Degradation of dioxin by four bacteria and their characteristics

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Introduction

Dioxins are very toxic chemicals and have recently been found in soils and rivers at several sites in Japan, which must be cleaned. Microorganisms that are able to degrade dioxins may be used as a safe and environmentally friendly method of restoring the ecosystem. Therefore it is important to know the capacity and vitality of microorganisms that degrade dioxins and dibenzofurans. This study isolated bacteria capable of degrading dioxins and dibenzofurans. We screened four possible dioxin-degrading bacteria, and isolated a useful bacterium.

Materials and methods

(1) Preparations of bacteria
We used four strains of bacteria, which were one strain selected among 11 pcb-degrading bacteria previously isolated , 2 strains isolated from rice plants and natto (fermented soybeans) isolated and 1 strain isolated from dioxin-polluted fields.
The strains were named SN-49910, SF-2000, SF-2001 and SF-2002.

(2) Identification of bacteria
The bacteria were identified by the homology of their 16S R-ribonucleic acid. The basic arrangement was determined by the PCR product using a direct unilateral primer by the epoxy termination method (ABI PRISM). The isolates were identified based on morphological and biochemical characteristics.

(3) chemicals
Dioxin chemical standards were consisted of:
1)Dibenzofurans(DBF),2)dibenzodioxines(DBD),3)monochlorodibenzodioxine (1Cl-DD), 4) monochlorodaibenzofuran (1Cl-DF),5) PCB (KC-400).6) Dioxins and PCBs were extracted from fly ash (A Dioxins Analytical Methods of Japan).

(4) Dioxins degradation experiment
Resting cell suspensions were prepared from cultures grown aerobically at 25 °C for 1 day. The cells were then harvested by centrifugation (10000rpm, 10 min), and then washed with phosphate buffer (20 mM, pH 7.1)., After that the harvested cells were suspended to give an OD of 2.0 at 600 nm in 20 ml of PAS medium containing each listed dioxin and other chemicals. The mixture was shaken for 5 days at 25 °C.

(5) Extraction and analysis of dioxins and PCBs

After incubation, the cultures of dioxins and other chemicals were stopped by heating to 70 °C for 20 min or by adding perchloric acid to a final concentration of 0.5 %. Then, 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-15A) with CB-5 capillary column (25 m by 0.25 mm internal diameter). We measured the dioxins and dibenzofurans degradation rate of four isolated strains. These fly ash extract solutions were analyzed by high-resolution GC-mass spectrometry (selected SIM mode) with JMS-SX102A and a fused silica SP-2331 capillary column (60 m by 0.22 mm i.d.). Operating parameters for the GC were as follows: injector 270, He carrier gas 0.8ml/min, split less. The oven temperature was initially maintained at 100 °C for 1 min and then increased to 250 °C at 8 °C /min and finally to 290°C at 4 °C /min and maintained at 290 °C for 5 min.

Result and discussion

(1) Identification of dioxin bacteria

SN-49910 is made up of aerobic Gram-negative rods, and other 3 strains is aerobic Gram-positive rods. The score by the 16SrRNA method was also identified anyway in the high homology of 900-1100, when this identification result was observed. The SN-49910 strain is *Proteus* sp., the SF-2000 and the SF-2001 strains were *Bacillus subtitles* of gram-positive bacteria. The SF-2002 strain was identified with *Bacillus* sp.

(2) Degradation of DBD, DBF and 1Cl-DBD

The degradation rate of the 4 strains on DBD, DBF of DBF and 1Cl-DBD 10ppm by is shown in table 1. In DBD, the SF-2000 degraded 98.42%, and SF-2002 degraded 98.43% of DBF, and SF-2001 degraded 85.32%. These strains showed a high ability to degrade non-chlorinated DBD and DBF.

Table 1 Degradation of DBD, DBF and 1Cl-DBD(%)

	SN-49910	SF-2000	SF-2001	SF-2002
DBD	98.33	98.42	98.21	98.18
DBF	79.53	81.43	87.44	98.43
1Cl-DD	64.31	70.21	85.32	78.5

(3) Degradation rate of KC-400

Degradation rate of KC-400 by the 4 strains is shown in table 2. SF-2001 degraded 91.40 (10ppm) and 97.26% (100ppm) of KC-400. The strain also was degraded more than 87.5% of 3,4', 3,4'-Tetrachlorobiphenyl(coplanar-PCB) in KC-400.

Table 2. Degradation rate of KC-400 (10 and 100ppm) by 4 strains

	SN-49910	SF-2000	SF-2001	SF-2002
KC-400 10ppm	72.94	90.32	91.40	83.71
KC-400 100ppm	64.30	75.12	97.26	87.32

(4) Degradation of PCBs and dioxins extracted in fly ash

PCBs extracted in fly ash composed 35ppm. TCDD and TCDF were TEQ-690ng/g and TCDFs were TEQ-1900ng/g in Extract 2. The degradation rate of PCB and dioxins in fly ash were degraded by 4 strains. The results were shown in tables 3. Of the 4 strains, SF-2001 showed the highest average degradation rate in PCB extracted in fly ash. Tow strains were degraded 97.26 and 87.86% of PCBs, which are higher than the degradation rate of a culture with KC-400 1 ppm added. The degradation of each isomer differed for each strain. The degradation rate of PCBs, TCDD and TCDF were good except for that of the SF-2000. The degradation rate of the SF-2001 was good, and exceeded 90% on TCDD and TCDF.

Table 3. The degradation rate of PCB and dioxins in fly ash were degraded by 4 strains.

	SN-49910	SF-2000	SF-2001	SF-2002
Ext.1(PCB)	97.01	35	94.86	97.6
TCDD	79.56	25.63	97.2	88.61
TCDF	80.21	12.3	92.01	62.35

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