A PCR-based specific assay reveals a population of bacteria within the *Chloroflexi* associated with the reductive dehalogenation of polychlorinated biphenyls

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Polychlorinated biphenyls (PCBs) accumulate and persist in sediments posing a risk to human health and the environment. Highly chlorinated PCBs are reductively dechlorinated in anaerobic sediments and two bacteria, designated o-17 and DF-1, from a novel phylogenetic group that reductively dechlorinate PCBs have recently been identified. However, there is a paucity of knowledge about the distribution, diversity and ecology of PCB-dechlorinating bacteria due to difficulty in obtaining pure cultures and the lack of detection by universal PCR 16S rRNA gene primer sets in sediments. A specific PCR primer was developed and optimized for detection of o-17/DF-1 and other closely related bacteria in the environment. Using this primer set it was determined that bacteria of this group were enriched in sediment microcosms from Baltimore Harbour concurrent with active dechlorination of 2,2’9,3,4,4’-hexachlorobiphenyl. Additional 16S rRNA gene sequences that had high levels of similarity to described PCB dechlorinators were detected in sediments from the Elizabeth River tributary of Chesapeake Bay, which had confirmed PCB-dechlorinating activities. Phylogenetic comparison of these detected 16S rRNA gene sequences revealed a relatively diverse group of organisms within the dehalogenating *Chloroflexi* that are distinct from the *Dehalococcoides* spp. Results from this study indicate that reductive PCB dechlorination activity may be catalysed by a previously undescribed group of micro-organisms that appear to be prevalent in PCB-impacted sites.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are bioaccumulative and toxic compounds that are environmentally stable and can adversely affect human health and the environment (ATSDR, 2000). As PCBs are hydrophobic, they strongly associate with clays and silt that ultimately migrate into the marine sediments as a result of runoff from tributaries and industrial activities along ocean coastlines (Brown et al., 1987). Anaerobic dechlorination is a significant process in the environment because it results in the sequential reduction of more extensively chlorinated PCBs to lesser chlorinated congeners or complete dechlorination to biphenyl (Wiegel & Wu, 2000). Many of the lesser chlorinated congeners are also substrates for cleavage of the biphenyl rings and subsequent mineralization by aerobic bacteria (Abraham et al., 2002).

Anaerobic reductive dechlorination of PCBs has been observed in laboratory microcosms and in the environment (Bedard & Quensen, 1995), but many attempts to isolate and identify the microbial catalysts responsible have been unsuccessful. Two anaerobic PCB-dechlorinating micro-organisms, o-17 and DF-1, were identified for the first time using an approach that combined classical enrichment protocols in a defined sediment-free medium with genetic screening of the microbial communities (Cutter et al., 2001; Pulliam Holoman et al., 1998; Watts et al., 2001; Wu et al., 2000).
2002b). Examination of the PCB dechlorinators revealed growth-linked reductive dechlorination of selected PCB congeners (Cutter et al., 2001; Wu et al., 2002a) and chlorinated ethenes and chlorinated benzenes (Milliken et al., 2004; Watts et al., 2004; Wu et al., 2002a). More recently Fennell et al. (2004) reported that *Dehalococcoides* ethenogenes 195 co-metabolically dechlorinates 2,3,4,5,6-pentachloroethene and other aromatic organochlorines with tetrachloroethene as primary electron acceptor. The range of dechlorination reactions performed by o-17, DF-1 and *Dehalococcoides* spp. implies a diverse range of catalytic activities resulting in organohalide reduction. However, despite similarities in the dechlorinating capabilities of the o-17/DF-1 group and *Dehalococcoides* spp. these microorganisms are phylogenetically distinct at the 16S rRNA gene level with less than 90% sequence similarity.

