Suyin YANG[1], Atsushi SHIBATA[2], Naoko YOSHIDA[2], Arata KATAYAMA[1,2]

Nagoya University, Graduate School of Engineering[1]

Nagoya University, EcoTopia Science Institute[2] Japan

ANAEROBIC BIODEGRADATION OF PHENOL DERIVATIVES

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1. Introduction

Phenol and phenol derivatives (such as chlorophenols and alkylphenols) are among the most common water/groundwater contaminants. In addition, they contribute 40% to 80% of the total COD in effluents from wastewater treatment. These phenolic compounds are highly toxic, especially, chlorophenols which have been extensively used as wood preservatives, herbicides, and fungicides, are suspected carcinogen. Since environments polluted with phenols often become anoxic, where organic matter is degraded through microbial activity, and oxygen is unavailable due to its low solubility in water and low rate of transportation, removal of phenolic compounds using anaerobic microorganisms is a viable and cost-effective remediation method for phenolic compounds-containing water and waste-water.

Anaerobic degradation of phenolic compounds has been reported under denitrifying, sulfate-reducing, iron-reducing or methanogenic conditions [1, 2]. Some pure phenol-degrading cultures using nitrate or sulfate as electron acceptors have been isolated [3, 4]. A dissimilatory Fe(III)-reducing isolate (GS-15) have been reported capable to use phenol [5]. No bacterium degrading chlorophenol (CP) has been isolated under denitrifying or Fe(III)-reducing conditions. Moreover, little substantial information about the microbial populations responsible for the CP degradation is available even for mixed cultures.

In this study, first, we attempted to establish the enrichment cultures from paddy soil dedrading phenol and monochlorinated phenols under anaerobic conditions using different electron acceptors. Secondly, the microbial communities of the enrichment cultures were characterized, respectively.

2. Materials and Methods

2.1. Enrichment method of phenolic compounds-degrading anaerobic cultures

A paddy soil without any contamination was used to enrich phenol and monochlorophenols-degrading cultures obtained from a rice field located at Kamajima in Aichi Prefecture, Japan. The soil sample was sieved by 2 mm of sieve, suspended in tap water, and then stored at 22 °C until use. Approximate 30 g of soil was added to 600 ml of serum bottle containing 50 ml of sterile water with 1mg of phenol under a stream of nitrogen gas. During three years of incubation, phenol-water solution was repeat added once phenol was depleted. And then, 5 ml of this soil slurry was transferred to 60 ml of serum bottle filled with 40 ml of sterile anaerobic salt medium with pH about 7.0. Nitrate, sulfate, or amorphous Fe(III) oxide (FeOOH) stock solution as electron acceptor and phenol, 3-chlorophenol (3-CP), or 4-chlorophenol (4-CP) as electron donor were added to the serum bottle, respectively. All the samples were incubated without shaking at 30 °C. After substrates were significantly depleted, 20% of subculture was serially inoculated to fresh medium to get enrichment culture.

2.2. Chemical and phylogenetic analytical methods

Phenol, 3-CP, and 4-CP were extracted and analyzed using a GC-MS equipped with a DB-5MS column. Fe(II) was measured spectrophotometrically by a 1,10-phenanthroline method. Sulfide was analyzed with spectrophotometric methylene blue method. DNA was extracted from the enrichment cultures by using the ISOIL DNA extraction kit (Nippon Gene Co., Tokyo, Japan) according to the manufacturer's instruction. 16S rDNA gene fragments were amplified and separated by applying PCR (Polymerase Chain Reaction)-DGGE (Denaturing Gradient Gel Electrophoresis). Major bands were excised from the DGGE gel, purified by using a Wizard Plus Minipreps DNA Purification System (Promega, Madison, WI, USA) and subcloned with pT7Blue Perfectly Blunt Cloning Kit using the manufacturer's protocol (Novagen, Madison, WI, USA) and the DNA sequences were analyzed by a GENETYX program (Version 7, Software Development CO., Tokyo, Japan).

