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# Isolation and Characterization of *Pseudomonas* sp. P2 Degrading Polychlorinated Biphenyls (PCBs)

**KIM, JUNG-HO*, SANG-KI CHOI, MOON-KI PARK, YOUNG-HO KIM[1], SEUNG-KYO SUH[2], CHEOL-JOO WOO[3], AND HEUI-DONG PARK[3]**

*Department of Environmental Science, Kyungsan University, Kyungsan 712-240*
*[1]Department of Food Science and Technology, Yeungnam University, Kyungsan 712-749*
*[2]Department of Environmental Management, Shinil Junior College, Taegu 706-020*
*[3]Department of Food Science and Technology, Kyungpook National University, Taegu 702-701, Korea*

The bacterial strain P2 degrading polychlorinated biphenyls (PCBs) was isolated from the soil around the *Shinchun* stream in Taegu after enrichment culture in a media containing biphenyl as the sole carbon source. The isolate was identified as a strain of *Pseudomonas* sp. based on its morphological and physiological characteristics. The optimal conditions of initial pH of media and temperature for growth were 7.0 and 30°C, respectively. Degradation of biphenyl and PCBs was confirmed by GC during the culture of *Pseudomonas* sp. P2 in a media containing them at a concentration of 500 mg/l. It was observed that *Pseudomonas* sp. P2 could degrade 97.0% of biphenyl and 60.0% of PCBs after 160 h culture.

Among the organic chemicals, polychlorinated biphenyls (PCBs) are one of the most widely studied pollutants in terms of environmental contamination and the focus of much great social concern (31). Theoretically PCBs can be represented by as many as 210 different compounds containing from 1 to 10 chlorine atoms on a biphenyl ring (11). PCBs were first synthesized by Schmidt and Schulz (28), and have been produced commercially since 1929 (31).

PCBs have been the subject of much concern because of their worldwide distribution in the environment. Studies on the microbial degradation of PCBs are essential to understand their behavior in the environment. However, the fact that PCBs are not a single compound but a mixture of different isomers is a major barrier to study of the interaction between PCBs and microorganisms (24).

Although PCBs are chemically inert and stable, there have been a number of reports on their microbial degradation. Many microorganisms degrading PCBs have been isolated by the use of traditional techniques such as by growing on PCBs as the sole carbon source (26). Clark *et al.* (7) isolated *Alcaligenes odorans* and *Alcaligenes denitrificans* degrading PCBs from soil and river sediment. Kong and Sayler (20) suggested that bacteria isolated from river sediment could degrade monohalogenated biphenyls under natural conditions. Ahmed and Focht (1) also reported the degradation of dichlorobiphenyls by *Achromobacter*. Bedard *et al.* (3) has studied the degradation of Aroclor 1248 by *Alcaligenes eutrophus* H850 and *Corynebacterium* sp. MB1. Other PCBs-degrading bacteria have been isolated such as *Acinetobacter* (9, 19, 30), *Alcaligenes* (4, 15), *Achromobacter* (25), *Arthrobacter* (10), *Bacillus* (25), *Corynebacterium* (5), and *Pseudomonas* sp. (12, 13, 17, 23).

Since PCBs are good conductors of heat and bad conductors of electricity, they have been used as an insulating oil in transformers and capacitors (8). About 205 tons of insulating oil containing PCBs are still in use in Korea (6).

Many studies have been performed to isolate microorganisms degrading PCBs, but little information is available regarding the microorganisms degrading PCBs within insulating oil. Therefore, this study was carried out to screen and isolate strains degrading PCBs, and to investigate the biodegradability of PCBs within insulating oil.

## MATERIALS AND METHODS

### Media

The medium for isolation and cultivation of bacteria degrading PCBs was composed of 1,000 mg/l $(NH_4)_2SO_4$, 1,000 mg/l $KH_2PO_4$, 200 mg/l $MgSO_4 \cdot 7H_2O$, 100 mg/l NaCl, 10 mg/l $FeSO_4 \cdot 7H_2O$, 20 mg/l $CaCl_2 \cdot 2H_2O$, and its initial pH was adjusted to 7.0 (15). Biphenyl was used as a

*Corresponding author
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carbon source for solid culture, which was added to the media in vapor form. For liquid culture, biphenyl or PCBs in an insulating oil impregnated with Aroclor 1242 (18, 22) were added to the media directly at a final concentration of 500 mg/l.

