Cloning and sequencing of the gene encoding the enzyme for the reductive cleavage of diaryl ether bonds of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in *Geobacillus thermodenitrificans* UZO 3

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Abstract
We have previously reported that a cell-free extract prepared from *Geobacillus thermodenitrificans* UZO 3 reductively cleaves diaryl ether bonds of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), a dioxin with the highest toxicity, in a sequential fashion producing 3',4',4,5-tetrachloro-2-hydroxydiphenyl ether (TCDE) as the intermediate, and 3,4-dichlorophenol (DCP) as the final reaction product. The detection of TCDE implicated the discovery of an unprecedented dioxin-degrading enzyme that reductively cleaves the diaryl ether bonds. In this study, we report the cloning and sequencing of the dioxin reductive etherase gene *dreE* which codes for the 2,3,7,8-TCDD-degrading enzyme. We showed that *dreE* was expressed in *Escherichia coli* and that the product of the expression could reductively cleave diaryl ether bonds of 2,3,7,8-TCDD to produce TCDE. Furthermore, we established that the amino acid sequence encoded by *dreE* was homologous to an enzyme with yet unknown function that is encoded by a gene located in the riboflavin (vitamin B2) biosynthesis operon in *Bacillus subtilis*. We also showed that the amino acid sequence possesses a coenzyme A (CoA) binding site that is conserved in the N-acyltransferase superfamily. For the first time, the degradation of 2,3,7,8-TCDD at the molecular level using a enzyme of bacterial origin has been demonstrated.
Keywords: Bioremediation, Dioxin, 2,3,7,8-TCDD, Cloning

1. Introduction

We have previously reported that a cell-free extract prepared from *G. thermodenitrificans* UZO 3 reductively cleaves diaryl ether bonds of 2,3,7,8-TCDD in a sequential fashion producing 3',4',4,5-tetrachloro-2-hydroxydiphenyl ether (TCDE) as the intermediate, and 3,4-dichlorophenol (DCP) as the final reaction product (Suzuki et al., 2016). The structure of the TCDE was identified by GC-MS analysis using a chemically-synthesized authentic compound. The detection of TCDE intermediate implicated the discovery of an unprecedented dioxin-degrading enzyme that reductively cleaves the diaryl ether bonds of 2,3,7,8-TCDD in a manner that is similar to glutathione-S-transferase (GST), a reduction cleavage enzyme that uses GSH as electron donor (Masai et al., 1991). The aim of this study was to elucidate the 2,3,7,8-TCDD degradation mechanism in *G. thermodenitrificans* UZO 3 as well as to demonstrate the degradation of 2,3,7,8-TCDD at the molecular level by cloning the gene encoding the degradation enzyme.

2. Resuls and Discussion

2.1. Cloning and sequence of the gene for dioxin reductive etherase dreE

Transformants in the gene library of *G. thermodenitrificans* UZO 3 were screened for degradation activity using 2,7,-dichlorodibenzo-*p*-dioxin (2,7-DCDD) as substrate. The enzymatic activity was evaluated by the detection of 4',5-dichloro-2-hydroxydiphenyl ether (DCDE), a degradation intermediate (Suzuki et al., 2011, 2012). As a result, we have cloned the gene for dioxin reductive ethrase *dreE*. The result of sequencing of the *dreE* gene showed that it contained 351 bp, coding 116 amino acid residues to express a 13,673 Da protein (Fig. 1). Homology search using BLAST showed that *dreE* encodes a protein that is 63% homologous to the protein encoded by *ribT*, a gene with unknown function and which is the furthest downstream in the gene of the riboflavin (vitamin B2) biosynthesis operon of *Bacillus subtilis* (Mironov et al., 1994). Moreover, domain analysis showed that a functional domain conserved in the N-acyltransferase

superfamily was found in residues 44–93 of the 116 amino acid sequence coded by *dreE* and further indicated that residues from 72 to 74 (Ile-Ser-Val) and from 84 to 85 (Gly-Lys) were likely to form a CoA binding site (delCardayre et al., 1998).

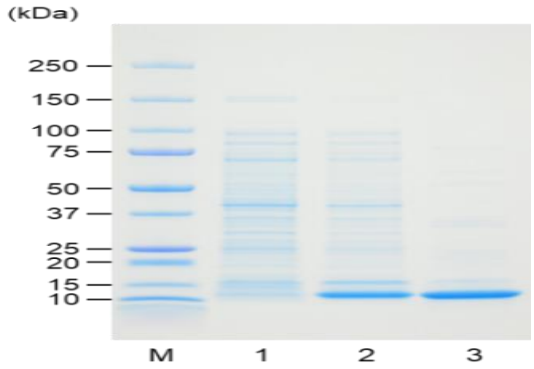

Figure 1. SDS-PAGE analysis of the *dreE* gene expression. Line M: molecular size markers; line 1: a protein fraction from *E. coli* BL21(DE3) cells with the pET28b(+) plasmid without *dreE* gene insertion (10 µg of protein); line 2: protein fraction from *E. coli* BL21(DE3) cells with the pETE plasmid, grown in the presence of 1 mM IPTG (10 µg of protein). lines 3: fractions of the DreE protein heated and centrifuged to remove the denatured *E. coli* proteins (10 µg of protein).