3. Results

3.1. Degradation of phenolic compounds by anaerobic cultures

Fast phenol degradation (50 μM per one week) was observed after three years of incubation with original soil slurry. During serially transfer cultivation, even though phenol degradation rate became rather slower under sulfate-reducing conditions but phenol degradation capacity maintained stably after four times of transfer. Finally, approximate 50 μM

of phenol was degraded in three weeks under sulfate-reducing conditions, and 200 μM of phenol was degraded in one week under iron-reducing conditions.

3-CP (50 μM) was degraded under iron-reducing conditions after one year of incubation, but not in sulfate-reducing conditions during fist time of incubation. Unfortunately, no 3-CP degradation occurred in iron-reducing culture after re-addition of 3-CP within 150 days of incubation. 4-CP degradation was observed under both sulfate-reducing and iron-reducing conditions with three months of lag phase. Re-addition of 4-CP led to a faster degradation (50 μM of 4-CP was degraded in two weeks) only under iron-reducing conditions, indicating that 4-CP-degrading culture were enriched. In following sequentially transfer cultivation, the 4-CP-degrading capability was lost with iron as electron acceptor after four times of transfer into freshly prepared medium, which might be caused by the reduction and disappearance of soil in the medium as biocarrier or supplying some organic matter. Thus, glass beads (0.5 mm) or 1ml of autoclaved soil slurry was added into medium to try to maintain degradation capacity. Finally, 4-CP degradation was maintained and enhanced with the addition of 1 ml of autoclaved soil slurry but not glass beads under iron-reducing conditions during the following transfers.

Degradation of 4-alkylphenol as one of phenol derivatives was observed under anaerobic conditions. In addition, corresponding anaerobic strain was isolated using nitrate as electron acceptor.

3.2. Phylogenitic characterization of enrichment cultures

The PCR-DGGE profile of the phenol-degrading sulfate-reducing and iron(III)-reducing cultures and 4-CP-degrading iron-reducing culture during serially- transferred cultivation were compared with that of the control cultures without the addition of phenol or 4-CP. There was no apparent difference in band pattern between active culture and control culture, which may because that 20% of culture transferred to new medium without phenol or original DNA from 1 ml of autoclaved soil slurry was also amplified during PCR. Sequence analysis of three clones derived from DGGE bands of sulfate-reducing phenol-degrading cultures fell within the *Deltaproteobacterium*, one showed 92% similarity with reported phenol-degrader. Two of four bacterial DGGE in fragments from phenol-degrading iron-reducing culture had 87-88% of similarity with the member of *Geobacter*. One of them was reported with the capacity degrading oil. *Geobacter* was detected in 4-CP-degrading iron-reducing cultures showing 86% similarity with uncultured clone related to hydrocarbon and chlorinated solvent degrader. *Firmicutes* were detected in both phenol-degrading and 4-CP-degrading iron-reducing cultures.

4. Conclusions

Microorganisms with capacity degrading phenolic compounds are widely distributed in anoxic environments. In addition, distinct microbial populations are enriched or isolated with different compounds as electron acceptors by sequentially transfer cultivation. These enrichment cultures could be considered as inocula for treatment of phenolic compounds-contaminated water. A detailed characterization of these anaerobic microbial communities is needed to fully understand their role during the degradation of these phenolic compounds under anaerobic conditions.

References

[1] Kazumi, J. Haggblom, M.M. Young, L.Y. Degradation of monochlorinated and nonchlorinated aromatic compounds under iron-reducing conditions. Appl Environ Microbiol, 1995, 61:4069-4073.

[2] Häggblom, M.M. Young, L.Y. Chlorophenol degradation coupled to sulfate reduction. Appl Environ Microbiol, 1990, 56:3255-3260.

[3] Bak, F. Widdel, F. Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium* phenolicum sp. nov. Arch microbial, 1986, 46:177-180.

[4] Boopathy, R. Isolation and characterization of a phenol-degrading, sulfate-reducing bacterium from swine manure. Bioresource technology, 1995, 54:29-33.

[5] Lovey, D.R. Lonergan, D.J. Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron-reducing organism, GS-15. Appl Environ Microbiol, 1990, 56:1858-1864.