Isolation and Characterization of Bacteria Degrading Polychlorinated Biphenyls

Sang-Ki Choi*, Jung-Ho Kim[1]
Dept. of Environmental Science, Keimyung University
[1]Faculty of Environmental Science and Engineering, Kyungsan University

1. Introduction

Among the organic chemicals, polychlorinated biphenyls (PCBs) is one of the most widely studied chemicals in terms of environmental contamination and toxicology with a great social concern[(1)]. Theoretically, PCBs can be represented as many as 210 different compounds containing from 0 to 10 chlorine atoms on a biphenyl[(2)]. PCBs was first synthesized by Schmidt and Schulz[(3)], and has been produced commercially since 1929[(1)] .

Since PCBs is good conductors of heat and bad conductors of electricity, the insulating oil contained PCBs can be applied to transformers and capacitors[(4)]. About 205 ton of insulating oil containing PCBs are still in use of stored in Korea[(5)].

Many studies were done to isolate microorganisms degrading PCBs, but information for microorganisms degrading PCBs within insulating oil is relatively little. Consequently, this study was carried out to screen and isolate strains degrading PCBs, and to investigate the biodegradability of PCBs within insulating oil.

2. Materials and Methods

2-1. Microorganisms and Medium

Strains were isolated from the soil on the Sinchun stream in Taegu. The medium for isolation and cultivation of bacteria degrading PCBs was composed of 1000 mg/L $(NH_4)_2SO_4$, 1000 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[(6)]. Biphenyl as a carbon source was added to

the medium with vapor form for solid medium. 500 mg/L biphenyl or 500 mg/L PCBs as a carbon source was added to the medium for liquid medium.

2-2. Cultivation

By using 150 rpm shaking incubator, the isolated strain was grown at 30℃ for 12 h in 30 mL nutrient broth medium for seed culture. For testing to degrade biphenyl or PCBs, the initial cell mass in the liquid medium containing 500 mg/L biphenyl was adjusted to 70 mg/L, and the initial cell mass in the liquid medium containing 500 mg/L PCBs was adjusted to 130 mg/L. And then, the culture was incubated for 160 h under the same conditions. The determination of cell mass was monitored by filtrating with filter membrane (0.22 ㎛ of pore size). After filtration, cell was dried at 90℃ for 2 h, and determined the cell mass (mg/L).

2-3. Identification

The morphological aspect of isolated strain was determined by a microphotograph (×1000). According to Bergey's manual of systematic bacteriology, the isolated microorganism was identified based on investigating its morphological, physiological, and nutritional characteristics[7].

2-4. Analysis of Biphenyl and PCBs

An analysis of biphenyl and PCBs was performed with the methods of perchlorination that PCBs were converted to decachlorinated biphenyl (DCB). Biphenyl and PCBs in medium were extracted with hexane by a magnetic stirrer for 1 h. The extracted biphenyl and PCBs were perchlorinated to DCB by $SbCl_5$[8].

DCB was analyzed by Varian 3300 gas chromatography equipped with ^{63}Ni electron capture detector[9].

3. Results and Discussion

3-1. Isolation and Identification

Strain P2 were showed a shape of rod of on microphotograph (×1000). Strain P2, which can not grow at 4℃ but can grow at 41℃, indicates to be aerobic, gram negative, and motile. The strain P2 can produce yellow pigment. Oxidase was expressed positive. Arginine dihydrolase and urease

were expressed negative. The strain P2 was negative to voges-proskauer and denitrification. In addition, the strain P2 did not form H_2S and indole, and could not liquify gelatin.

The strain P2 could utilize various nutritional materials 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 acid of alanine, serine, leucine, lysine, glycine, and proline were utilized as energy source by strain P2. No growth was observed in inuline and inositol. These results suggested that the isolated strain P2 should be identified as a strain of *Pseudomonas* sp.

3-2. Degradation of Biphenyl

The growth of *Pseudomonas* sp. P2 was confirmed by means of cell mass in the medium at different time intervals. The growth was varied from 70 mg/L to 620 mg/L for 160 h incubation.

To confirm the degradability of biphenyl, the residual biphenyl in medium was measured by gas chromatography after perchlorination. The chromatogram was demonstrated to the standard decachlorinated biphenyl (DCB) with 6.5 min retention time. After 110 h incubation, the degradation of biphenyl was compared with the peak height of initial biphenyl. The peak height of DCB was decreased after 110 h incubation.

3-3. Degradation of PCBs

The biodegradability of PCBs within insulating oil was further examined by *Pseudomonas* sp. P2. PCBs was obtained directly from insulating oil impregnated with Aroclor 1242[10,11]. The cell mass showed the relationship between the growth of *Pseudomonas* sp. P2 and degradability of PCBs was varied from 130 mg/L to 280 mg/L for 160 h incubation.

To confirm the degradability of the PCBs, the residual PCBs in medium was measured by gas chromatography after perchlorination. The peak height of DCB was decreased after incubation. These results indicated that PCBs was apparently utilized as a carbon source by *Pseudomonas* sp. P2 isolated in this work.

The degradation of PCBs was compared with the concentration of initial PCBs. After 40 h incubation, *Pseudomonas* sp. P2 could utilize 2.0% of 500 mg/L PCBs. *Pseudomonas* sp. P2 could degrade 49.0% at 80 h, 60.0% at

120 h, and 60.0% at 160 h, respectively. PCBs was not significantly altered after 100 h incubation. PCBs with substituted chlorines on a biphenyl was degraded slowly at over 4.75 mg/L per h on 0.29 day^{1} of μ_{max} by *Psdudomonas* sp. P2.

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