Although isolation of the dechlorinating microbes is essential to fully understand the physiology and biochemistry of the PCB dechlorination process, molecular techniques can yield important information about these micro-organisms and their role in the environment. Previous attempts to identify the dechlorinating species in the microbial community required construction of a large number of universal 16S rRNA gene libraries and sequencing of hundreds of restriction fragment length polymorphism (RFLP) patterns to try to identify the microbial catalyst involved (Pulliam Holoman et al., 1998). However, as the dechlorinating species are minority components of the total sediment microbial community, even in microcosms highly enriched with PCBs, it is difficult to identify species that catalyse the dechlorination reaction within a diverse microbial community (Pulliam Holoman et al., 1998). The creation of a specific PCR assay would allow the direct detection of the o-17/DF-1 group within a diverse microbial community and provide a means of identifying potential PCB dechlorinators in sediments.

A selective PCR approach for 16S rRNA genes has been used previously to examine the geographical distribution of *Dehalococcoides* spp. in sites impacted by chlorinated ethenes (Cuppen et al., 2003; Hendrickson et al., 2002; Loffler et al., 2000). Using these *Dehalococcoides*-specific primers in our laboratories we were unable to detect 16S rRNA gene sequences with high similarity to the o-17/DF-1 group, which so far are the only micro-organisms known to link growth with reductive dechlorination of PCBs. As this group would be overlooked with assays using *Dehalococcoides*-specific PCR primers, our aim was to develop a complementary set of primers that would detect the o-17/DF-1 group of PCB dechlorinators in the environment. Primers were developed from 16S rRNA gene sequences for the two currently described PCB-dechlorinating microorganisms, o-17 and DF-1 (Watts et al., 2004), which we previously identified from estuarine sediments (Cutter et al., 2001; Wu et al., 2002b). The o-17 co-culture, which contains the dechlorinating bacterium o-17 and a non-dechlorinating *Desulfovibrio* sp., reductively dechlorinates PCB congeners with single-flanked ortho-chlorines and double-flanked meta-chlorines on the biphenyl rings. The DF-1 co-culture, which contains the dechlorinating bacterium DF-1 and a non-dechlorinating *Desulfovibrio* sp., reductively dechlorinates PCB congeners with double-flanked chlorines. A role for the *Desulfovibrio* spp., which remains in low numbers (<1%) relative to the dechlorinators, has not been determined (Cutter et al., 2001; Wu et al., 2002b).

We describe the development of a PCR based assay that utilizes 16S rRNA gene primers for the detection of a group of bacteria capable of PCB dechlorination. In this study we have applied the primers to sediment-free PCB-dechlorinating cultures, sediment microcosms enriched with PCB and finally PCB-impacted environmental samples to determine their suitability for detection of PCB-dechlorinating bacteria. It is proposed that this set of primers used in combination with *Dehalococcoides*-specific primers (Hendrickson et al., 2002) will provide a more rigorous assessment of the presence of microbes associated with the anaerobic reductive dechlorination of PCBs in organochlorine-impacted sediments.

**METHODS**

**Sediment samples.** Baltimore Harbour and Elizabeth River sediments were sampled with a Ponar petite grab sampler as described by Berkaw et al. (1996). Exact locations for these sampling sites were Baltimore Harbour 39° 16′-8′ N, 76° 36′-1′ W and Elizabeth River 37° 2′-0′ N, 76° 30′-6′ W. Sediments were stored anaerobically under nitrogen prior to use.

