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Molecular Characterization of Polychlorinated Biphenyl-Dechlorinating Populations in Contaminated Sediments

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Polychlorinated biphenyl (PCB)-dechlorinating microorganisms were characterized in PCB-contaminated sediments using amplified ribosomal DNA restriction analysis (ARDRA). The sediments were prepared by spiking Aroclor 1248 into PCB-free sediments, and were inoculated with microorganisms eluted from St. Lawrence River sediments. PCB-free sediments inoculated with the same inoculum served as the control. Four restriction fragment length polymorphism (RFLP) groups in the eubacterial and two in the archaeal domain were found exclusively in PCB-spiked sediment clone libraries. Sequence analysis of the four eubacterial clones showed homology to *Escherichia coli*, *Lactosphaera pasteurii*, *Clostridium thermocellum*, and *Dehalobacter restrictus*. The predominant archaeal sequence in the PCB-spiked sediment clone library was closely related to *Methanosarcina barkeri*, which appear to support earlier findings that methanogens are involved in PCB dechlorination. When the dot-blot hybridization was performed between the sediment DNA extract and the probes designed with eubacterial RFLP groups, the intensity of two of eubacterial RFLP groups, which showed high sequence homology to *C. pascui* and *D. restrictus*, was highly correlated with the number of dechlorinating microorganisms suggesting these two members intend to contribute to PCB dechlorination.

***Keywords*:** polychlorinated biphenyls, reductive dechlorination, dechlorinating bacteria, amplified ribosomal DNA restriction analysis, restriction fragment length polymorphism

Although microbial reductive dechlorination of polychlorinated biphenyls (PCBs) has been known to occur in contaminated sediments for a decade, its microbiology is poorly understood mainly because of the failure to isolate microorganisms responsible for the process (Bedard and Quensen, 1995; Wiegel and Wu, 2000). Our microcosm studies conducted with inocula from the St. Lawrence River sediment, which is primarily contaminated with Aroclor 1248, have demonstrated that there are at least two populations with different dechlorinating competence (Sokol *et al*., 1998; Kim and Rhee, 1999; Cho *et al*., 2000). When methanogens were inhibited with 2-bromoethanesulfonate (BES) in sediments amended with Aroclor 1248, the extent of dechlorination by St. Lawrence River sediment microorganisms was reduced by about half, indicating that some methanogens are involved in PCB dechlorination (Kim and Rhee, 1999). Using a combination of the dilution fractionation and most probable number (MPN) technique, we were able to distinguish populations of different competence and also quantify the population size (Cho *et al*., 2000).

Although traditional cultivation-based methods have failed to isolate or characterize the microbial populations involved in PCB dechlorination, molecular analyses can be used to characterize many 'unculturable' microbial populations. An amplified ribosomal DNA restriction analysis (ARDRA) involving generation of a clone library from PCR-amplification of sediment DNA can be used to identify eubacterial and archaeal 16S rDNA sequences that are associated with PCB-contaminated sediments. It has been reported that the analysis of microbial community in the absence of isolation with molecular techniques is effective for identifying phylogenetic groups which is associated with PCB dechlorination (Holoman *et al*., 1998; Watts *et al*., 2001). Phylogenetic information gained from these sequence analyses is useful not only in the characterization of dechlorinating microorganisms, but also in determining dechlorination potential of contaminated sediments and monitoring the effectiveness of bioremediation efforts.

Our early investigations on population growth using the MPN technique have clearly shown that PCB-dechlorinating microorganisms can grow only in PCB-contaminated sediments (Kim and Rhee, 1997). Therefore, the present study has investigated sediment microbial populations in PCB-contaminated and PCB-free sediments using ARDRA. Assuming no PCR-amplification template bias, we expect that restriction fragment length polymorphism (RFLP) groups detected at increased frequency in clone libraries generated from PCB-contaminated sediment DNA will be PCB dechlorinating microorganisms due to their growth in these sediments. From ARDRA, we designed DNA probes from each RFLP type

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and then compared the frequency of each with the number of PCB-dechlorinating microorganisms using DNA-DNA hybridization.

Materials and Methods

Culture preparation

PCB-free sediments were air-dried and sifted through a 150-μm sieve. Sediments were spiked with PCBs by coating with Aroclor 1248 in hexane to yield a final concentration of 300 μg/g sediment, and then hexane was evaporated. PCB-free and PCB-spiked sediments were separately made into slurries by adding reduced synthetic mineral medium (Balch *et al*., 1979). Slurries were dispensed into 120 ml serum vials, sealed with Teflon®-coated rubber stoppers and aluminum crimp seals, and autoclaved for 40 min on three successive days.

