JOURNAL OF BIOSCIENCE AND BIOENGINEERING
Vol. 104, No. 4, 268–274. 2007
DOI: 10.1263/jbb.104.268

© 2007, The Society for Biotechnology, Japan

Effects of Inhibitors on Anaerobic Microbial Consortium with Enhanced Dechlorination Activity in Polychlorinated Biphenyl Mixture

Daisuke Baba,[1] Naoko Yoshida,[2] and Arata Katayama[1,2]*

Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8603, Japan[1] *and Division of Integrated Research Projects, EcoTopia Science Institute, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8603, Japan*[2]

Received 24 November 2006/Accepted 10 July 2007

Characterization was carried out on the anaerobic microbial consortium with enhanced degradation activity toward polychlorinated biphenyls in Kanechlor-300 and Kanechlor-400 mixtures in a burnt soil (BS) culture. The addition of molybdate to the BS culture resulted in the accumulation of less-chlorinated biphenyls such as 4,4′-dichlorinated biphenyl and 2,3′,4-trichlorinated biphenyl; however, no such accumulation occurred without molybdate supplementation. No significant effect was observed in individual congeners in the BS culture supplemented with 2-bromoethane sulfonic acid. Analyses involving both the polymerase chain reaction-denaturing gradient gel electrophoresis of partial 16S rRNA genes and respiratory quinones showed that the predominant microorganisms in the BS culture were anaerobic *Firmicutes*, while sulfate reducers of the phyla *Deltaproteobacteria*, *Firmicutes* and *Chloroflexi* were absent in the culture amended with the inhibitors. No positive correlation was observed between the dechlorination activity and a PCR-based detection of gene fragments of known dechlorinating bacteria. These results suggest that sulfate reducers played an important role in the enhanced anaerobic dechlorination of PCBs in the BS culture.

[**Key words:** anaerobic dechlorination, polychlorinated biphenyls, microbial consortium, inhibitor, *Firmicutes*, *Deltaporteobacteria*]

Polychlorinated biphenyls (PCBs) are widely present at low concentrations in the environment, particularly under anaerobic conditions. To remediate environments contaminated with PCBs, inexpensive *in situ* remediation technology is desired. The utilization of microbial activities that degrade PCBs under anaerobic conditions (1) has attracted attention as an inexpensive *in situ* bioaugumentation technology.

As for PCB-dechlorinating microorganisms, *Dehalococcoides ethenogenes* 195, classified into the phylum *Chloroflexi*, was reported to dechlorinate 2,3,4,5,6-pentachlorinated biphenyl (2). Two anaerobic PCB-dechlorinating uncultured microorganisms, namely, DF-1 and *o*-17, also classified into *Chloroflexi*, have been shown to have a correlation between their growth and the reductive dechlorination of PCBs (3, 4). In addition, Yan *et al.* (5) have reported a PCB-dechlorinating culture including *Dehalococcoides* sp. and uncultured bacteria within the phylum *Chloroflexi*. However, the dechlorination activities of these bacteria reported previously were evaluated using only a few PCB congeners. To remediate the actual environments contaminated with PCB mixtures such as Kanechlor, it is necessary to be able to degrade PCB mixtures composed of more than 40 PCB congeners. Many attempts to enrich the microbial consortia with enhanced PCB-degrading activities have been carried out under different incubation conditions (1). The significant dechlorination of a wide range of PCB congeners has not yet been achieved using single isolates; it has been achieved using consortia. However, the successful consortia whose high degrading activities have been maintained in laboratories are very limited: the methanogenic granule in upflow anaerobic sludge blanket reactor (6), the culture originating from Hudson river sediment contaminated with PCBs (7) and the burnt soil (BS) culture originated from a gley paddy soil without contamination history reported in our previous paper (8). The PCB-degrading activity was strongly enhanced using BS in our culture, but was not enhanced significantly in the paddy soil (PS) culture by altering the incubation conditions such as variety of electron donors, variety of electron acceptors, temperature and pH (8). To develop an *in situ* bioaugumentation technology, it is expected that BS will be used as a microbial carrier to support the anaerobic PCB-degrading activity and that the BS culture will be used as a microbial source. The characterization of the BS culture is essential for the development of the remediation technology, but it has not been carried out yet.