**Anaerobic cultures.** Anaerobic cultures were prepared and maintained as described by Watts et al. (2001). Briefly, a defined sediment-free, low-sulfate estuarine salts medium (E-CI) was prepared according to Berkaw et al. (1996) with the exclusion of Na₂S·9H₂O. The medium was anaerobically dispensed into 10 ml aliquots into 20 ml Balch tubes, which were sealed under N₂/CO₂ (80:20) with butyl rubber stoppers secured with aluminum crimp seals. The medium was autoclaved for 20 min and the final pH was 6.8. All subsequent additions were performed aseptically in an anaerobic glove box under an N₂/CO₂ (80:20) atmosphere. Sodium formate (10 mM) was added to the medium as a potential carbon and energy source. Enrichment cultures were prepared by inoculating sterile E-CI medium with sediments or a 1 ml inoculum from an active, PCB-dechlorinating culture. The congeners 3,5-dichlorobiphenyl, 2,3,4,5-tetrachlorobiphenyl or 2,2′,3,4′,5′-hexachlorobiphenyl (AccuStandard) were solubilized in 10 μl acetone and added to the enrichment cultures at a final concentration of 50 p.p.m. Sterile controls were prepared by autoclaving sediment microcosms twice for 1.5 h, 48 h apart. The PCB congener was added after the second autoclaving. After addition of PCB all cultures were sealed with sterile Teflon-lined butyl rubber stoppers. All cultures were incubated in the dark at 30°C. To confirm that growth of bacterium DF-1 was concurrent with the dechlorination of PCB, two sets of DF-1 cultures were initiated; one set with 2,3,4,5-tetrachlorobiphenyl added, the other control set with no PCB added. Triplelicate cultures were sacrificed for DNA extraction and PCR analysis at selected time points over the course of 30 days.

**Analytical techniques.** PCBs were analysed as described by Berkaw et al. (1996). Enrichment culture samples were extracted with hexane (HPLC grade; Fisher Scientific) and the organic fraction was passed through a Florisil/copper (4:1) column. PCBs were
analysed using a Hewlett Packard 5890 series II gas chromatograph equipped with a DB-1 capillary column (30 m × 0.25 mm × 0.25 μm; JW Scientific) and a Ni65 electron capture detector (ECD). Individual PCB congeners were identified by matching the GC retention times with those of authentic standards (99% purity; AccuStandard) and quantified with a 16-point calibration curve for each congener (Wu et al., 2000).

**Primer development.** The 16S rRNA gene sequences of the PCB dechlorinators o-17 and DF-1 were aligned using ClustalX (Thompson et al., 1997) with 25 other closely related bacterial 16S rRNA gene sequences. Primers were designed to hybridize with unique sequences in a variable region of the aligned 16S rRNA molecules. Trichloroethylene (TCE)- and tetrachloroethylene (PCE)-dechlorinating *Dehalococoides* spp. were included in the alignment as these species have the highest sequence similarity to the o-17/DF-1 PCB-dechlorinating micro-organisms (Fig. 1). This alignment, over a 1400 bp region, revealed that the o-17/DF-1 group had several conserved regions in common with the *Dehalococoides* group as well as a number of unique sequence regions. A specific primer, Dehal1265R, was developed with high similarity values to o-17 and DF-1 16S rRNA gene sequences and was tested *in silico* using the GenBank and RDP databases. This specific primer was then paired with a universal forward primer (Edwards et al., 1989), yielding a PCR product of 1215 bp with DF-1.

**Extraction of genomic DNA.** DNA was extracted according to the procedure described by Pulliam Holoman et al. (1998). Briefly, 1 ml aliquots of culture samples or sediment samples were subjected to bead beating with a Fastprep Cell Disruptor (Qbiogene) and phenol/chloroform extractions followed by purification electrophoresis in a 1-3% (w/v) low-melting-point agarose gel containing 2% (w/v) polyvinylpyrrolidone. The chromosomal DNA was excised from the gel and recovered with a Promega Wizard PCR Prep Kit according to the manufacturer’s instructions.

**PCR.** Total microbial community DNA was PCR-amplified using the universal primer set 519F and 1406R (Lane et al., 1985) to confirm that DNA was suitable for PCR using conditions described previously (Watts et al., 2001). DNA samples were screened for the presence of *Dehalococoides* spp. with forward primer DHC 1 and the reverse primer DHC 1377 as described by Hendrickson et al. (2002). Total DNA was screened for 16S rRNA gene sequences from PCB-dechlorinating micro-organisms with universal forward primer 14F (5'-AGAGTTTGATCCTGGCTCAG-3') (Edwards et al., 1989) and specific reverse primer Dehal1265R (5'-GCTATTCCTACCT- GCTGTTAC-3'). PCR (50 μl total volume) was performed as described previously (Watts et al., 2001) using the GeneAmp PCR kit with Tag DNA polymerase (PE Applied Biosystems) in a PTC200 thermal cycler (MJ Research) and the following cycle parameters: an initial denaturation step of 1 min at 94°C, followed by 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 62°C, elongation for 30 s at 72°C, with a final extension step of 5 min at 72°C. Correct size and yield of PCR products were confirmed on a 0-8% (v/v) TAE agarose gel (Fisher Biotech).

