Cell growth was observed after 14 d.

HPLC Analysis

Concentrations of BPA and 4-HAP were determined with HPLC using a Chemcosorb 5-ODS-UH column (2.1–mm diameter, 150-mm length; Chemco, Osaka, Japan). The samples were centrifuged at 12,500*g* for 5 min and 20 μL of the supernatant was injected. The mobile phase was acetonitrile/water (40/60 [v/v]) and the flow rate was 0.3 mL/min. The retention times of BPA, 4-HAP, and 4-hydroxy-benzoic acid (4-HBA) were 6.6, 2.3, and 1.4 min, respectively. The eluent was monitored with a photodiode array ultraviolet (UV)-visible detector (SPD-M10Avp; Shimadzu, Kyoto, Japan). To determine the concentrations of BPA and 4-HAP, the wavelength

Table 1
Taxonomic Properties of Isolated BPA-Degrading Bacteria[a]

	BP-2CK	BP-21DK	BP-22DK
Shape	Rod	Rod	Rod
Size (μm)	0.8 × 1.5–2.0	0.8 × 2.0–2.5	0.8–0.9 × 2.0–2.5
Gram staining	+	variable	–
Spore	+	+	+
Motility	+	+	+
Aerobic growth	+	+	+
Anaerobic growth	+	+	+
Growth at 50°C	+	+	+
Catalase	+	+	+
Oxidase	+	+	+
Gelatinase	–	+	+
O-F test	–/–	–/–	–/–
Nitrate reduced to nitrite	–	–	–
Hydrolysis of			
Casein	+	+	+
Hippurate	–	–	–
Utilization of			
D-Glucose	+	+	+
L-Arabinose	+	+	–
D-Xylose	+	+	–
D-Mannitol	+	+	+
L-Rhamnose	–	+	+
Trehalose	+	–	+
Gentiobiose	+	–	+

[a]+, positive; –, negative.

was set at 280 nm. UV absorption spectra were obtained in the range of 190–360 nm.

Results

Isolation and Characterization of BPA-Degrading Microorganisms

By using various kinds of kimchi as the source of microorganisms, three colonies forming a halo were isolated from BPA-NB agar plates. Two of the colonies were isolated from the same kimchi sample and named BP-21DK and BP-22DK. The other strain was isolated from another kimchi sample and named BP-2CK. All isolates were found to show BPA-degrading activity in a liquid medium. BPA in the culture supernatant of BPA-PBY medium was completely removed after 7 d of cultivation, and almost no BPA was recovered by extraction from the cells.

Isolated strains were then subjected to identification tests. Table 1 presents the morphologic and biochemical characteristics of BP-2CK, BP-21DK, and BP-22DK. All three strains were rod shaped, endospore form-

ing, aerobic, and catalase positive, suggesting that these strains belong to the genus *Bacillus*. Several characteristic differences were observed among them. Partial nucleotide sequences (500 bp) of the 16S rDNA of all strains exhibited 100% similarity with that of *B. pumilus*, respectively. Therefore, we determined BP-2CK, BP-21DK, and BP-22DK to be *B. pumilus*, although some properties, such as anaerobic growth and hippurate-degrading activity, were different from the typical characteristics of the standard strain *(14)*.

Degradation of BPA by BPA-Degrading Bacteria

Figure 1 presents the time courses of BPA degradation by the isolated strains. Each strain could degrade BPA in BPA-PBYS medium accompanying cell growth. Complete degradation of 10 ppm of BPA was achieved by BP-2CK within 16 h. BP-21DK and BP-22DK also degraded 10 ppm of BPA completely within 24–36 h.

All of the strains were not able to grow in MS medium containing BPA or glucose as a sole carbon source. In addition, no degradation of BPA was observed in the resting cell assays. For effective degradation of BPA, the addition of nutrients such as peptone, beef extract, and yeast extract was necessary. We selected BP-2CK and the concentrations of these nutrients were optimized. When compared with the BPA degradation after 22 h of cultivation, the optimum concentrations of peptone and beef extract were found to be 0.5 and 0.25%, respectively (Table 2). When the concentrations of peptone and beef extract were <0.1 and <0.05%, respectively, BPA degradation did not continue to proceed. Excess amounts of these components also caused a decrease in the rate of BPA degradation. In the meantime, 0.1% yeast extract was sufficient for degradation of 10 ppm of BPA in 22 h. When the amount of yeast extract was reduced, a longer time was required for the complete degradation of BPA.