In this study, we characterized the anaerobic microbial

* Corresponding author. e-mail: a-katayama@esi.nagoya-u.ac.jp
phone: +81-(0)52-789-5856 fax: +81-(0)52-789-5857

consortium in the BS culture by the effects of inhibitors on the PCB-degrading activity, the polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) analysis of partial 16S rRNA genes, respiratory quinone analysis and specific PCR targeting members of dechlorinating microorganisms.

MATERIALS AND METHODS

PCBs A PCB mixture of Kanechlor-300 and Kanechlor-400 (Kanegafuchi Chemical Industry, presently Kaneka Corp., Osaka) at 1:1 (w/w) was prepared as an acetone solution of 1 g/*l*, and used as a stock solution.

PS and BS PS was collected from the plowing layer of gley lowland soil in the Kuridashi region of Aichi, Japan (FAO/UNESCO classification: Eutric Fluvisol), which had no history of PCB contamination. The PS was stored under flooded condition at 22°C in the dark until use after passing it through a 2-mm sieve. BS was prepared by air-drying and calcining PS at 550°C for 24 h. The properties of the PS and BS are reported elsewhere (8).

PCB-dechlorinating microbial source PCB-dechlorinating Kuridashi PS (9) was used as the microbial source.

Maintenance of PCB-degrading anaerobic consortium in BS medium The BS was sterilized by autoclaving at 121°C for 30 min three times, and 4 g of the sterilized BS was placed in a 100-ml glass vial and spiked with 100 μg of the PCB mixture to prepare 100 μl of the stock solution. Then, 15 ml of acetate/lactate (AL) medium was added under anaerobic condition using an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA) for microbial growth and PCB degradation. The composition of the AL medium was as follows (g/*l*): K_2HPO_4, 0.5; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.02; yeast extract, 0.5; sodium lactate, 10; sodium acetate, 8; and resazurin, 0.001 (pH 7.0, adjusted using by KOH). The vial was filled with 100% nitrogen and sealed with a Teflon-coated butyl rubber stopper. The prepared medium containing AL medium and BS was designated as BS medium. The PCB-degrading microbial source was inoculated into BS medium anaerobically at a rate of 5% (v/v) as a soil suspension and incubated statically at 30°C in the dark for 56 d, which was designed as BS culture. After the incubation, the residual PCBs in the BS culture were analyzed, and the BS culture was transferred to new BS medium at a rate of 5% (v/v). The PCB-degrading BS culture was maintained using this procedure of incubation and transfer. To evaluate the characteristics of the microbial consortium in the BS culture, the PS culture was also prepared and maintained using the same procedure as that used for the BS culture using PS instead of BS.

Effects of inhibitors on PCB degradation To examine the effects of inhibitors on the anaerobic PCB-degrading activity of the consortium, 2 mM sodium molybdate, an inhibitor of sulfate-reducing bacteria (10), or 2 mM 2-bromoethane sulfonic acid (BES), an inhibitor of methanogens (11), was added into the culture. After 56 d of incubation, the effects on the anaerobic PCB degradation were evaluated by comparing the amount of PCB residue in the BS culture with inhibitor with that in the culture without any inhibitors. Experiments were performed on duplicate samples.

Gas analysis The concentration of methane in the head space of the vial was determined using a gas chromatography system equipped with a flame ionization detector (GC-14B; Shimadzu, Kyoto).

Measurement of sulfate concentration in culture The concentration of sulfate in the culture was measured using an ion analyzer (IA-200; TOA-DKK Corp., Tokyo) according to the manufacture's instructions.