2.2. 2,3,7,8-TCDD-degrading activity of DreE

The results showed that despite the major presence of the unreacted 2,3,7,8-TCDD substrate in the reaction solution, significant formation of TCDE was detected (Fig. 2). In the control, TCDE was not detected. These results revealed that the enzymatic activity of DreE is a catalytic reaction in which reductive cleavage of the diaryl ether bonds of 2,3,7,8-TCDD occurs, producing TCDE as the reaction product (Fig. 3). we have identified the previously undefined gene for the 2,3,7,8-TCDD-degrading enzyme. We also clarified at the molecular level that the DreE, which is encoded as part of an operon implicated in riboflavin biosynthesis, reductively cleaves the diaryl ether bonds of 2,3,7,8-TCDD (Suzuki et al., 2018).

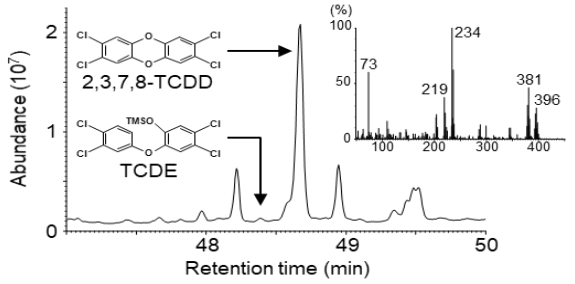

Figure 2. GC-MS analysis of the reaction mixture for 2,3,7,8-TCDD degradation mediated by DreE. Full scan run of the reaction mixture of the detected intermediate TCDE, and its corresponding MS spectrum.

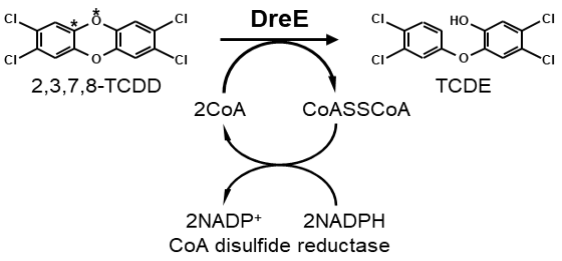

Figure 3. Novel proposed model for the reductive cleavage of diaryl ether bond of 2,3,7,8-TCDD by *G. thermodenitrificans* UZO 3. Asterisks indicate the positions where hydrogen is added.

References

delCardayre S.B., Stock K.P., Newton G.L., Fahey R.C. and Davies, J.E. (1998) Coenzyme A disulfide reductase, the primary low molecular weight disulfide reductase from *Staphylococcus aureus*, *Jourbnal of Biological Chemistry*, **273**, 5744-5751.

Masai E., Katayama Y., Kawai S., Nishikawa S., Yamasaki M. and Morohoshi N. (1991), Cloning and sequence of the gene for a *Psedomonas paucimobilis* enzyme that cleaves β-aryl ether, *Journal of Bacteriology*, **173**, 7950-7955.

Mironov V.N, Kraev A.S., Chikindas M.L., Chernov B.K., Stepanov A.I., and Skryabin K.G. (1994), Functional organization of the riboflavin biosynthesis operon from *Bacillus subtilis* SHgw, *Molecular and General Genetics*, **242**, 201-208.

Suzuki Y., Nakamura M., Otsuka Y., Suzuki N., Ohyama K., Kawakami T., Sato K., Kajita S., Hishiyama S., Fujii T., Takahashi A. and Katayama Y. (2011), Novel enzymatic activity of cell-free extract from thermophilic *Geobacillus* sp. UZO 3 catalyzes reductive cleavage of diaryl ether bonds of 2,7-dichlorodibenzo-*p*-dioxin, *Chemosphere*, **83**, 868-872.

Suzuki Y., Nakamura M., Otsuka Y., Suzuki N., Ohyama K., Kawakami T., Sato K., Kajita S., Hishiyama S., Fujii T., Takahashi A. and Katayama Y. (2012), Development of a highly sensitive assay for enzyme-mediated reductive degradation of polychlorinated dibenzo-*p*-dioxin, *Environmetal Toxicology and Chemistry*, **31**, 1072-1075.

Suzuki Y., Nakamura M., Otsuka Y., Suzuki N., Ohyama K., Kawakami T., Umeka Y., Maninang J.S., Izawa-Sato K., Hishiyama S., Inoue K., Kameyama T., Takahashi A. and Katayama, Y. (2016), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) degradation by the thermophilic *Geobacillus* sp. UZO 3, *Journal of Environmental Biotechnology*, **15**, 105-108.

Suzuki Y., Nakamura M., Otsuka Y., Suzuki N., Ohyama K., Kawakami Izawa-Sato K., Navarro R.R., Hishiyama S., Inoue K., Kameyama T., Takahashi A. and Katayama Y. (2018), Cloning and sequencing of the gene encoding the enzyme for the reductive cleavage of diaryl ether bonds of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in *Geobacillus thermodenitrificans* UZO 3, *Journal of Bioscence and Bioenginnering*, **126**, 488-496.