**Specificity and detection limits of the primer set.** Specificity of the primer was examined with genomic DNA from the following species: *Pseudomonas stutzeri*, *Pseudomonas putida*, *Nocardia asteroides*, *Bacillus subtilis*, *Vibrio cholerae*, *Streptomyces lividans*, *Micromonaspora purpurea*, *Methanosarcina acetivorans*, *Rhodococcus rhodochrous*, *Escherichia coli* and *Dehalococoides* et al (genomic DNA and 16S rRNA gene clone). Sensitivity of the primer set was examined by PCR amplification of serially diluted cloned 16S rRNA gene from DF-1 with the specific primer set using the reaction conditions described above. PCR products were then separated by gel electrophoresis. The image was captured using a Gel Doc XR documentation system and analysed by examining background corrected pixel intensity using the Quantity One 1-D analysis software (Bio-Rad). When no increase in pixel intensity could be recorded the PCR assay was considered saturated.

**Amplified rDNA restriction analysis (ARDRA).** Microcosms dechlorinating 2,2',3,4,4',5'-hexachlorobiphenyl and a no PCB control were screened by ARDRA using primers 14F and Dehal1265R. Triplicate PCR products were combined and purified using the QiAqick PCR purification kit (Qiagen). Plasmid libraries were generated in vector pCR2.1 (Invitrogen) according to manufacturer’s instructions. Eighty-five clones from the dechlorinating microcosm and 45 clones from the no PCB control tested positive for inserts. PCR fragments were generated from these clones using primers 14F and Dehal1265R and screened by restriction analysis after digestion with the endonucleases *Hae*III and *Hinf*I (Pulliam Holoman et al., 1998). DNA restriction fragments were separated by gel electrophoresis on a 3% Trevigel at 25 V for 3 h at 0°C.

**Sequencing and analysis.** A Qiagen Plasmid Mini Kit was used to purify plasmid DNA according to manufacturer’s instructions. The purified plasmid was used as the template for dye terminator cycle sequencing on an ABI 373 Automated sequencer (PE Applied Biosystems). The sequence was examined for errors and edited using DNAMAN (Lynnon BioSoft), then checked for chimeras formation using the CHECK_CHIMERA program of the Ribosomal Database Project (Maidak et al., 2000). The sequence was submitted to the National Center for Biotechnology Information’s (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) and the Ribosomal Database Project (Maidak et al., 2000) to determine percentage similarity with other 16S rRNA molecules. Partial 16S rRNA gene sequences were manually compiled and aligned using PHYLIP software (Chun, 1995). Evolutionary trees were generated using the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash., 1967) and maximum-parsimony (Kluge & Farris, 1969) algorithms in the PHYLIP software package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining and
Fitch–Margoliash methods were generated as described by Jukes & Cantor (1969). The robustness of the inferred tree topologies was evaluated after 1000 bootstrap resamplings of neighbour-joining data.

RESULTS AND DISCUSSION

Primer specificity and detection limits

Primer specificity was examined by performing PCR with a wide range of microbial genomic DNA from Gram-negative and Gram-positive bacteria, including other known dechlorinating species and archaeal species (data not shown). PCR was optimized and PCR products were detected only with o-17 or DF-1 DNA as templates. No PCR products were detected with other bacteria, including closely related Dehalococcoides spp., indicating that the primers are suitable for detecting the o-17/DF-1 group in a microbial community.