Analysis of PCB residues The PCB residues were extracted from the culture with a chloroform-methanol (2:1) mixture and purified using a silica gel column and a deactivated Florisil column with *n*-hexane. The purified PCB fractions were subjected to analysis using a Shimadzu gas chromatograph GC-2014 (Kyoto) equipped with a ^{63}Ni electron capture detector and a HT8 capillary column (0.22 mm inner diameter, 50 m length, 0.25 μm film thickness; SGE, Austin, TX, USA). Forty-four PCB congeners were identified, including congeners overlapping on the chromatogram. The detection limit was 35.7 ng for the individual PCB congeners in the culture spiked with 100 μg of the Kanechlor-300/400 mixture. The recovery rate for total PCBs was 92.9%±5.5% (w/w) among all the cultures, whereas the recovery rate for each congener was at least 83.6%±2.5% (w/w). Neither monochlorinated biphenyls nor biphenyls were detected under this condition. The analytical data was corrected using the recovery rate for total PCBs and the individual congeners. The degradation activity was evaluated as the percent reductions in total PCBs and in individual PCB congeners by comparing the amount of PCB residue remaining in the culture with that remaining in the sterilized control after incubation: (the residual amount of PCB in each condition sample)/(the residual amount of PCB in sterilized sample). The dechlorination ratio was calculated as (the mol percent reduction in total chlorine atoms)/(the mol percent reduction in total PCBs), which indicated that dechlorination occurred at the ratio higher than 1. In the sterilized control after the incubation, no degradation was observed, as shown by the total amount of PCB residue of 99.1±3.4 mol% with the lowest residual amount of the individual PCB congeners of 83.6±2.5 mol%. The statistical significance of the effects of the inhibitors on PCB degradation were evaluated by analysis of variance (F-test) followed by a Bonferroni multiple comparison test. The procedure used to analyze the PCB residues is described elsewhere in details (8).

Respiratory quinone analysis Respiratory quinones were extracted from the culture with a chloroform-methanol (2:1) mixture at the same time that the PCB extraction was carried out. The respiratory quinones in the extract were fractionated with Sep-Pak Plus Silica cartridges (Waters, Milford, MA, USA), where the menaquinone fraction was eluted with *n*-hexane containing 2% diethyl ether, and the ubiquinone fraction was eluted with *n*-hexane containing 10% diethyl ether; both fractions were dried under a nitrogen stream. The quinone fractions, which were dissolved in 100 μl of acetone, were analyzed using a high performance liquid chromatography system equipped with a photodiode array detector and two ZORBAX ODS columns connected in series (4.6 mm inner diameter, 250 mm length; DuPont, Wilmington, DE, USA). The mobile phase (1.0 ml/min) was a 9:2 mixture of methanol and isopropyl ether. The analytical procedure used for respiratory quinones was described previously in detail (12).

DNA extraction and PCR-DGGE analysis The genomic DNAs of the microbial consortia in the cultures were extracted and purified using an ISOIL extraction kit (Nippon Gene, Tokyo) according to the manufacturer's instructions. PCR was used to amplify partial 16S rRNA genes from the purified DNA for DGGE analysis using a thermal cycler (Takara-Bio, Shiga). Bacterial 16S rRNA genes were amplified with a primer set from 341F to 518R (13). Archaeal 16S rRNA genes were also amplified using a primer set from 344F to 518R (14). The thermal program was as follows: 94°C for three min (initial denaturation), 94°C for 30 s, 55°C for 30 s and 72°C for one min (35 cycles for amplification) and then a final extension step of 72°C for 10 min. The reaction mixture was prepared as 100 μl of solution in a 200-μl tube as follows: the individual primers, 50 pmol/μl; *ExTaq* DNA polymerase (Takara-Bio), 2.5 U; *ExTaq* buffer (Takara-Bio), 10 μl; dNTP mixture (2.5 mM each dNTP), 10 μl; DNA template (25–35 ng), 1–2 μl; and distilled water, 74 μl. The amplified PCR products (about 0.2 μg) were subjected to DGGE analysis using a gradient, ranging from 40% to 70%, of the denaturants of formamide and urea in the polyacryl-

Download English Version:

https://daneshyari.com/en/article/22475

Download Persian Version:

https://daneshyari.com/article/22475

Daneshyari.com