**Isolation of PCBs-degrading Bacteria**

PCBs-degrading bacteria were isolated from the soil around the *Shinchun* stream in Taegu. Enrichment culture for the PCBs-degrading bacteria was carried out by two cycles of culture at 30°C for one week in a liquid media containing PCBs as the sole carbon source. Single colonies were isolated on a solid media containing biphenyls because of their slow growth on PCBs, and were tested their abilities to degrade PCBs in liquid media.

**Cultivation**

Bacteria were grown at 30°C for 12 h in 30 ml nutrient broth for seed culture using a shaking incubator (150 rpm). To test their ability to degade biphenyl or PCBs, the bacteria were inoculated to 80 mg/l of cell mass in a liquid media containing biphenyl, and to 130 mg/l in a liquid media containing PCBs. The culture was, then, incubated for 160 h under the same conditions. The cell mass (mg/l) was monitored by weighing dried cells after filtration of culture broth through a membrane filter (0.22 μm of pore size) and drying at 90°C for 2 h.

**Identification**

The isolated microorganism was identified according to Bergey's manual of systematic bacteriology based on its morphological, physiological, and nutritional characteristics (21).

**Analysis of Biphenyl and PCBs**

The analyses of biphenyl and PCBs contents were performed with the perchlorination method (14). Biphenyl and PCBs in the insulating oil, media or culture broth were extracted with hexane on a magnetic stirrer for 1 h. The extracted biphenyl and PCBs were perchlorinated by $SbCl_5$ to decachlorinated biphenyl (DCB) (16, 32). And then, DCB content was analyzed by Varian 3300 gas chromatography equipped with $^{63}Ni$ electron capture detector. The stainless column (2 m long × 0.6 cm i.d.) packed with 3% OV-17 on 80/100 chromsorb was used. Each sample was chromatographed isothermally at 250°C with nitrogen gas as a carrier at a flow rate of 60 ml/min. The temperatures of injector and detector were 260°C (2, 27, 29).

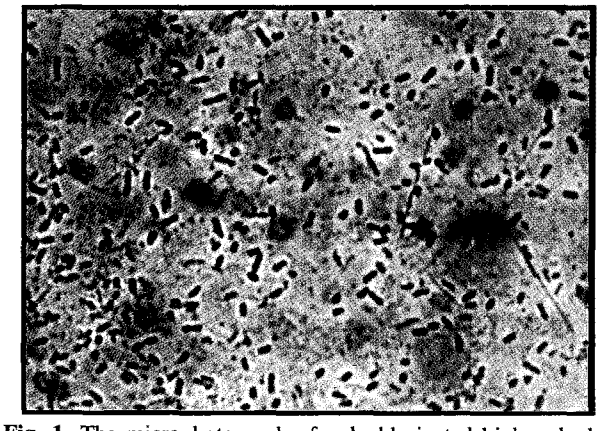

**Fig. 1.** The microphotograph of polychlorinated biphenyls-degrading bacteria *Pseudomonas* sp. P2 (× 1000).

## RESULTS AND DISCUSSION

**Isolation and Identification**

The colonies grown in the presence of biphenyl as the sole carbon source were obtained after enrichment culture from the soil around the *Shinchun* stream in Taegu. Ten types of colonies designated from P1 to P10 appeared on solid medium. Among them, strain P2 showed the highest growth and ability to degrade PCBs. Therefore, strain P2 was selected for further characterization in this work.

Fig. 1 showed the shape of strain P2 on microphotograph (× 1000), which is rod. The morphological and physiological characteristics of strain P2 were summarized in Table 1. Strain P2 could not grow at 4°C but grew at 41°C. It was found to be aerobic, gram negative, and motile. It could produce yellow pigment. Oxidase test was positive, but arginine dihydrolase and urease test was negative. It was negative to Voges-Proskauer. Starch hydrolysis and denitrification were shown to be positive. In addition, neither $H_2S$ and indole formation nor gelatin liquefaction was observed.