Sensitivity of the Dehal1265R primer was examined by a dilution experiment. The threshold for detection by gel electrophoresis of PCR products was 100–1000 16S rRNA gene copies per PCR mixture when 8 μl was loaded onto a gel, which is similar to the detection range reported by Hendrickson et al. (2002) for the detection of Dehalococcoides spp. 16S rRNA gene copies.

Analysis of PCB-dechlorinating cultures

Application of the specific primers to PCB-dechlorinating co-cultures o-17 and DF-1 confirmed that 16S rRNA gene sequences from these cultures formed a phylogenetically distinct cluster separate from other dechlorinating bacteria (see Fig. 2). The o-17/DF-1 group has the highest 16S rRNA gene sequence similarity to the Dehalococcoides group. However, this similarity is 89–90 % over 1200 bp, which indicates that o-17/DF-1 and similar species are at least in a separate genus from Dehalococcoides. The phylogenetic analysis indicates that o-17 and DF-1 form a distinct grouping separate from Dehalococcoides based on defined co-cultures and 16S rRNA gene sequences from non-cultured micro-organisms (Fig. 2).

These primers were also applied to a highly enriched Baltimore Harbour sediment culture that reductively dechlorinates 3,5-dichlorobiphenyl to 3-chlorobiphenyl (Cutter et al., 1998). The PCR products were cloned and analysed and only one sequence, designated m-1, was detected. The m-1 sequence, which has 92–94 % similarity...
to Dehalococcoides spp., forms a separate branch between Dehalococcoides spp. and the o-17/DF-1 group (Fig. 2). The m-1 16S rRNA gene sequence was only detected in cultures that actively dechlorinated 3,5-dichlorobiphenyl; the sequence was not detected when 3,5-dichlorobiphenyl was omitted from the culture. The results indicate that the specific 16S rRNA gene primer assay can detect PCB-dechlorinating o-17/DF-1-like bacteria that selectively dechlorinate different PCB congeners.

**Detection of growth-linked PCB dechlorination**

To determine if there was a relative increase in the specific PCR product concurrent with dechlorination activity, a set of DF-1 co-cultures dechlorinating 2,3,4,5-tetrachlorobiphenyl were monitored over 30 days. Dechlorination activity appeared to lag for 5 days and was then detected at a constant rate after day 10 (Fig. 3). This pattern of dechlorination activity is routinely observed in DF-1 cultures. The results indicated a relative increase in the PCR product detected corresponding to the onset of dechlorination of 2,3,4,5-tetrachlorobiphenyl between 0 and 10 days (Fig. 3). Although dechlorination continued after 10 days, the PCR signal remained constant due to saturation of the PCR assay. In contrast, an increase in PCR product was not detected in cultures without PCB. These results confirm that dechlorination of 2,3,4,5-tetrachlorobiphenyl is linked to an increase in the relative number of DF-1 16S rRNA gene copies. These results are consistent with previous PCR-denaturing gradient gel electrophoresis (DGGE) studies (Cutter et al., 2001; Wu et al., 2002b), in which DGGE band intensities increased with PCB dechlorination activity, and with results described by Hendrickson et al. (2002), in which 16S rRNA genes PCR-amplified with specific primers for Dehalococcoides spp. increased in intensity during active reductive dechlorination of tetrachloroethene. However, the overall trend is similar in both studies in that the onset of dechlorination activity is linked to a rapid increase in specific PCR product. Similarly, Cupples et al. (2003) used competitive PCR to show that 16S rRNA genes of the chloroethene-dechlorinating strain VS increased in cultures that actively dechlorinated cis-dichloroethene and vinyl chloride. Overall the data described herein and by others indicate that an increase in PCR-based 16S rRNA gene signal is indicative of active dechlorination and presumably growth within a microbial community. Moreover, the results suggest that detection of the o-17/DF-1 group in an environmental sample provides a presumptive test for anaerobic PCB dechlorination potential in a PCB-impacted site.

