DEGRADATION OF 3Cl-DIOXIN AND THE DIOXIN PATHWAY BY TWO BACTERIA

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Introduction

In 2000, we reported four bacteria that decompose PCB, DF and DD. Therefore, we investigated the degradation of metabolites of 3Cl-DBD, DF and DD by these bacteria.

Materials and methods

1. Preparations of bacteria

Two strains of bacteria were used: *Proteus* sp., and *Bacillus* sp.

2. Chemicals and standards

1) Dibenzofurans (DF), 2) Dibenzodioxines (DD), 3), Three chloride dibenzodioxin (3Cl-DD), 5) Benzaldehyde, 6) Benzyl alcohol, 7) Dichlorobenzoic acid, 8) Chlorobenzoic acid.

3. Dioxins degradation experiment

Resting cell suspensions were prepared from cultures grown aerobically at 25℃ for 1 day. The cells were harvested by centrifugation (10000rpm, 10 min), and washed with a phosphate buffer (20 mM, pH 7.1). The harvested cells were then suspended to give an OD of 2.0 at 600 nm in 20 ml of PAS medium containing each it of listed dioxin and other chemicals. The mixture was shaken for 72 hours at 25℃.

3) Extraction and analysis of dioxins

After incubation, the reaction were stopped by heating to 70℃ for 20 min or by adding perchloric acid to a final concentration of 0.5 %. Subsequently, 10 % SDS 1 ml was added, and the dioxins were extracted from the cells with 4 volumes of mixed solvent (*n*-hexane: diethyl ether, 6:4). The samples were shaken vigorously for 10 min with a reciprocating shaker. The phases separated without centrifugation, and the extract samples were removed by drying over Na_2SO_4. Samples of extracted PCBs were analyzed on GC/MS (Shimadzu GC-17A) using a CB-5 capillary column (25 m by 0.25 mm internal diameter). Operating parameters for the GC/MS were as follows: injector 270℃, He carrier gas 0.8ml/min, splitless. The oven temperature was initially maintained at 40℃ for 1 min and then increased to 200℃ at 10℃/min and finally to 250℃ at 4℃/min and maintained at 290 ℃ for 5 min.

Results and discussion

(1) Degradation of 3Cl-DD

The degradation rate of the 2 strains on 3Cl-DD by 10ppm is shown in table 1. After one hour, SN-49910 degraded 8ug, and SF-2001 degraded 4ug of 3Cl-DD, and after 72 hours, SN-49910

degraded 172.4ug and SF-2001 degraded 156.6ug.

Table 1 Time course of degradation % of 3Cl-DD by the two strains

	Hours			
	0	1	24	72
SN-49910	0	4	72.4	86.2
SF-2001	0	2	57.1	78.3

2) Intermediary metabolites of 3Cl-DD and DF degraded by the SN-39910 strain.
We investigated the degradation of DD and DF by the SN-49910 strain. Benzoic acid, benzaldehyde and benzyl alcohol were detected. For 3Cl-DD, chrolobenzoic acid and dichlorobenzoic acid were detected. We are evaluating other intermediate metabolites to determine the metabolic pathway.

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