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Complete Detoxification of Short Chain Chlorinated Aliphatic Compounds: Isolation of Halorespiring Organisms and Biochemical Studies of the Dehalogenating Enzyme Systems

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Research Objective

Widespread use and careless handling, storage and disposal practices, have lead to the dissemination of chlorinated short chain aliphatics into groundwater systems. These compounds are toxic and the presence of chlorinated ethenes and chlorinated propanes in the environment is of public concern. Halorespiration is a newly recognized anaerobic process by which certain bacteria use chlorinated compounds as terminal electron acceptors in their energy metabolism. In contrast to co-metabolic dechlorination, which is fortuitous, slow, and without benefit to the organisms, halorespiration, characterized by high dechlorination rates, is a specific metabolic process beneficial to the organism. Our goals are to isolate and characterize organisms which use chlorinated ethenes (including tetrachloroethene [PCE], trichloroethene [TCE], cis-dichloroethene [cis-DCE], and vinyl chloride [VC], or 1,2-dichloropropane [1,2-D]) as electron acceptors in their energy metabolism. Better understanding of the physiology and phylogeny of the halorespiring organisms as well as the biochemistry of the dehalogenating enzyme systems, will greatly enhance our knowledge of how these organisms can successfully be employed in the bioremediation of contaminated sites.

Research Progress and Implications

This report summarizes the results of 1.5 years of a 2-year project.

Anaerobic microcosms were established using a variety of geographically distinct sediments. In several microcosms complete dechlorination of PCE to ethene (ETH), and 1,2-D to propene was observed. Upon subsequent transfers to anaerobic medium, four sediment-free, methanogenic enrichment cultures were obtained that dechlorinated PCE to ETH, and two cultures that dechlorinated 1,2-D to propene. 2-Bromoethanesulfonate (BES), a well known inhibitor of methanogens, did not inhibit the dechlorination of 1,2-D to propene or the dechlorination of PCE to cis-DCE. However, the complete dechlorination of PCE to VC and ETH was severely inhibited. We could also show that BES inhibited the dechlorination of chloroethenes in cultures without methanogens. Therefore, BES should not be used to attribute dechlorination activities to methanogens.

The non-methanogenic cultures obtained after the BES treatment were subsequently transferred to medium without BES, and complete dechlorination of PCE to ETH occurred as in the original microcosms. Subcultures were further enriched with PCE, cis-DCE, VC, or 1,2-D as the only available electron acceptor and with acetate, or acetate plus hydrogen as the only available electron donor(s). To date, these cultures have undergone over 30 transfers. Three cultures that had originally dechlorinated PCE to ETH, but were then enriched with cis-DCE or VC, lost their ability to dechlorinate PCE. This finding indicates that different populations are involved in the complete dechlorination of PCE to ETH in these cultures. Interestingly, one sediment yielded cultures that maintained their ability to dechlorinate PCE even after enrichment with cis-DCE or VC. Using molecular tools (16S rDNA targeted PCR, TA cloning of 16S rDNA genes, ARDRA analysis and sequencing) we showed that the PCE-dechlorinating culture consisted of two distinct populations. One of them was isolated in pure culture yet showed no dechlorinating activity, indicating that the other population, or the interaction of the two, was responsible for the complete dechlorination of PCE to ETH in the co-culture. Hydrogen was the only electron donor shown to support dechlorination.

We are currently focused on isolating this interesting dechlorinator, and on the taxonomic characterization of both members of the consortium.

Reductive dechlorination of cis-DCE or VC to ETH in the four cis-DCE/VC enrichment cultures unable to dechlorinate PCE, was supported by a variety of electron donors, including hydrogen. In a bicarbonate-buffered medium in the presence of cis-DCE or VC as electron acceptor, hydrogen was consumed below concentrations of 0.5 ppmv. In contrast, the same cultures consumed hydrogen to threshold concentrations around 300 ppmv in the absence of the chlorinated ethene. These findings suggest that both cis-DCE and VC can be used as terminal electron acceptors by at least one population in our highly enriched cultures.