Effect of BPA Concentration on BPA Degradation

As shown in Fig. 2, 25 ppm of BPA was effectively degraded by all three strains. Higher concentrations of BPA caused inhibition of their BPA-degrading activity. BP-22DK successfully degraded 50 ppm of BPA, whereas BPA degradation by BP-2CK and BP-21DK proceeded very slowly and stopped within 21 and 5 d, respectively. All strains showed neither degradation activity nor cell growth in the presence of 100 ppm of BPA.

Effect of NaCl Concentration on BPA Degradation

B. pumilus is known to be salt tolerant and possible to grow in the presence of at least 7% NaCl *(14)*. Therefore, the tolerance of the isolated strains to NaCl was examined. When BP-2CK, BP-21DK, and BP-22DK were inoculated into PBY medium without BPA and containing different concentrations of NaCl, each strain exhibited growth in the medium containing up to 19, 14, and 14% NaCl, respectively. In view of their high salt

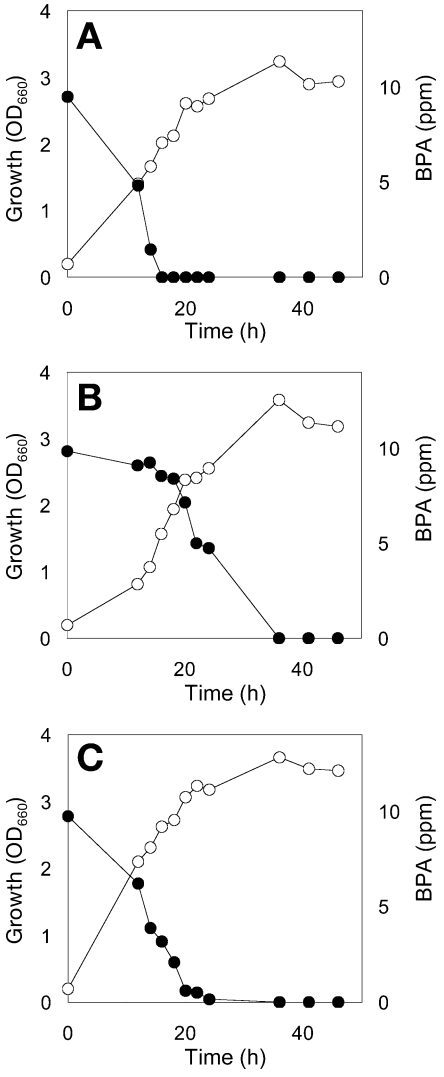

Fig. 1. Degradation of BPA in BPA-PBYS medium by **(A)** BP-2CK, **(B)** BP-21DK, and **(C)** BP-22DK. Each strain was cultivated in 100 mL of BPA-PBYS medium. ○, Growth; ●, BPA concentration.

tolerance, the effect of NaCl concentration on BPA degradation was examined. All three strains exhibited BPA-degrading activity in the medium containing 10% NaCl, and BP-22DK degraded BPA more efficiently than BP-2CK and BP-21DK. Figure 3 presents the effect of NaCl concentration on BPA degradation by BP-22DK. As the concentration of NaCl increased, the degradation rate became much slower. BPA degradation as well as cell growth was never seen when the concentration of NaCl became up to 12.5%.

Table 2
Effects of Peptone, Beef Extract, and Yeast Extract on Degradation of BPA by BP-2CK[a]

Peptone (g/L)	Beef extract (g/L)	Yeast extract (g/L)	Growth (OD_{660})	BPA remaining (%)[b]
0	0	1	0.66	72
1	0.5	1	1.20	46
2.5	1.25	1	1.80	9
5	2.5	1	2.98	ND
10	5	1	4.45	34
5	2.5	0	1.41	22
5	2.5	0.2	1.72	5
5	2.5	1	2.85	ND
5	2.5	2	3.50	ND
5	2.5	5	4.95	ND

[a]BP-2CK was cultivated for 22 h in 100 mL of a medium containing the same components as BPA-PBYS medium except that the amounts of peptone, beef extract, and yeast extract were changed.
[b]ND, no BPA was detected.