**Detection of dechlorinating bacteria in PCB-enriched sediment microcosms**

The o-17/DF-1 group-specific primers were tested for their ability to monitor PCB-dechlorinating bacteria in Baltimore Harbour sediment microcosms with 2,2′,3,4,4′,5′-hexachlorobiphenyl. These microcosms had very slow rates of dechlorination and therefore provided a realistic test of these primers for detection of indigenous dechlorinating populations within the diverse sediment communities. The congener 2,2′,3,4,4′,5′-hexachlorobiphenyl was selected as it is a major component of Aroclor 1260 and in a previous study was found to be reductively dechlorinated in Baltimore Harbour sediment microcosms (Wu et al., 1998).

These cultures were examined after 240 days incubation when the mean amount of parent congener remaining was 19-7 mol% (with a standard error from three replicates of 2-1 %). The sequential reductive dechlorination products from this reaction were 2,2′,4,4′,5-pentachlorobiphenyl and 2,2′,4,4′-tetrachlorobiphenyl, which resulted from dechlorination of double- and single-flanked meta chlorines, respectively. As a negative control, the analysis was also performed on enrichment cultures that contained no added PCB. The DNA was extracted and the 16S rRNA genes were amplified by PCR using the Dehal1265R specific primer followed by ARDRA.

The phylogenetic analysis of these sequences is shown in Fig. 2. It can be observed that operational taxonomic unit (OTU) sequences are found throughout the o-17/DF-1 group. This is not surprising as the microcosms were developed using Baltimore Harbour sediment. However, the ARDRA of these cultures revealed a microbial community shift with the addition of PCB to the microcosms. When no PCB was added to the enrichment cultures, six different RFLP patterns or OTUs with similarity to o-17/DF-1 were found in relatively equal distribution (7–19 % of the clone library). These sequences were designated OTU 1–15. The addition of PCB to the cultures changed the community
composition, resulting in detection of 10 OTUs (OTUs 1–10). However, ARDRA indicated that there was a significant increase of a single OTU (OTU 1) in the community. This OTU accounted for 60% of the clone library of our microbial community, while other OTUs were found at a lower frequency (3–7%). This enriched OTU 1 has the greatest sequence similarity to o-17 (Fig. 2). Its phylogenetic placement and apparent enrichment in the presence of PCB suggests that the micro-organism represented by OTU 1 catalyses the observed dechlorination activities. Furthermore, not all putative dechlorinators detected in the no PCB control became enriched with the addition of PCB. This observation suggests that some of the species detected are not involved in that specific PCB dechlorination reaction.

Sequencing revealed that each OTU detected from these sediments had greatest sequence similarity to the o-17/DF-1 group (96–99%) and only 91–95% similarity to cultured and uncultured Dehalococcoides spp. In addition, when the primer set was applied directly to another set of 2,3,4,5-tetrachlorobiphenyl-dechlorinating enrichments in a previous study (Watts et al., 2004), the primers detected a number of unique 16S rRNA gene sequences (designated N in Fig. 2). The majority of these 2,3,4,5-tetrachlorobiphenyl microcosm sequences cluster closely with DF-1 and o-17; however, a number of these sequences are more closely related to environmental clones from Elizabeth River.

The o-17/DF-1-specific primers preferentially detected only non-Dehalococcoides sequences within the Chloroflexi. As this dechlorinating group represents a small fraction of the total microbial diversity in sediments, less than 1% based on ARDRA (Pulliam Holoman et al., 1998), the ability to selectively detect this population within the total microbial community is essential for examining its distribution and diversity. Due to community composition (von Wintzingerode et al., 1997) and PCR bias (Suzuki & Giovannoni, 1996) these minority populations are often overlooked in ARDRA using universal bacterial primer sets.

**Detection of PCB-dechlorinating populations in Chesapeake Bay Watershed**

Sediments collected from two PCB-impacted tributaries within the Chesapeake Bay, Baltimore Inner Harbour in the Patapsco tributary and a site from the Elizabeth River in the James River tributary, were examined for the presence of 16S rRNA gene sequences within the o-17/DF-1 PCB-dechlorinating group. Both sites have a history of PCB impact as a result of heavy industrial and shipping activities (Baker et al., 1997). DNA was extracted from both sites and tested with universal PCR primers, to ensure that the DNA was suitable for PCR, followed by analysis with specific PCR primers to detect dechlorinating species. Specific PCR products were cloned and sequenced for comparative sequence analysis.

