Assessment of PCB-dehalogenating Potential
In Coastal Sediments

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Molecular probes developed during the course of this ONR funded project were successfully used for both qualitative and quantitative assessment of PCB dechlorinating bacteria in PCB impacted sites. These probes are currently being used to determine the diversity of PCB dechlorinating bacteria required to dechlorinate commercial PCB mixtures and to assess the distribution of PCB dechlorinating species in PCB impacted sites of interest to the Navy.

PCBs, bioremediation, marine sediments, natural attenuation, microbial catalysts
FINAL REPORT

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GRANT TITLE: Collaborative Research: Assessment of PCB-biodehalogenating
potential in coastal sediments

REPORT PERIOD: 30 September 2001 – 29 September 2002

OBJECTIVE: The overall objective of this research is to confirm the ability to
use molecular probes as tools for assessing the PCB dehalogenating potential
extant in coastal sediments. Studies outlined in this proposal will validate
the application of molecular probes as a presumptive test for assessing PCB
dechlorinating potential based on microbial enumeration coastal sediments.
Ultimately the goal is to use molecular probes to monitor and assess the
effectiveness of natural or bioaugmented PCB-dechlorination in situ.

APPROACH: The approach is as follows: (i) develop molecular probe assays for
assessing PCB-dechlorinating potential in PCB impacted environments; (ii)
screen for diversity and distribution of microbes that catalyze PCB
dechlorination in selected environmental samples, (iii) assess the effects of
sedimentary contaminants on PCB dechlorination by GC analysis and molecular
probes assays. An effective monitoring assay combined with information on how
dechlorination is affected by mixed contaminant wastes will provide
fundamental information required to develop bioremediation strategies to promote
intrinsic PCB transformation in impacted sites such as marine harbor sediments
and dredge deposition sites.

Dr. Kevin Sowers (PI) and Dr. Joy Watts (co-PI), both of UMBI, developed the
molecular screening tools used in this study. Dr. Harold May (PI), Dr.
Qingzhong Wu, Mr. Greg Miller and Mr. Matt Brigmon, all of MUSC, have
conducted the enrichment and isolation studies for identification of PCB
dechlorinating bacteria and assessment of toxic effects of compounds on
dechlorination.

ACCOMPLISHMENTS:
Objective 1. Development of molecular probe assays for assessing PCB-
dechlorinating potential in PCB impacted environments. Assays for monitoring
PCB dechlorinating bacteria in the environment had been precluded by the
inability of laboratories to isolate and identify the microbial catalysts by
conventional microbiological methods. With prior ONR supported research the
PIs identified for the first time two sediment free anaerobic PCB
dechlorinating cultures by using an approach that combined classical
enrichment protocols with screening of the
microbial 16S rDNA communities (Pulliam
Holoman et al., 1998; Watts et al, 2001,
Cutter et al., 2001, and Wu et al., 2002).
Using these cultures, primers were
developed by obtaining 16S rDNA sequence
for PCB dechlorinating microorganisms (O-
17 and DF-1) in defined culture
conditions in our laboratory. These
sequences were aligned using ClustalW
(Higgins et al., 1996) with other closely
related bacteria and regions of

Figure 1. Specific primer
locations in 16S rDNA gene
variability were examined for the suitability of probe formation. TCE and PCE dechlorinating microorganisms from the Dehalococcoides ethenogenes genus were included (Fig. 1). It was important that the specific probes do not detect species of the D. ethenogenes genus as this group has not been detected in PCB dechlorinating cultures. The specific primer was developed and tested in silico using the Genbank and RDP databases, the specific primer only produced high similarity values to o-17 and DF-1. The specific primer was paired with a universal forward primer (Rainey et al. 1992) and this combination yielded a product of 1198 bp. To examine the specificity of the primers, PCR reactions were performed with a wide range of other microbial DNA (Gram negative, Gram positive, Archaea and other known dechlorinators including Desulfotobacteium spp.). PCR reactions were optimized so that the primers generated PCR products with only the PCB dechlorinators o-17 or DF-1 in our assay (Fig. 2). To examine the PCR assay sensitivity microcosms were constructed containing 1 g of sediment and different amounts of o-17 16S rDNA. The total DNA was extracted from the sediment using the method described in Pulliam Holoman et al., 1996. Briefly, sediment was treated by shaking in SDS lysis buffer with glass beads for 30s at a setting of 5.5 using a Fastprep FF120 instrument (ThermoSavant). DNA was extracted using a modified phenol chloroform procedure. The PCR assay was able to detect 16S rDNA easily for the microcosms containing 8500 ng/g - 85 ng/g, the band is faint but still visible in the 8.5 ng/g microcosm (Fig. 3). This experiment indicated that our detection level is at 8.5 ng/g 16S rDNA per microcosm. This level of sensitivity is within the commonly reported range for detecting microorganisms in environmental samples.

Objective 2. Screening for diversity and distribution of microbes that catalyze PCB dechlorination in selected environmental samples. In order to optimize and assess the effectiveness of the probes for detecting PCB dechlorinating microorganisms in sediment, analyses were conducted with highly enriched sediment microcosms and with environmental samples impacted to varying degrees with PCBs.