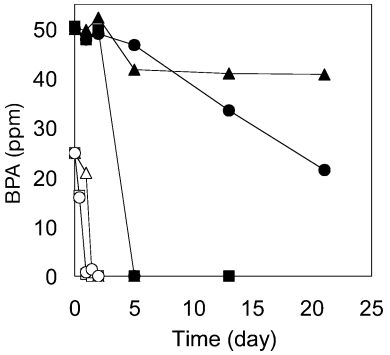

Fig. 2. Effect of BPA concentration on degradation of BPA by BP-2CK (○, ●), BP-21DK (△, ▲), and BP-22DK (□, ■). Each strain was cultivated in 100 mL of a medium containing the same components as BPA-PBYS medium except that the amount of BPA was changed to 0.025 g/L (○, △, □) or 0.05 g/L (●, ▲, ■).

Degradation Intermediates Produced During BPA Degradation

The isolated strains efficiently degraded BPA under the cultivation conditions employed, and remarkable accumulation of the metabolites was not observed with HPLC. However, temporally formation of trace amounts of unidentified compounds was observed in some cultivation experiments. Figure 4A provides a typical HPLC chromatogram of the culture supernatant of BP-21DK. Large amounts of the culture components were eluted at retention times shorter than 2 min, and formation of less hydrophobic compounds including 4-HBA could not be confirmed because of their interfer-

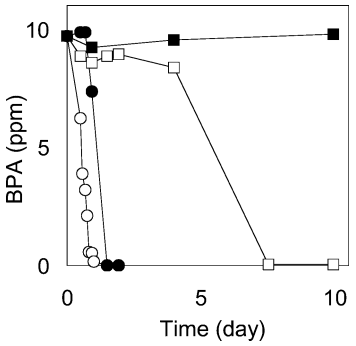

Fig. 3. Effect of NaCl concentration on degradation of BPA by BP-22DK. BP-22DK was cultivated in 100 mL of a medium containing the same components as BPA-PBYS medium except that the amount of NaCl was changed to 50 g/L (○), 75 g/L (●), 100 g/L (□), or 125 g/L (■).

ence. Some peaks of unidentified compounds temporally appeared during BPA degradation but disappeared in the prolonged cultivation. Figure 4B presents UV absorption spectra of the peak at retention times of 3.6 min (compound A), 5.3 min (compound B), and 6.6 min (BPA). The absorption maxima of compounds A (313 nm) and B (314 nm) were longer than the maximum of BPA (278 nm). 4-HAP was not detected throughout BPA degradation by each strain. In addition, no degradation of 4-HAP was observed when all three strains were cultivated in HAP-PBY medium, although they exhibited significant growth in the medium and OD_{660} reached approx 2.5.

Discussion

There have been several reports on BPA-degrading microorganisms isolated from the sludge taken from treatment plants receiving BPA-containing wastewater *(10,11)*. Our previous studies *(12,13)* indicate that BPA-degrading microorganisms are widely distributed in the natural environments instead of being restricted in the polluted regions. From these results, we planned to focus our attention on screening for novel BPA-degrading microorganisms from fermented foods. Although microorganisms isolated from foods have been used for various purposes including food processing and biopreservation *(15,16)*, little attention has been paid to the application of food microorganisms in the degradation of xenobiotics. However, it will be a very significant approach because construction of safer microbial treatment systems is possible by using generally regarded as safe microorganisms having xenobiotics-degrading activity.

We used kimchi, a traditionally fermented food prepared with various vegetables, as the source of microorganisms. In the course of screening, the sample suspension was directly spread on the rich agar medium containing BPA without enrichment procedure, and some colonies were selected using halo formation as the indicator of BPA-degrading ability. As a result, novel BPA-degrading bacteria that belong to *B. pumilus* were

