Anaerobic Transformation of Chlorinated Dioxins by Microorganisms

Lorenz Adrian[1], Ute Lechner[2]

[1]Fachgebiet Technische Biochemie, Technische Universität Berlin
[2]Institut für Mikrobiologie, Martin-Luther-Universität Halle-Wittenberg

1 Introduction

Bacteria of the genus *Dehalococcoides* are the first known bacteria able to transform highly chlorinated dioxins. Here we describe dechlorination of dioxins by these bacteria and discuss perspectives of their use for in situ remediation of contaminated soils and waters.

Organohalide compounds are part of the global halogen cycle, i.e. they are formed by a variety of natural or anthropogenic halogenation processes and, on the other hand, can undergo different degradation processes. Depending on the prevailing redox and nutritional conditions bacteria express a range of degradation and dehalogenation reactions to use these compounds as carbon and/or energy source[16]. However, especially polychlorinated dioxins and furans (PCDD/Fs) are very persistent. Once PCDD/Fs enter the environment they tend to partition into organic matter. Aquatic sediments and soils constitute a typical sink. Because also many other organic compounds accumulate in sediments that can be oxidized by aerobic bacteria these sediments tend to be anaerobic.

Reductive dehalogenation under anaerobic conditions is the only biological process observed so far, which transforms polyhalogenated compounds. In this process bacteria catalyze the stepwise substitution of chlorine by hydrogen atoms. As has been shown for polychlorinated biphenyls, reductive dechlorination can reduce the overall burden and toxicity in river sediments[10]. Lower chlorinated products can be degraded aerobically, thus reductive dehalogenation might be the initial step for complete degradation. Reductive dehalogenation can be a cometabolic process, but for several pure cultures it has been identified as the terminal electron accepting process in an anaerobic respiration. The reducing equivalents stem from electron donors like hydrogen, acetate or formate. Reductive dehalogenase, a membrane-bound corrinoid enzyme, catalyzes the final step in this redox process. A proton gradient is generated and drives ATP synthesis. The redox potentials of redox pairs of PCDD congeners and their respective less chlorinated products range from + 300 to + 490 mV[19], thus, suggesting their suitability as electron acceptors in dehalorespiration.

Reductive dechlorination of dioxins has been studied in microcosms and anaerobic enrichment cultures with inocula obtained from freshwater or estuarine sediments. The fate of spiked congeners such as hexa-, hepta- or octachlorinated dioxins (HeCDD, HpCDD, OCDD)[1,7] or 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TeCDD)[6,9,12,23] was analyzed. A variety of pathways of chlorine removal was observed, which ranged from complex dehalogenation activities, which removed chlorines from peripheral as well as lateral positions[7] to dehalogenation reactions restricted to the removal of chlorines from positions flanked by chlorines at both sides[12]. Barkovskii and Adriaens[7]

assumed from the comparison of pasteurized and non-pasteurized cultures that subpopulations of dehalogenating bacteria may contribute different regioselectivities to the overall dechlorination pathway observed in a mixed culture.
We investigated the anaerobic transformation of polychlorinated dioxins by mixed and pure bacterial cultures.

2 Material and Methods

Inocula were taken from anaerobic sludge and directly transferred to anaerobic, vitamin amended, bicarbonate buffered, mineral media that were reduced with sulfide or Ti(III) citrate as described[3,11]. Dioxins were added as electron acceptor and hydrogen as electron donor. For enrichments a mixture of pyruvate, fumarate, benzoate, formate and acetate was used as co-substrate. No fermentable substances were present in cultures with the pure strain CBDB1 and acetate was used as a C-source. All cultivations were done under strict anaerobic conditions avoiding any oxygen contaminations. Isolation of strain CBDB1 was done using dilution series in anaerobic agarose-solidified medium as described[3].
Dioxins were identified and quantified by GC and GC-MS[11].

3 Results and Discussion

Reductive dehalogenation of dioxins by mixed cultures
Mixed bacterial cultures were enriched from Spittelwasser sediment using 1,2,3,4-TeCDD as the only electron acceptor. Fig. 1 depicts a typical dechlorination pathway showing both lateral and peripheral dechlorination reactions. The ability of simultaneous removal of peripheral and lateral chlorines led to the formation of both intermediates 1,2,4- and 1,2,3-trichlorodibenzo-*p*-dioxin (TrCDD) from 1,2,3,4-TeCDD, and of 2,3- and 1,3-dichlorodibenzo-*p*-dioxins (DiCDD) from the intermediate 1,2,3-TrCDD[12]. However, repeated subcultivation of this primary enrichment culture with one of the intermediates, 1,2,4-TrCDD, selected for peripherally dechlorinating bacteria. The enrichment cultures were tested after five subcultivations (1:10, vol/vol) for the regiospecifity of chlorine removal from 1,2,3-TrCDD. Specific dechlorination at a peripheral position was indicated by the formation of 2,3-DiCDD as the only product (Fig. 1).

