CHARACTERISTICS OF DEGRADATION OF DIOXINS BY MIX-BACTERIA

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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 useful 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 mix bacteria capable of degrading dioxins, dibenzofurans and coplanar PCB in soil from a polluted site. We screened a few possible dioxin-degrading bacteria.

Materials and methods

(1) Preparations of bacteria

We used mixbacteria, which were used to make a compost of farm animal excreta.
The strains were named MIX-2002.

(2) Identification of bacteria

The bacteria were identified by the homology of their l6S R-ribonucleic acid and other methods. 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) Dioxin-polluted soil

We used dioxin-polluted soil from Kanagawa Prefecture, Japan, for this experiment.

(4) Dioxin degradation experiment

Inorganic liquids containing mix bacteria were prepared from cultures grown aerobically at 25 ºC for 90 days. The cultures were sometimes turned over 1 time per 7 days using a shovel, and a small amount of water was added.

(5) Extraction and analysis of dioxins and Co-PCB

After incubation, 1g was gathered from each culture, followed by heating to 70 ºC for 20 min, or by adding perchloric acid to a final concentration of 0.5 %. Then, the dioxins were extracted from the cells with 4 volumes of ethyl acetate.

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 a GC/MS (Shimadzu GC-15A) with a CB-5 capillary column (25 m by 0.25 mm internal diameter). We measured the dioxins, dibenzofurans and Co-PCB degradation rate of the mix culture. These 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.) and Immunoassay method. The operating parameters for the GC were as follows: injector 270 ºC, He carrier gas 0.8ml/min, split less. The oven temperature was initially maintained at 100 ºC for 1 min and then increased to 250 ºC at 8 ºC/min and finally to 290 ºC at 4 ºC/min and maintained at 290 ºC for 5 min.

We used the standard method of the Japanese Environmental Agency to analyze for polluted soils.

Result and discussion

(1) Identification of mix bacteria

MIX-2002 is made up of 7 aerobic Gram-negative 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 MIX-2001-1strain is *Allucarygenes* sp., the MIX-2002-2 and the MIX-2002-3 strains were *Bacillus* sp. of gram-positive bacteria. The MIX-2002-4 strain was identified as Colyneform.

(2) Degradation of dioxins in the polluted soil (pg/g)

The degradation percentage of the dioxin-polluted soils by mix 2002 is shown in table 1. These strains showed a high ability to degrade all of the dioxins.

Table 1. Degradation of dioxins in the polluted soil (pg/g)

	start	90days	Degradation %(TEQ/G)
Total Dioxins	3300	1016	69.2
Total Dibenzofurans	5500	3063	44.3
Total Co-PCBs	460	235	48.9
Total	9260	4314	Average 53.4

(3) Time course of degradation of dioxins(pg-TEQ/g) by MIX 2002

The degradation rate of MIX 2002 on dioxin-polluted soil is shown in table 1. After 30 days, MIX 2002 degraded 2400 pg-TEQ and after 60 degraded 4100 pg-TEQ/g.

Table 1. Time course of degradation percentage (pg TEQ/g) of polluted soil by MIX 2002

Time(Day)	0	30	60	90
MIX 2002	0	26.1	44.6	64.1

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