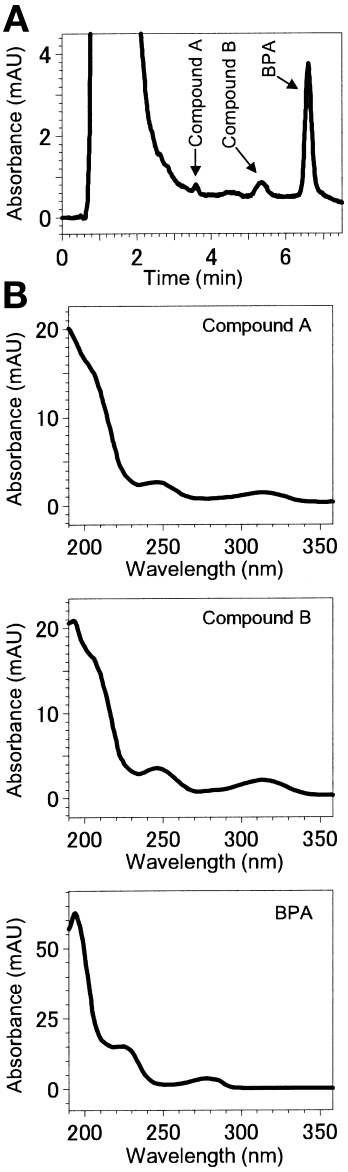

Fig. 4. HPLC analysis of culture supernatant of BP-21DK. **(A)** Chromatogram of culture supernatant of BP-21DK. BP-21DK was cultivated in 100 mL of a medium containing the same components as BPA-PBYS medium except that the amounts of peptone and beef extract were changed to 2.5 and 1.25 g/L, respectively. The culture supernatant after 16 h of cultivation was subjected to HPLC analysis. **(B)** UV absorption spectra of compound A, compound B, and BPA.

isolated. To our knowledge, this is the first report of BPA degradation by the genus *Bacillus*. Although rigorous scientific testing is needed to establish the safety of the isolated strains, *B. pumilus* is usually considered safe and used as probiotics for human use *(17)*. The isolated strains BP-2CK,

BP-21DK, and BP-22DK were unable to grow in MS medium containing BPA or glucose as a sole source of carbon, or to degrade BPA in MS medium containing BPA. According to *Bergey's Manual of Systematic Bacteriology (14)*, many strains belonging to the genus *Bacillus* require growth factors and *B. pumilus* requires biotin and amino acids. Thus, the addition of nutrients was effective for efficient growth as well as BPA degradation. This should be one possible reason that the genus *Bacillus* with BPA-degrading activity has not been isolated with the conventional enrichment techniques using a minimum medium containing BPA as the sole carbon source. Biodegradation of BPA has been mainly investigated in nutrient-poor conditions such as in surface water, and there have been few reports on biodegradation of BPA in nutrient-rich conditions. The isolated strains efficiently degraded BPA in the rich medium, suggesting that they are effective for nutrient-rich pollution such as nutrient-rich sewage. This may be because they were isolated from fermented foods, nutrient-rich environments.

The isolated strains successfully degraded 25–50 ppm of BPA. These concentrations are high enough for the treatment of BPA-contaminated environments, although there have been other reports of BPA-degrading bacteria showing activity at concentrations higher than 100 ppm *(11–14)*. When the concentration of BPA in a medium became higher, growth of the isolated strains was inhibited. In addition, their salt tolerance when BPA was present in a medium was lower than when BPA was absent. In the screening experiments, the number of colonies formed on BPA-NB agar plates was much smaller than that on agar plates without BPA (data not shown), suggesting that the inhibitory effect of BPA is widely observed in the other microorganisms. BPA is generally regarded as readily biodegradable, but insufficient degradation of BPA was observed in some biodegradation tests *(1,18)*. The intrinsic toxicity of BPA may be one of the possible factors leading to these results.

High concentrations of inorganic salts are often present in industrial wastewaters containing BPA. For example, aromatic polycarbonates are mainly prepared by the interfacial polycondensation of BPA with phosgene. In this process a large quantity of inorganic salts such as NaCl is produced as byproducts, and dilution with a large amount of water is necessary for the biotreatment of BPA. The isolated strains were found to degrade BPA in a medium containing 10% NaCl. No other BPA-degrading bacterium is reported to exhibit BPA-degrading activity in the presence of such a high concentration of inorganic salts, although *S. yanoikuyae* BP-7, isolated from seawater, showed activity in the presence of 3% NaCl *(12)*. This finding is significant because construction of the small-scale system is possible by adopting salt-tolerant BPA-degrading microorganisms in the wastewater treatment plants. Usually kimchi is manufactured by seasoning vegetables with a high concentration of salt as well as other ingredients such as salted fish, garlic, and hot red pepper. This procedure may account for the high salt tolerance of the strains isolated from kimchi.

