REMOVAL OF 1,2,3,4-TCDD and 1,2,3,4-TCDF BY SOIL MICROORGANISM(S)

Hyo-Bong Hong , In-Hyun Nam, Sung-Deuk Choi, and Yoon-Seok Chang

School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang, Korea

Introduction

Various xenobiotic chemicals are released intentionally or unintentionally into the environment. Public and scientific concern about their release has increased efforts to remove the pollutants from the environment with safe and effective. These efforts made the scientists identify new bacterial strains capable of degrading such persistent and recalcitrant compounds as carbon and energy sources, or at least of attacking by co-metabolism and new possibilities of bioremediateion of contaminated soil or aquifer [3,6,7]. However, many toxic compounds strongly resist biotic degradation. Especially, chlorinated, aromatic compounds, like the polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/PCDFs), show extremely high persistence in soil and sediments. For example, the half-life of 2,3,7,8-TCDD in soil and sediment extends up to ten years[3]. The persistence of PCDD/DF in the environment, however, does not mean that microbial transformation could not be occurred. As their low concentration in soils and their strong sorption on soil particles, both reducing the bioavailability may also be responsible for their recalcitrance[9]. In fact, several laboratory studies reported evidence for microbial degradation of PCDDs and PCDFs though the degradation rate was very slow[1,4,5]. Whereas degradation of chlorinated dibenzofuran (DF) or dibenzo-*p*-dioxin (DD) has yet been reported only as co-metabolic transformation, Fortnagel et al. recently succeeded in isolation of bacterial strains, which utilize as DF and DD sole carbon source and energy [2]. However, there has not been considerable information on degradation of 1,2,3,4-TCDD or TCDF, yet. In the case of 1,2,3,4-TCDF and TCDD, all chlorines are collected in one benzene ring. The metabolic intermediates by known dioxygenation (Lateral or Angular) can be more harmful than mother molecules.

There have not been yet reports presenting biological removal of 1,2,3,4-TCDD or 1,2,3,4-TCDF, yet. Here, we report some microorganisms isolated from contaminated soil can degrade the 1,2,3,4-TCDD and TCDF.

Methods and Materials

Soil samples were taken in the vicinity of timber mill (Kyounju, Korea) and a industrial incinerator (Yangsan, Korea). The soil samples were put in the sterilized 50-ml of plastic centrifuge. There was no refrigeration or freezing during the transportation or storage at the lab. 0.5 g of soil samples were weighted and added to phosphate buffer of which pH was adjusted to 7.0. Particles were removed by centrifugation (1000 rpm for 10 min). One hundred ul of liquid obtained at the followed procedures was inoculated to 100 ml of nutrient broth and incubated in the shaking incubator for overnight (160 rpm). One hundred ul of liquid culture was streaked on nutrient solid media. After three days of incubation, colonies were inoculated to minimal salts medium containing dibenzofuran as carbon source. The twelve strains selected based upon growth in MSM containing DF were inoculated to 50 ml of minimal salt medium (MSM) containing

0.01% of yeast extract and 10 ng of 1,2,3,4-TCDF and TCDD per ml. Then, they were incubated in the rotary shaking incubator (160 rpm in 30°C), and 5 ml of samples was taken periodically to measure 1,2,3,4-TCDF remained in the samples. MSM used in this study consisted of 2.2 g of Na_2HPO_4, 0.8 g of KH_2PO_4, 3.0 g of NH_4NO_3, and 0.01 g of $MgCl_2.6H_2O$ per liter without EDTA. The initial pH was 7.0. In order to add dioxins as crystalline form, mixed stock solution of 1,2,3,4-TCDD and TCDF (1 ug each in 10 ml of acetone) was prepared. Then, Five hundred ul of the solution was transferred to the flasks and the solvent was removed by N_2 gas blowing. Then, autoclaved liquid media was added, and the media was heated in water bath of which temperature was 60℃ for 30 min. The subsequent analysis showed that this procedure caused no loss of dioxin and no contamination from another microorganisms. In addition to the cultures containing dioxins as crystalline form, separate culture media with dioxin and acetone were also prepared. In this case, 500 ul of stock solution was directly added to culture media after filtration. For the determination of bacteria growth, the optical density at 600 nm and total protein concentration were measured.

Ten ml of culture from the samples was taken and 5 ml of them was used for the UV-VIS analysis. The rest of them were used for the measurement of remaining 1,2,3,4-TCDD and TCDF. In order to recover 1,2,3,4-TCDD and TCDF adsorbed by biomass, 5 ml of concentrated H_2SO_4 was added to break down the cells and organic compounds. The procedure was repeated until the color of the solution became transparent. Then, five ml of toluene was added, and the total solution was shaked vigorously for 2~3 min. The recovery rate was adjusted with dibenzofuran.

A GC-ECD with autosampler (HP-6890, Hewlett-Packard, Wilmington, DE) and UV-VIS spectrophotometer (Cary 3bio,Victoria, Australia) were used in the analysis. Gas chromatogram was made on a GC with nitrogen as a carrier gas by using an ECD and fused silica capillary column (HP-5, 30 m). The initial temperature, 70°C, was maintained for 3 min, and then the temperature was increased to 260°C by 10°C/min and was held at 260°C for 25 min

In order to measure the content of protein in the culture, 10 ml of culture solution was taken. After three times of washing with 0.9% of NaCl solution, 1 ml of NaOH (1M) was added and heated at 95°C for 5 min. After cooling down the solution under the room temperature, the debris was removed by centrifugation (14,000 × g for 10 min). The protein content of supernatant was measured by Bradford method with bovine serum albumin as standard.

