Degradation of tributyltin by marine microorganisms in sediment collected from Tokyo Bay

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INTRODUCTION

The environmental concern about the pollution of organotin compounds has increased considerably in recent years. Tributyltin (TBT) was most widely used as effective antifouling paints, wood preservatives and so on. The release of TBT from antifouling paints has been shown worldwide to be marine contamination and toxic to non-target biota such as gastropod. TBT has been considered as one of endocrine disrupter chemicals, responsible for the imposex syndrome in the female in several species of aquatic organisms. However, there have been a few researches concerning microbial breakdown of the discharged TBT and isolation of organotin-degrading microorganism. Recently Kawai et al.¹⁾ reported that tributyl phosphate (TBP)-degrading terrestrial *Pseudomonas* strain also had ability to degrade TBT. No report, however, has been yet available concerning TBT-degrading marine organisms. Therefore, we investigated TBT-degrading activities of the microbes in the sediment collected from various areas and found a notable activity from Tokyo Bay. In addition, two types of bacteria that degraded TBT were isolated from the sample to apply them to marine bioremediation.

MATERIALS AND METHODS

Materials

Yeast Nitrogen Base (YNB) was purchased from Difco Laboratories, Detroit, MI, USA. Tributyltin chloride (TBTCl) and triphenyltin chloride (TPenTCl) were obtained from Tokyo Kasei (Tokyo, Japan). TPenTCl was used as internal standard to correct for loss of extraction procedure and injection volume. Stock solutions of TBTCl and TPenTCl were prepared by dilution with hexane. The solvent used for the extraction (hexane, methanol, ethylacetate and diethyl ether) of these organotins were analytical-reagent grade.

Collection of samples and culture conditions

Sediment samples were collected from various sea areas such as Tokyo Bay, Sagami Bay and Off-Boso during the KT-00-9 cruise on the research vessel Tansei-maru of the Ocean Research Institute, University of Tokyo using a multiple-corer sediment sampler. YNB medium prepared with artificial water containing 100 µg/L TBT was used for the isolation of TBT-degrading microbes. About 10 g of sediment sample was suspended in 100 ml of the culture medium and an aliquot of 2 ml was inoculated to 20 ml of the same medium and incubated at 27°C for 21 days in the dark (first enrichment culture). After incubation, an aliquot of 200 µl of the broth was transferred to fresh medium (20 ml) and further incubated under the same conditions (second and third enrichment culture). Control experiments without sediment sample were also performed in order to detect possible non-biological elimination under the same conditions. In addition, autoclaved sediment samples were used as control during enrichment culture to inspect whether the degradation was participated by microorganism or not.

Analytical procedure for TBT

Sample preparation for culture media containing sediment sample was carried out according to the procedure shown in Fig. 1²⁾. 0.2 ng of TPenCl was added to culture medium as internal standard. Gas chromatography (GC) was performed on a Hewlett-Packard (Avondale, PA, USA) 5890 series II gas chromatograph equipped with a flame photometric detector (FPD) with a 611.6 nm filter. The GC column used was a fused-silica capillary column (15 m×0.25 mm id) coated with DB-1 (0.1 µm film thickness) (J&W Scientific, Rancho Cordova, CA, USA).

16S rDNA analysis

Total bacterial DNA was obtained by ordinary method.³⁾ Subsequently, a partial region of the 16S rDNA was amplified PCR using Taq DNA polymerase (Takara

Shuzo, Kyoto) and primer set, corresponding to positions 8-27 (forward primer) and 1492-1510 (reverse primer) was used. The thermal profile involved 25 cycles: 94 °C for 60 s, 60°C for 60 s, and 72°C for 90 s, with a final polymerization at 72°C for 5 min. The reaction mixture was analyzed with an Applied Biosystems 373A DNA sequencer (Perkin-Elmer, Foster City, CA). The obtained nucleotide sequences were subjected to the similarity search program BLAST.

Fig. 1 Sample preparation procedure for TBT in culture medium

RESULTS AND DISCUSSION

Degradation of TBT in the sediment samples collected from each station

Degradation of TBT in the sediment samples collected from each sample station is shown in Table 1. The ratio of degradation of TBT in third enrichment culture was summarized in Table 1. TBT-degrading activity was detected in 6 stations. The strongest activity (72.7%) was detected in St. 4 sediment sample, suggesting that TBT-degrading microorganisms exist widely in the marine environment.

Table 1 Degradation of TBT in the sediments samples collected from each station

St. No.	Sea area	Latitude (N)	Longitude (E)	Depth (m)	Percentage of TBT degraded
1	Tokyo Bay	35° 34.0'	139° 59.0'	31	0

2	Tokyo Bay	35° 30.1'	139° 52.4'	15	13.3
3	Tokyo Bay	35° 27.1'	139° 50.1'	15	0
4	Tokyo Bay	35° 21.0'	139° 47.0'	12	72.7
5	Tokyo Bay	35° 11.3'	139° 44.9'	134	0
6	Sagami Bay	35° 04.5'	139° 39.1'	122	33.3
7	Sagami Bay	35° 00.1'	139° 20.0'	1504	40.0
8	Off-Bos o	34° 29.8'	139° 05.8'	6517	53.3
9	Off-Bos o	34° 33.1'	141° 54.0'	6347	53.3

Isolation and characterization of TBT-degrading microorganisms

From third enrichment culture collected from St. 4 sediment sample, two microorganisms were isolated on YNB medium. These strains were named as HA-T and HB-N and inoculated into the same medium containing 100 µg/L (pure culture conditions) and degradation of TBT was determined. These strains could degrade TBT independently. HA-T strain could degrade 60 µg/L. Whereas, HB-N strain degrade about 97 µg/L of TBT after 21 days incubation. From 16S rDNA gene sequence analysis, HA-T strain was found to have a high homology with *Halomonas aquamarina* (97%). On the other hand, HB-N strain had close similarity with *Halomonas alimentaria* (98%). Further physiological and biochemical characterization as well as the application on the marine bioremediation is under way.

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