The biodegradation route of BPA was proposed in strain MV1 *(19)*, in which the degradation of BPA primarily occurs via 4-HAP and 4-HBA. 4-HAP was also the major intermediate produced in BPA degradation by *S. yanoikuyae* (unpublished results). However, formation of 4-HAP was not observed in the course of BPA degradation by the isolated strains. Furthermore, growing cells of all three strains exhibited no 4-HAP-degrading activity. These results suggest that the BPA degradation pathway in the isolated strains is different from that proposed in strain MV1. Formation of unidentified compounds, which may be the intermediates, was observed during BPA degradation. They seemed to be hydrophobic compounds possessing longer conjugation, when considering the longer absorption maxima. Attempts are currently in progress to investigate the conditions for accumulation of these compounds sufficient for structural analyses.

Conclusion

Novel BPA-degrading bacteria that are applicable for the treatment of BPA were isolated. They will be effective especially for pollution with high concentrations of BPA, salts, and nutrients. Efforts in constructing and evaluating the BPA-removal system with these isolates are in progress.

References

1. Staples, C. A., Dorn, P. B., Klecka, G. M., O'Block, S. T., and Harris, L. R. (1998), *Chemosphere* **36,** 2149–2173.
2. Sajiki, J. and Yonekubo, J. (2003), *Chemosphere* **51,** 55–62.
3. Cousins, I. T., Staples, C. A., Klecka, G. M., and Mackay, D. (2002), *Hum. Ecol. Risk Assess.* **8,** 1107–1135.
4. Voordeckers, J. W., Fennell, D. E., Jones, K., and Haggblom, M. M. (2002), *Environ. Sci. Technol*. **36,** 696–701.
5. Kang, J. H. and Kondo, F. (2005), *Chemosphere* **60,** 1288–1292.
6. Yamamoto, T., Yasuhara, A., Shiraishi, H., and Nakasugi, O. (2001), *Chemosphere* **42,** 415–418.
7. Alexander, H. C., Dill, L. W., Smith, L. W., Guiney, P. D., and Dorn, P. (1988), *Environ. Toxicol. Chem.* **7,** 19–26.
8. Takahashi, S., Chi, X. J., Yamaguchi, Y., et al. (2001), *Mutat. Res.* **490,** 199–207.
9. McLachlan, J. A. (2001), *Endocr. Rev.* **22,** 319–341.
10. Lobos, J. H., Leib, T. K., and Su, T.-M. (1992), *Appl. Environ. Microbiol.* **58,** 1823–1831.
11. Ike, M., Jin, C.-S., and Fujita, M. (1995), *Jpn. J. Water Treat. Biol.* **31,** 203–212.
12. Kanaoka, H., Horie, T., Sakai, K., and Ohe, T. (2002), *Japan Kokai Tokkyo Koho* 2002-142757.
13. Monri, M., Sakai, K., and Ohe, T. (2003), *Japan Kokai Tokkyo Koho* 2003-24050.
14. Sneath, P. H. A. (1986), in *Bergey's Manual of Systematic Bacteriology*, vol. 2, Sneath, P. H. A., Mair, N. S., Sharpe, M. E., and Holt, J. G., eds., Williams & Wilkins, Baltimore, pp. 1104–1207.
15. O'Sullivan, L., Ross, R. P., and Hill, C. (2002), *Biochimie* **84,** 593–604.
16. Aoki, H., Uda, I., Tagami, K., Furuya, Y., Endo, Y., and Fujimoto, K. (2003), *Biosci. Biotechnol. Biochem.* **67,** 1018–1023.

17. Duc, L. H., Hong, H. A., Barbosa, T. M., Henriques, A. O., and Cutting, S. M. (2004), *Appl. Environ. Microbiol.* **70,** 2161–2171.
18. Chemicals Inspection and Testing Institute Japan. (1992), Biodegradation and bioaccumulation: data of existing chemicals based on the CSCL Japan, Japan Chemical Industry Ecology-Toxicology and Information Center, Tokyo, Japan.
19. Spivack, J., Leib, T. K., and Lobos, J. H. (1994), *J. Biol. Chem.* **269,** 7323–7329.