Table 2 showed the nutritional characteristics of strain P2. The strain P2 could utilize various carbon sources

**Table 1.** Morphological and physiological characteristics of the isolated strain P2.

| Characteristics | Strain P2 |
|---|---|
| Gram stain | -[a] |
| Shape | Rod |
| Motility | Motile |
| Pigment production | + |
| Growth at 4°C | - |
| Growth at 41°C | + |
| Oxidase | + |
| Arginine dihydrolase | - |
| Urease | - |
| V-P test | - |
| Denitrification | + |
| $H_2S$ formation | - |
| Indole formation | - |
| Gelatin liquefaction | - |
| Starch hydrolysis | + |

[a] +, positive; -, negative.

**Table 2.** Nutritional characteristics of the isolated strain P2.

| Characteristics | Strain P2 | Characteristics | Strain P2 |
|---|---|---|---|
| Adonitol | +[a] | Rhamnose | + |
| Arabinose | + | Ribose | + |
| Cellobiose | + | Sorbitol | + |
| Citrate | + | Starch | + |
| Erythritol | + | Succinate | + |
| Ethanol | + | Sucrose | + |
| Fructose | + | Xylose | + |
| Galactose | + | Alanine | + |
| Glucose | + | Serine | + |
| Glycerol | + | Leucine | + |
| Lactate | + | Lysine | + |
| Lactose | + | Glycine | + |
| Malate | + | Proline | + |
| Maltose | + | Inuline | − |
| Mannitol | + | Inositol | − |
| Mannose | + | | |

[a] +, positive; -, negative.

such as adonitol, arabinose, cellobiose, citrate, erythritol, ethanol, fructose, galactose, glucose, glycerol, lactate, lactose, malate, maltose, mannitol, mannose, rhamnose, ribose, sorbitol, starch, succinate, sucrose, and xylose. Amino acids including alanine, serine, leucine, lysine, glycine, and proline could be utilized by strain P2. No growth was observed on inuline and inositol. These results suggest that the isolated strain P2 should be a strain of *Pseudomonas* sp.

**Effects of pH and Temperature**

Fig. 2 showed the effects of the initial pH of media on the growth of *Pseudomonas* sp. P2. The growth was monitored after culture in the media containing biphenyl whose pH was adjusted to a range between 5 and 8. *Pseudomonas* sp. P2 showed the maximum growth at pH 7.0. The relative growth at pH 5.0 and 8.0 were 54.5 and 66.1%, respectively, compared with that obtained at pH 7.0.

The effects of temperature on the growth of *Pseudomonas* sp. P2 is shown in Fig. 3. The growth was monitored at a range of temperatures between 20 and 40°C. Maximum growth was obtained at 30°C, which is twice as high as that at 20°C.

**Degradation of Biphenyl**

Fig. 4 shows the relationship between cell growth and the degradation of biphenyl during culture of *Pseudomonas* sp. P2 in the media containing 500 mg/l biphenyl. The cell growth of *Pseudomonas* sp. P2 was measured by means of cell mass in the culture broth. The cell mass increased from 80 mg/l to 610 mg/l for 160 h culture. *Pseudomonas* sp. P2 could degrade 9.8% of 500 mg/l biphenyl after 40 h culture. It degraded 40.0% of biphenyl at 80 h, 87.0% at 120 h, and 97.0% at 160 h.