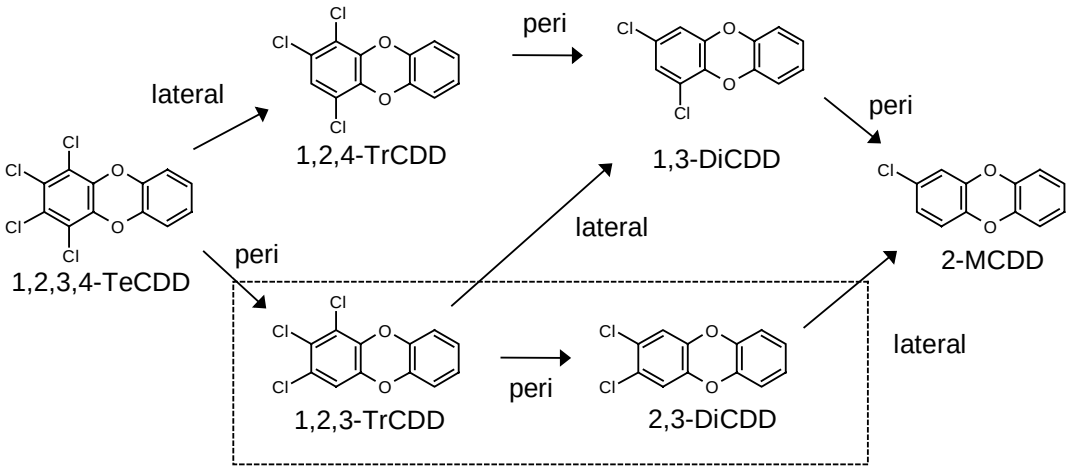

Fig. 1: Pathway of reductive dechlorination of 1,2,3,4-TeCDD by enrichment cultures from Spittelwasser sediment. The pathway was elucidated by subcultivation with individual less chlorinated dioxins. Box: Pathway of peripheral (peri) dechlorination of 1,2,3-TrCDD selected by sequential subcultivation with 1,2,4-TrCDD. Removal of chlorine from peripheral or lateral positions is denoted.

A complex dechlorination pattern of a historical dioxin contamination was also observed in a study by Albrecht et al. during anaerobic incubation of contaminated sediment and demonstrated (i) the ability of intrinsic bacteria to dechlorinate sediment-associated dioxins and (ii) the formation of 2,3,7,8-TeCDD from peripheral dechlorination of OCDD and its further lateral dechlorination to 2-monochlorodioxin (MCDD)[4].

In our studies, dioxin-dechlorinating enrichment cultures could be maintained with 1,2,4- or 1,2,3-TrCDD (25 – 50 µM) as electron acceptor over many successive transfers indicating growth of the dechlorinating bacteria. However, due to the low concentration of dioxins the number of dechlorinating bacteria was low (2.5×10^3 ml^{-1} compared to a total cell number of 10^8 ml^{-1}). With molecular methods (PCR with species-specific primers) many different bacteria previously shown to be able to dechlorinate divers chlorinated compounds could be detected in the cultures However, only one of these bacterial genera *–Dehalococcoides* - was detected in all dioxin dechlorinating cultures tested, indicating their involvement in dioxin dechlorination. By DNA sequencing it was found that the 16S rDNA gene of the organism in the dioxin dechlorinating culture was identical with the 16S rRNA gene of a previously described chlorobenzene dechlorinating bacterium, *Dehalococcoides* strain CBDB1[3]. Therefore, strain CBDB1 was studied for its capacity to transform chlorinated dioxins under anaerobic conditions.

Dechlorination of chlorinated dioxins by Dehalococcoides *sp. strain CBDB1*
Strain CBDB1 was initially isolated for its ability to reductively dechlorinate 1,2,3- and 1,2,4-trichlorobenzene (TrCB) using hydrogen as electron donor and acetate as carbon source[3]. However, hexa- and pentachlorobenzene also served as electron acceptors. Growth with these chlorinated benzenes resulted in growth yields of about 2.1 and 2.9 g protein (mol Cl^-)$^{-1}$, respectively[20]. The three tetra- and two trichlorinated benzenes were also dechlorinated.