Results and Discussion

One hundred and eighty strains of soil microorganisms were isolated from the nutrient broth cultivation. Twelve of these strains showed that they were already adapted to the minimal salt media containing dibenzofuran. However, only four of them survived in the media containing the 1,2,3,4-TCDD and/or 1,2,3,4-TCDF and acetone. The survival of microorganisms was confirmed by smearing the microorganisms on nutrient agar and increasing the optical density at 600 nm. Without the addition of acetone, microorganism growth was very slow, and deviations in the growth rate and removal rate were up to 50% or more. The very low solubility of 1,2,3,4-TCDD and TCDF is assumed to be responsible for this significant diversity, and it is assumed that the addition of acetone helps the dioxins to be distributed more effectively throughout the aqueous culture. In addition, the growth of microorganisms indicates that acetone can be utilized as the carbon source. The results of a GC-ECD analysis showed that the microorganisms could remove 10~83% of 1,2,3,4-TCDD and 47~93% of 1,2,3,4-TCDF in a month, depending on the microorganisms used (Figs. 1 and 2). The UV-VIS spectrometer analysis (Table 1) and increase in protein concentration (data not shown) also indicate that the microorganisms utilize 1,2,3,4-TCDD or TCDF as the carbon source. However, the specificity to dioxins differs significantly depending

on the microorganism, as shown for KJ1 and KJ2 in Fig 2, and it is not clear whether these microorganisms can utilize dioxins as the sole carbon source.

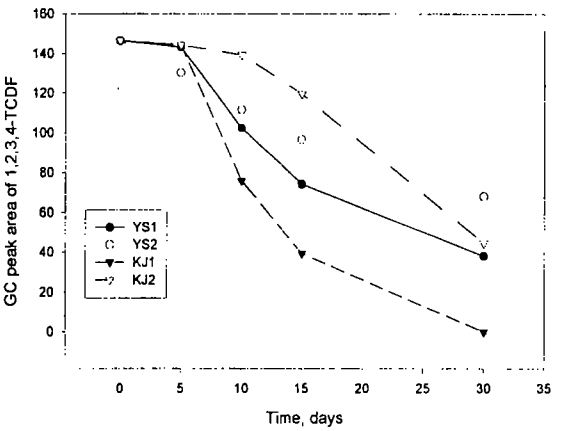

Fig 1. Removal of 1,2,3,4-TCDF by microorganisms from soil samples of Yangsan (YS) and KyoungJu (KJ) City, Korea

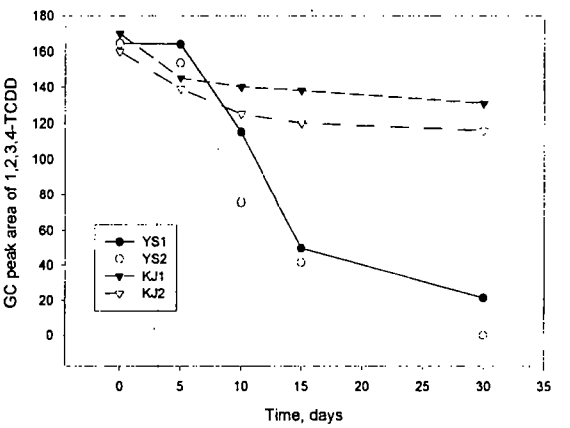

Fig 2. Removal of 1,2,3,4-TCDD by microorganisms from soil samples of Yangsan (YS) and KyoungJu (KJ) City, Korea

Table 1. Change of optical density (O.D) during the growth of microorganisms. The values were adjusted with that of 0 day and control. All values are means for triplicate cultures.

Day / Strain	0	5	10	15	30
YS1	0.000	0.004	0.201	0.483	0.468
YS2	0.000	0.003	0.312	0.369	0.345
KJ1	0.000	0.003	0.220	0.423	0.442
KJ2	0.000	0.003	0.201	0.179	0.103

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation(KOSEF) through the Advanced Environmental Monitoring Research Center at Kwangju Institute of Science and Technology.

References

1. Cerniglia, C. E., Morgan J. C. and Gibson D.T. (1979) Biochem. J. 180, 175.
2. Forntnagel P., Harms H., Wittich R., Krohn S., Meyer H., Sinnwell V., Wilkes H. and Francke, W. (1990) Appl. Environ. Microbiol. 56, 1148.
3. Halden R. U., and Dwyer D. F. (1997) Bioremed. J. 1, 11.
4. John R. P., Kolanda A and Arjan R.W. (1998) Chemosphere 37, 1915.
5. Klecka G. M, and Gibson D. T. (1980) Appl. Environ. Microbiol. 39, 228.
6. Monna L., Omori T. and Kodama T. (1993) Appl. Environ. Microbiol. 59, 285.
7. Parons J.R., and Storms M. (1998) Chemosphere 19,1297.
8. Thomas P, Kenneth N. T and Wittich, R.M. (1998) Appl. Environ. Microbiol. 64, 3798.
9. Wittich, R. M. (1999) Appl. Environ. Microbiol. 49, 489.