**Degradation of PCBs**

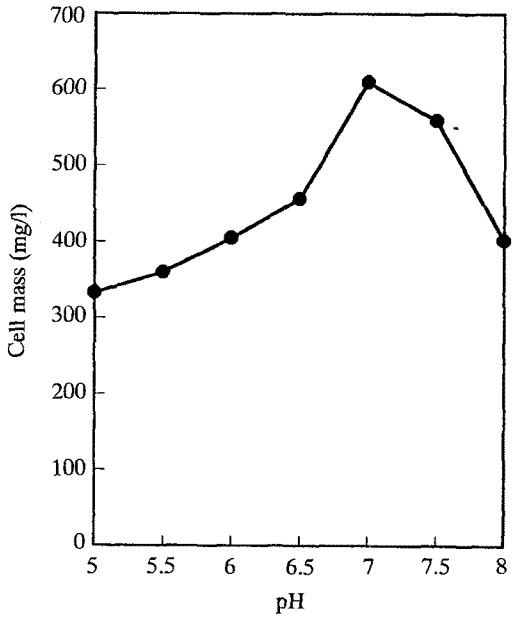


**Fig. 2.** Effect of initial pH of the media on the growth of *Pseudomonas* sp. P2.
Growth is shown as dry cell mass (mg/l) after 100 h culture in a media containing 500 mg/ml biphenyl as a carbon source.

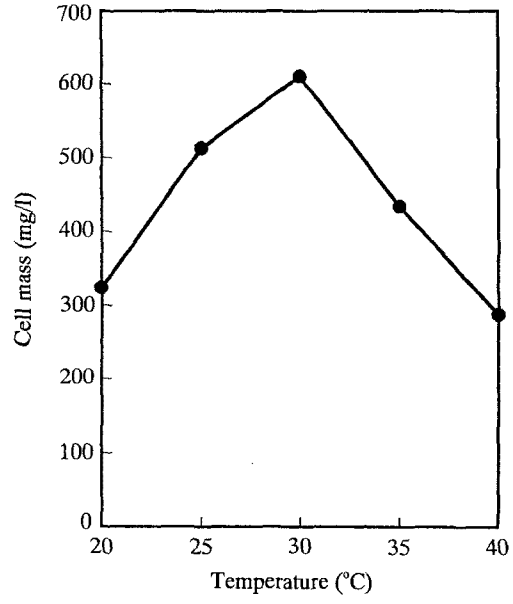


**Fig. 3.** Effect of temperature on the growth of *Pseudomonas* sp. P2.
Growth is shown as dry cell mass (mg/l) after 100 h culture in a media containing 500 mg/ml biphenyl as a carbon source.

In order to confirm PCBs degradation, the residual PCBs content was measured by gas chromatography during culture of *Pseudomonas* sp. P2 in a media containing

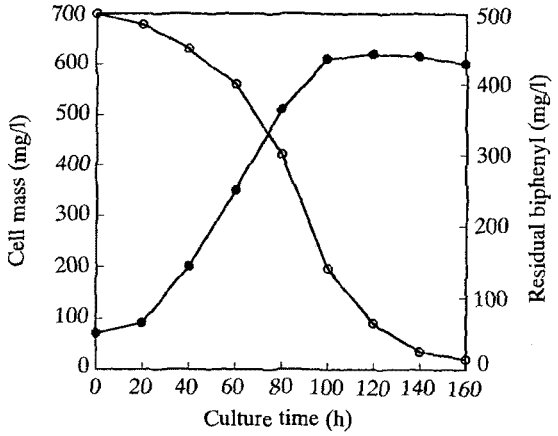


**Fig. 4.** Time course of cell growth and biphenyl degradation during the culture of *Pseudomonas* sp. P2 in a media containing 500 mg/l biphenyl as a carbon source.
●—●, Cell mass (mg/l); ○—○, Residual biphenyl (mg/l).

