EFFECT OF MICROBIAL INHIBITORS ON ANAEROBIC DEGRADTION OF DDT

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Introduction

In this study, a mixed anaerobic culture enabling to dechlorinate DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane] was obtained from river sediment in Taiwan. For understanding the effect of these microorganisms on DDT dechlorination, microbial inhibitors BESA (2-bromo-ethanesulfonate) and molybdate, for inhibiting methanogenic and sulfate-reducing bacteria, respectively, were chosen to investigate the interaction between specific microbial communities and their degradation activities. Besides, a molecular technique, denaturing gradient gel electrophoresis6 (DGGE), based on analyzing the 16S rDNA of bacteria, was used for monitoring the bacterial community structure.

Methods and Materials

Chemicals: *p, p'*-DDT with 98% purity was purchased from Riedel-deHaën Co, Germany. HPLC-graded solvents used in this experiment, including *n*-hexane and acetone were purchased from E. Merck Co, Germany.

Culturure: The sediment was collected from the Er-Jen River, a serious contaminated river located at southern Taiwan. Anaerobic mixed culture was prepared by mixing sediment (100g) and culture medium (400 mL) in a 1-L serum bottle to make a slurry-like culture under a modular atmosphere controller system (dwscientific Co, England) filling with N_2, H_2, and CO_2 gases (85:10:5).

Batch procedures: The batch degradation of DDT were performed by adding 5 mL of anaerobic mixed culture to a 125-mL serum bottle containing 45 mL of culture medium, and then 10 μg/mL of *p,p'*-DDT was spiked to serum bottles. In the inhibitor study, BESA or molybdate was additional added in final concentrations of 5 mM and or 50 mM, respectively.

Residue analysis: The residue of DDT in sample culture was extracted by *n*-hexane, and then analyzed by GC-ECD.

DNA extraction: At regular intervals, 3 mL of anaerobic mixed cultures was taken and extracted by using an UltraClean Soil DNA kit (MO BIO Laboratories, Inc).

PCR-DGGE: Polymerase chain reaction (PCR) was carried out with a thermal cycler 9700 (Applied Biosystem, USA). Bacterial 16S rDNA was specifically amplified by using the primer pairs as described by Gelsomino et al.[1]. A GC-clamp of 40 bases, as described by Muyzer et al.[2], was attached to the 5'-end of the forward primer. DGGE analysis was performed using a D-Code universal mutation detection system (Bio-Rad, Hercules, Calif.). PCR product (20 μL) was loaded onto a 7% (w/v) denaturing gradient polyacrylamide gel consist of 40% to 60% of formamide and urea from up side through bottom. The electrophoresis was run at 60°C in 1X TAE for 12 hours at a constant voltage of 75V. After the electrophoresis, gel was stained with SYBR Green I nucleic acid gel stain, visualized on an UV transilluminator, and photographed with a digital camera.

Table 1: The half-life of *p,p'*-DDT in the addition of inhibitors under anaerobic conditions

Treatment	*p,p'*-DDT	
	k	$t_{1/2}$(days)
Control	0.13	5.15
5 mM BESA	0.09	7.69
50 mM BESA	0.1	7.1
5 mM Molybdate	0.1	6.8
50 mM Molybdate	0.09	7.4
50 mM BESA+Molybdate	0.07	10.4

k: rate constant
t1/2: half-life

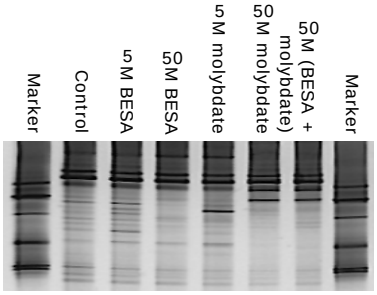

Figure 1: PCR-DGGE analysis of 16S rDNA sequence fragments obtained from the DDT-degrading consortium incubated with microbial inhibitors (BESA or molybdate) for 15 days.

Conclusion

Our results showed the dechlorination of *p,p'*-DDT was delayed while microbial inhibitors added, and 50mM of both BESA and molybdate added lead a higher degree of inhibition than any other treatment in DDT-dechlorination. This result implied that dechlorination of *p,p'*-DDT was involved in a complicated interaction between microbial communities.

The DGGE analysis of the 16S rDNA fragments obtained from mixed cultures indicated that microbial community structure was shifted during the incubation periods.

Addition of 5 mM of BESA did not affect the DDT-degrading bacterial community structure, but the bacterial community structure was slightly changed by adding 50 mM of BESA. This result implied that the inhibition of microbial DDT-degrading ability by BESA was due to the suppression of methanogenic bacteria. The supplementation of 5 or 50 mM of sulfate-reducing bacteria inhibitor molybdate caused in changing of bacterial community structures.

References

1.Gelsomino A., Keijzer-Wolters A.C., Cacco G., van Elsas J.D. (1999) J. Microbiol. Method. 38, 1-15.
2.Muyzer G., De waal E.C., Uitterlinden A.G. (1993) Appl. Environ. Microbiol. 59, 695-700.