PCB dechlorinating microcosms were started with 13 different PCB congeners and Aroclor 1260 (in triplicates with sterile controls), in addition to a set of control cultures without PCB. Baltimore Harbor sediments were added as inocula. The congeners are chosen on the basis that they are present in Aroclor 1260 by more than 3 weight % according to Frame et al. (1996) and that Baltimore Harbor sediments have the capacity to dechlorinate these congeners (Wu et al., 1997). After 8 months of incubation cultures with 6 of the congeners showed dechlorination. The pattern of this dechlorination is seen in Fig. 4. Most of the dechlorination is occurring in the meta position, but there is evidence of both para and ortho dechlorination. These cultures have been transferred and will be assayed again to confirm the pathways of dechlorination. Preliminary analysis with the specific probe of the cultures enriched with PCB 151 detected three 16S rDNA genes with highest sequence similarity to PCB dechlorinators o-17. Since most of the funding period was required to develop the cultures, studies utilizing these cultures will be
continued during the course of a subsequently funded ONR project (N00014-03-1-0035). However, the preliminary results suggest that this is a valid approach for determining which microorganisms catalyze specific patterns of PCB dechlorination. Ultimately this experiment will enable us to determine the diversity of PCB dechlorinating species required to dechlorinate a commercial PCB mixture (e.g., Aroclor 1242, 1260, etc.).

Analysis of PCB dechlorinating cultures revealed that phylogenetically this group appeared to be distinct from other dechlorinating bacteria (Fig. 5). The Dehalococcoides group has the highest sequence similarity to the PCB dechlorinators and from our analysis they form a distinct and separate group (cultured isolates and non-isolated 16S sequences).

To examine environmental samples Baltimore Harbor, sediment was collected from a number of sites within the bay. DNA was extracted and subjected to the specific PCR. PCR products were cloned, sequenced and analyzed from sites in the Inner Harbor; no PCR products were detected from the Outer Bay samples. These sequences appeared to be closely related to the laboratory PCB dechlorinating enrichment cultures although distinct from the D. ethenogens dechlorinating group (see Figure 5). Although this assay is presumptive, as 16S rDNA does not infer functional activity, it is interesting that Baltimore Inner Harbor (a highly contaminated PCB site) contained closely related sequences to PCB dechlorinating microorganisms in the laboratory. However, from the less impacted Outer Harbor region no specific PCR products were detected indicating that these sequences may be linked to the presence/absence of PCBs in the environment.

Objective 3. Assessing the effects of sedimentary contaminants on PCB dechlorination by GC analysis and molecular probes assays. Effects of various known environmental organic and inorganic co-contaminants of PCBs were tested on PCB dechlorinator DF-1. Among the organic compounds tested two classes of organochlorines (chlorobenzenes CBZ and chlorinated ethenes CE) support growth and dechlorinating activity. Further analysis of the dechlorinating capabilities of DF-1, show that this microorganism is capable of dechlorinating hexa-CBZ → penta-CBZ → 1,2,3,5-CBE → 1,3,5-CBZ (Fig. 6), which is the predominant pathway reported in microcosm studies. This was the first identification of an organism that could complete this CBZ pathway and was the first observation linking PCB and CBZ dechlorination to the same organism. Several other organic co-contaminants, including several organochlorines, which occur in the environment from biogenic, sources such as fungi were not attacked by the PCB dechlorinating culture. More recently the PIs have discovered that DF-1 dechlorinates PCE to trichloroethene (TCE) and cis and trans dichloroethene.
(cDCE and tDCE) (unpublished, see below). For the first time dechlorination of these three different classes of organochlorines has been demonstrated with one organism. The effects of the chromium, copper, lead and zinc, contaminants currently found in Baltimore Harbor, were tested to determine the effects of these heavy metals on the dechlorinating activity of DF-1. Cultures were sampled over the course of 6 months and analyzed for activity. Preliminary results at the time of this report are inconclusive because the cultures are not fully developed, but the cultures will continue to be monitored as they mature and the results reported in the current ONR-funded project.

**CONCLUSIONS:** For the first time molecular probes have been developed for assessment of presumptive dechlorinating potential in PCB impacted sites. Preliminary results indicate that PCB dechlorinating populations are only detected in PCB impacted sites. Results also suggest that putative PCB dechlorinating microbes have highest similarity with the known PCB dechlorinating species DF-1 and o-17 and that they cluster in a discreet phylogenetic branch. PCB dechlorinating microorganisms also have the ability to utilize the chloroethenes and chlorobenzenes, which often occur as co-contaminants of PCBs.

**SIGNIFICANCE:** Molecular probes developed during the course of this ONR funded project were successfully used for both qualitative and quantitative assessment of PCB dechlorinating bacteria in PCB impacted sites. These probes are currently being used to determine the diversity of PCB dechlorinating bacteria required for the breakdown of commercial PCB mixtures and to assess the distribution of PCB dechlorinating species in PCB impacted sites of interest to the Navy.

**PATENT INFORMATION:** Information from this project contributed to a patent that relates to the use of microbes to catalyze dechlorination of PCBs.

**AWARD INFORMATION:** None.

**PUBLICATIONS:**