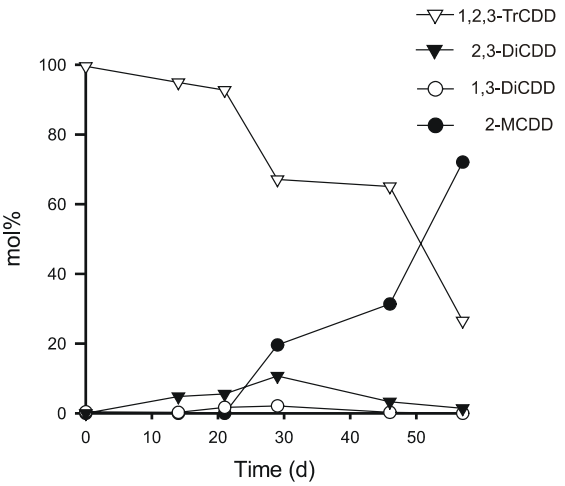

Fig. 2: Reductive dechlorination of 25 µM 1,2,3-TrCDD by *Dehalococcoides* sp. strain CBDB1.

CBDB1 was incubated with three different dioxin congeners - 1,2,3,4-TeCDD, 1,2,3- and 1,2,4-TrCDD – and all were reductively dechlorinated[11]. The dechlorination pathways were characterized by preferred removal of chlorine atoms from peripheral positions and, thus, resembled the pathway enriched with 1,2,4-TrCDD from Spittelwasser sediment (see above). Fig. 2 shows reductive dechlorination of 1,2,3-TrCDD by strain CBDB1, which released transiently more 2,3-DCDD than 1,3-DiCDD and finally 2-MCDD. In addition, the capacity of strain CBDB1 for the dechlorination of lateral chlorine atoms was demonstrated with spiked 2,3-DiCDD, which was transformed to 2-MCDD. The dioxin dechlorinating activity was maintained over several transfers (10 % vol/vol) into fresh medium, suggesting that the dioxins serve as electron acceptors for growth of strain CBDB1.
The ability of strain CBDB1 to dechlorinate environmentally relevant dioxins that carry chlorine substitutents at the positions 2, 3, 7 and 8 was demonstrated with 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) (Fig. 3). However, in contrast to the dechlorination of 1,2,3,4-TeCDD the reductive dechlorination of 1,2,3,7,8-PeCDD (3 µM) was very slow. Three % of the parent congener was transformed within 104 days. According to the preferred peripheral dechlorination, 2,3,7,8-TeCDD, the most toxic dioxin congener, was formed as the first product. However, due to its lateral dioxin-dechlorination activity, strain CBDB1 successively removed one chlorine atom from each of both rings resulting in a dichlorinated final product, which is less toxic and can even be degraded by aerobic bacteria.

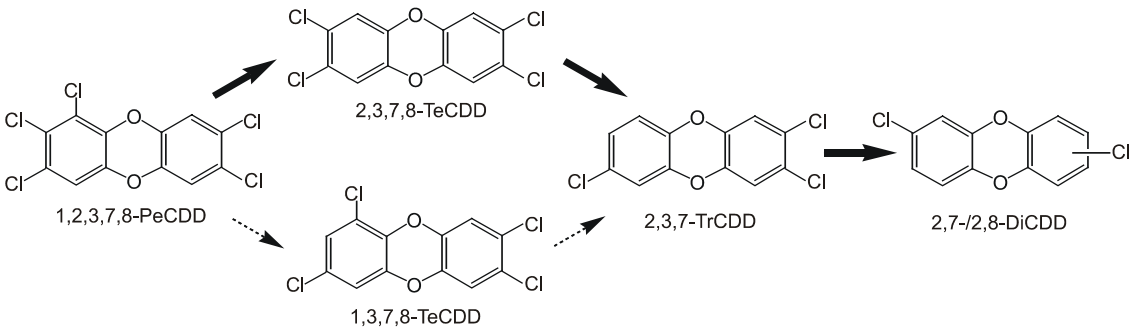

Fig. 3: Proposed pathway of reductive dechlorination of 1,2,3,7,8-PeCDD by *Dehalococcoides* sp. strain CBDB1. Thick arrows: main route.