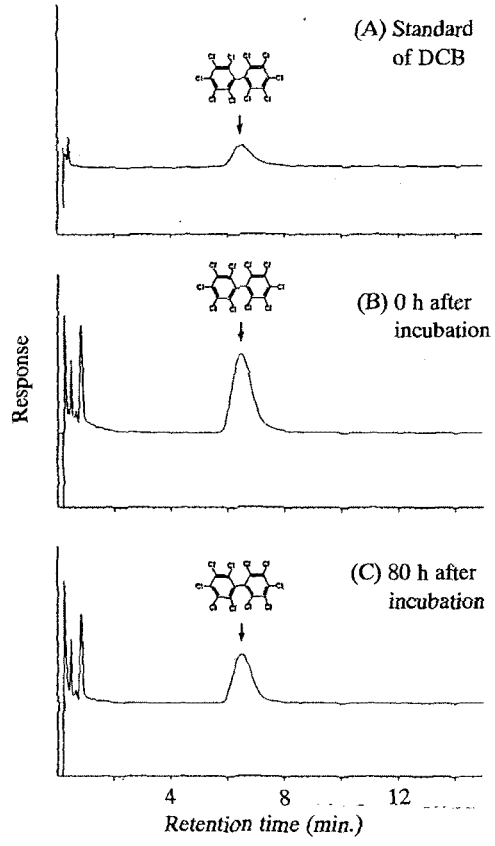


**Fig. 5.** GC-ECD chromatogram for the analysis of polychlorinated biphenyls (PCBs) in a media containing 500 mg/l PCBs during the culture of *Pseudomonas* sp. P2.
*Decachlorinated biphenyl* (DCB) (A) was applied to GC analysis as a standard. PCBs in the media (B) and the culture broth after 80 h cultivation (C) were extracted with hexane and perchlorinated with $SbCl_5$ to DCB, and then DCB was analyzed by GC.

500 mg/l PCBs. The gas chromatograms in Fig. 5 demonstrate the degradation of PCBs within insulating oil by strain P2. The gas chromatogram presented in Fig. 5 A represents the peak of standard decachlorinated biphenyl (DCB) with 6.5 min retention time. The gas chromatograms shown in Fig. 5 B and C were obtained with the perchlorinated form of PCBs in the media (B), and the culture broth after 80 h cultivation of strain P2 (C), respectively. The DCB peaks of Fig. 5 B and C were completely agreed with the standard DCB peak in Fig. 5A. The peak height of DCB was decreased significantly after 80 h culture of strain P2 (Fig. 5 C) compared with that in the media (Fig. 5 B). These results indicate that PCBs were apparently utilized as a carbon source by *Pseudomonas* sp. P2 isolated in this work.

The biodegradability of PCBs within insulating oil was further examined by *Pseudomonas* sp. P2. Fig. 6 shows the relationship between the growth of *Pseudomonas* sp. P2 and the degradation of PCBs added to the media. The cell mass was increased from 130 mg/l to 280 mg/l for 160 h culture. The residual PCBs content was determined during culture and compared with the initial concentration of PCBs in the media. After 40 h incubation, *Pseudomonas* sp. P2 could degrade 2.0% of 500 mg/l PCBs, and degrade 49.0% at 80 h, 60.0% at 120 h, and 60.0% at 160 h. No significant change in PCBs content was observed after 100 h culture. Degradation rate has been reported to be decreased remarkably as more chlorine was substituted in biphenyl. Liu(23) showed that Arochlor 1221 was degraded much faster than Aroclor 1254 by *Pseudomonas* sp. Wong and Kaiser(33) reported that unchlorinated biphenyl was degraded faster than 2-chlorobiphenyl and 4-chlorobiphenyl isomers. In this work, it was also observed that

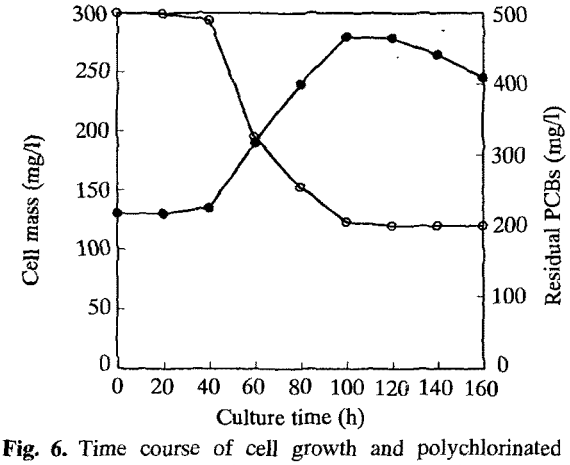


**Fig. 6.** Time course of cell growth and polychlorinated biphenyls degradation during the culture of *Pseudomonas* sp. P2 in a media containing 500 mg/L PCBs as a carbon source.
●—●, Cell mass (mg/l); ○—○, Residual polychlorinated biphenyls (mg/l).

unchlorinated biphenyl could be degraded faster than PCBs within insulating oil by *Pseudomonas* sp. P2 (Fig. 4, 6).

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