To date we have isolated two pure cultures of PCE dechlorinating organisms from these enrichments. One isolate, a strictly anaerobic organism designated strain BB1, dechlorinated PCE to cis-DCE using acetate, lactate, or pyruvate, but not hydrogen or formate, as electron donors. This is an important observation since it is generally believed that hydrogen is the ultimate electron donor supporting chlororespiration in anaerobic environments. Phylogenetic analysis showed that this organism belongs to the genus Desulfuromonas and its closest relative is another PCE to cis-DCE dechlorinating organism, Desulfuromonas ethenica strain TTB4. However, in addition to several key physiological features, strain BB1's16S rDNA sequence is 5% different from the sequence of strain TTB4 indicating that strain BB1 is a different species. Strain BB1's ability to grow by chlororespiration was confirmed by showing PCE dependent growth with acetate, and by determining the fe value. The fe value describes the fraction of electrons transferred from the electron donor (acetate) to the electron acceptor (PCE). Strain BB1 revealed an fe value of 0.66 indicating that 66% of the electrons from acetate were consumed in the reductive dechlorination of PCE to cis-DCE. Furthermore, we optimized the culture conditions to obtain sufficient cell material to assess PCE dechlorinating activity in cell-free systems. A test system to measure PCE dechlorination using gas chromatography was developed. The reductive dechlorination of PCE and TCE to cis-DCE could be measured with reduced methyl viologen as the electron donor. Dechlorination activity was membrane fraction associated with less than 1% of the total activity remaining in the soluble protein fraction, which suggested that the dechlorinating enzyme system is membrane-bound. The membrane-bound reductive dechlorinase could be solubilized with 1% Triton X-100, and up to 80% of the total initial activity could be recovered in the solubilized fraction.

The other isolate that dechlorinated PCE to TCE was designated strain Viet1. This strictly anaerobic organism was identified as a new member of the genus Desulfitobacterium. Strain Viet1 grew well on pyruvate, and a variety of electron donors, including hydrogen and formate, supported dechlorination. Therefore, sufficient cell material for the investigation of cell-free dechlorinating activity was available. The capacity for chlororespiration was confirmed by hydrogen threshold measurement, fe value determination, and PCE dependent growth.

Two microcosms which were enriched with 1,2-D, yielded sediment-free, nonmethanogenic cultures that completely dechlorinated 1,2-D to propene. These could be indefinitely transferred to mineral salts medium containing acetate as a carbon source and hydrogen as the electron donor. Hydrogen threshold measurements confirmed that chlororespiring organisms were responsible for the conversion of 1,2-D to propene. ARDRA analysis of a clone library from one of the enrichment cultures showed seven different patterns. Partial sequence analysis of the cloned 16S rDNA genes revealed that six of the seven clones had identical sequences with only one distinct sequence. We are currently analyzing the complete 16S rDNA sequences in order to determine the phylogenetic relationships of the organisms. This will permit the development of an efficient isolation procedure for the dechlorinating populations.

Planned Activities

(1) Determination of fe values and hydrogen thresholds to confirm the presence of chlororespiring organisms in all cultures. This ongoing work will be completed within the next 2 months.
(2) Phylogenetic analysis of the highly enriched mixed cultures that dechlorinate cis-DCE, VC, or 1,2-D, and the PCE to ETH dechlorinating co-culture. This work is in progress and will be

completed within the next 3 months. The goal is to purify and identify the dechlorinating populations.

(3) Purification of the PCE/TCE reductive dechlorinase from strain BB1, and the PCE reductive dechlorinase from strain Viet1. Since the dehalogenases from both organisms are membrane-bound, but can be solubilized by a detergent treatment, we anticipate to complete the purification and characterization of at least one dechlorinase within the next 6 months.

(4) Localization of the reductive dechlorinases in the highly enriched cis-DCE, VC, or 1,2-D dechlorinating cultures, as well as in the PCE to ETH dechlorinating co-culture. This task will be completed within the next 4 months.

Other Access To Information

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