The Baltimore Inner Harbour and Elizabeth River sediments yielded unique sequences that clustered with 16S rRNA gene sequences detected in Baltimore Harbour enrichment microcosms, adding to the considerable diversity of this phylogenetic group (Fig. 2). A previous report analysing PCE- and TCE-impacted sediments that dechlorinated chloroethene to ethene with Dehalococcoides-specific primer sets (Hendrickson et al., 2002) revealed only Dehalococcoides spp. Interestingly, PCR products were not detected in three sites tested by Hendrickson et al. (2002), which happened to be the only sites that partially dechlorinated PCE to 1,2-cis-dichloroethene. Both o-17 and DF-1 partially dechlorinate PCE to 1,2-cis-dichloroethene (Watts et al., 2004; Miller et al., 2005). Since the Specific-specific primers would not detect species within the o-17/DF-1 group, it is possible that these micro-organisms may have been the predominant chloroethene dechlorinators in these sites. The results of the current study indicate that a potentially diverse population of PCB dechlorinators exists in estuarine environments.

As putative PCB dechlorinators had been detected in these sediments previously, microcosms were initiated to confirm that actively dechlorinating micro-organisms were present. Dechlorination was assayed after 65 days. Microcosms from both sites exhibited active dechlorination of 2,3,4,5-tetrachlorobiphenyl with Elizabeth River sediment microcosms dechlorinating at a slightly faster rate than Baltimore Harbour sediment microcosms. At 65 days the Elizabeth River sediment microcosm had 72 ± 3 mol% original congener remaining and Baltimore Inner Harbour had 83 ± 3 mol% original congener remaining. No dechlorination was detected in sterile controls with 99.5 ± 0.17 mol% of original compound remaining after 65 days. These results indicated that active PCB dechlorinators were present in these sediment samples that tested positive for o-17/DF-1-like 16S rRNA gene sequences.

The application of probes to sediment samples from the Baltimore Inner Harbour and Elizabeth River both produced a strong PCR product with the o-17/DF-1-specific primers, but no signal was detected with Dehalococcoides-specific primers (Hendrickson et al., 2002). This may be due to our sampling sites being estuarine sediment environments rather than groundwater or freshwater samples used in prior studies. Alternatively, Dehalococcoides spp. could be present below the detection limits of the primer set due to the lack of chlorinated ethene contamination in these sites. The 16S rRNA gene sequences detected from Baltimore Harbour and Elizabeth River were most closely related to previously identified PCB dechlorinators (Fig. 2). This indicates that the specific primer set selectively detected 16S rRNA gene sequences from sediment populations closely related to PCB-dechlorinating micro-organisms. Although the exact metabolic function of these micro-organisms is unknown, their detection in sediments exhibiting PCB dechlorination activity strongly suggests that this group has a significant role in the reductive dechlorination of PCBs in sediments.

This study has focused on the development of a set of primers to facilitate analysis of a previously undetected
group in the environment that have been linked to anaerobic reductive dechlorination of PCBs. Environmental application of 6-o7/DF-1-specific primers has revealed a diverse population of dechlorinating species in PCB-impacted estuarine environments. As few dechlorinating species within this phylogenetic cluster have been described, the results suggest that the full range of dechlorinating capabilities by these microbes is largely untested. By combining 6-o7/DF-1 and Dehalococcoides-specific primer sets it is now possible to effectively detect and monitor a larger complement of species within the Chloroflexi with a potentially diverse range of dechlorinating activities. A rapid molecular screening approach for assaying the total dechlorinating potential of an environment provides a valuable monitoring tool for bioremediation feasibility studies and could provide essential information about the distribution and diversity of PCB dechlorinators in situ.

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