Conclusions

1. Mixed bacterial cultures catalyze diverse chlorodioxin-dehalogenation pathways. Some of these pathways lead to relatively harmless end products, which can undergo further biological degradation e.g. by aerobic bacteria. However, the possible formation of highly toxic products is a critical problem for a bioremediation approach but also for untreated sites where such dechlorination reactions can occur.
2. Bioaugmentation with suitable pure or mixed cultures is promising. This has recently been demonstrated in a tetrachloroethene-contaminated groundwater using a *Dehalococcoides*-containing inoculum that almost completely converted tetrachloroethene to ethene without accumulation of the toxic intermediate vinyl chloride[21].
3. With *Dehalococcoides* sp. strain CBDB1 the first bacterium is now known, that grows by dehalorespiration with dioxins. Learning from the physiology and biochemistry of this bacterium will help us to understand the role of these bacteria in the environment and to predict the fate of dioxin pollution.

4 Acknowledgement
This work was supported by grants of Deutsche Forschungsgemeinschaft and Land Sachsen-Anhalt.

5 References

1 Adriaens, P., and D. Grbic-Galic. 1994. Chemosphere 29:2253-2259.
2 Adrian, L., and H. Görisch. 2002. Res. Microbiol. 153:131-137.
3 Adrian, L., U. Szewzyk, J. Wecke, and H. Görisch. 2000. Nature 408:580-583.
4 Albrecht, I. D., A. L. Barkovskii, and P. Adriaens. 1999. Environ. Sci. Technol. 33:737-744.
5 Ballerstedt, H., J. Hantke, M. Bunge, B. Werner, J. Gerritse, J. R. Andreesen, and U. Lechner. 2004. FEMS Microbiol. Ecol. 47:223-234.
6 Ballerstedt, H., A. Kraus, and U. Lechner. 1997. Environ. Sci. Technol. 31:1749-1753.
7 Barkovskii, A. L., and P. Adriaens. 1996. Appl. Environ. Microbiol. 62:4556-4562.
8 Beurskens, J. E. M., G. A. J. Mol, H. L. Barrefeld, B. van Munster, and H. J. Winkels. 1993. Environ. Toxicol. Chem. 12:1549-1566.
9 Beurskens, J. E. M., M. Toussaint, J. de Wolf, J. M. D. van der Steen, P. C. Slot, L. C. M. Commandeur, and J. R. Parsons. 1995. Environ. Toxicol. Chem. 14:939-943.
10 Brown, J. F., D. L. Bedard, M. J. Brennan, J. C. Carnahan, H. Feng, and R. E. Wagner. 1987. Science 236:709-712.
11 Bunge, M., L. Adrian, A. Kraus, M. Opel, W. G. Lorenz, J. R. Andreesen, H. Görisch, and U. Lechner. 2003. Nature 421:357-360.
12 Bunge, M., H. Ballerstedt, and U. Lechner. 2001. Chemosphere 43:675-681.
13 Dolfing, J, and B.K. Harrison 1993. FEMS Microbiol. Ecol. 13:23-29.
14 Geyer, H. J. *et al*. 2000. *In* B. Beek (ed.), The handbook of environmental chemistry, vol. 2. Springer-Verlag, Berlin Heidelberg.
15 Götz, R., B. Steiner, P. Friesel, K. Roch, F. Walkow, V. Maaß, H. Reincke, B. Stachel, 1996. Organohalogen Compounds 27:440-444.
16 Häggblom, M. M., and I. D. Bossert (ed.). 2003. Kluwer Academic Publishers.
17 Holliger, C., G. Schraa, A. J. M. Stams, and A. J. B. Zehnder. 1992. Appl. Environ. Microbiol. 58:1636-1644.
18 Hölscher, T., H. Görisch, and L. Adrian. 2003. Appl. Environ. Microbiol. 69:2999-3001.
19 Huang, C.-L., B.K. Harrison, J. Madura, J. Dolfing, 1996. Environ. Toxicol. Chem. 15:824-836.
20 Jayachandran, G., H. Görisch, and L. Adrian. 2003. Arch. Microbiol. 180:411-416.
21 Lendvay, J. M. *et al*. 2003. Environ. Sci. Technol. 37:1422-1431.
22 Ohtake, F. *et al*. 2003. Nature 423:545-550.
23 Vargas, C., D. Fennell, and M. M. Häggblom. 2001. Appl. Microbiol. Biotechnol. 57:786-790.