Autoclaved vials with PCB-spiked sediment slurries were divided into three groups and treated as follows: molybdate (10 mM), BES (10 mM), and no addition of inhibitors. The vials were inoculated with sediment slurry from a single batch of previous Aroclor 1248 enrichment culture which had been set-up in Aroclor 1248-spiked sediments with microorganisms eluted from the Reynolds site in the St. Lawrence River (Sokol *et al*., 1998). Culture set-up and sampling was performed in an anaerobic chamber (Coy Laboratory Products, USA).

Counting of anaerobic bacteria

The number of PCB-dechlorinating microorganisms was estimated by MPN method described previously (Kim and Rhee, 1997). MPN vials were prepared with 0.25 g of PCB-free sediment and 9 ml of reduced synthetic mineral medium. The sediment in the MPN vials was spiked with a mixture of 2,3,4- and 2,5,3',4'-chlorobiphenyls (IUPAC number 21 and 70, respectively; 150 μg/g sediment of each congener). A dilution series (10^{-1} to 10^{-7}) of each sample was prepared by serially transferring a 1 ml portion of sediment slurry. From each dilution, five MPN vials were inoculated. The vials were then incubated statically for 15 weeks. After incubation, MPN vials showing dechlorination were counted as positive when dechlorination removed greater than 5% of the total chlorines. Sulfate-reducing bacteria (SRB) were determined by the blackening of sediments in the sample vials with the production of FeS. Methanogens were determined by analyzing the head-space gas of MPN vials for methane on a gas chromatograph with a thermal conductivity detector (Kim and Rhee, 1997). MPNs were calculated from the frequency of positive vials by using a Microsoft Excel program (Briones and Reicharst, 1999) and were normalized to gram dry weight of sediment.

PCB extraction and analysis

For congener-specific analysis, sediments were Soxhlet-extracted with hexane-acetone solvent mixture as described previously (Rhee *et al*., 1993; Sokol *et al*., 1998). The solvent extract was phase-separated by addition of distilled water, and the hexane layer was placed into a flask containing sodium sulfate. Hexane extracts were treated with a tetrabutylammonium-hydrogen sulfite reagent to remove elemental sulfur, and cleaned up on a deactivated Florisil column. Quantitative analysis of Aroclor 1248 was performed by using a gas chromatograph (Hewlett-Packard 5890II, Agilent, USA) equipped with a ^{63}Ni electron capture detector and a HP-5 fused silica capillary column (Agilent). The gas chromatography conditions used were described elsewhere (Rhee *et al*., 2001). The PCB congeners in the Aroclor 1248 extract were identified and quantitated using a calibration standard containing a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 μg/ml of each in hexane). Peaks were identified and calibrated as previously described (Rhee *et al*., 1993; Sokol *et al*., 1994). PCB extracts from MPN vials were identified and quantitated with a calibration standard composed of a mixture of 47 individually weighed authentic single congener standards (99% purity; AccuStandard, USA). Uninoculated PCB-spiked sediment controls, set up at the beginning of the experiment and sampled at every time point, were used to monitor extraction efficiency.

Extraction of DNA and PCR amplification

The cells in sediment-slurry samples were disrupted by a bead-beating method using sodium phosphate buffer with SDS (Lee *et al*., 1996; Holoman *et al*., 1998). Crude DNA in the supernatant was purified with phenol-chloroform, isopropanol precipitation, and low-melting-temperature gel purification with polyvinylpyrrolidone (Young *et al*., 1993).

Eubacterial universal primers: U519F; 5'-CAG-CMG-CCG-CGG-TAA-TWC-3' and U1406R; 5'-ACG-GGC-GGT-GTG-TRC-3' (Lane *et al*., 1985) and archaeal primers: A21F; 5'-TTC-CGG-TTG-ATC-CYG-CCG-GA-3' and A958R; 5'-YCC-GGC-GTT-GAM-TCC-AAT-T-3' (DeLong, 1992) were used for eubacterial and archaeal 16S rDNA amplification, respectively. For all PCRs, the final Mg^{2+} concentration was 2 mM, and 0.02 U of AmpliTaq® DNA polymerase (PE Biosystems, USA) per microliter was used. The PCR cycle for amplification of sediment DNA consisted of a 4 min pre-cycle at 94°C, 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and polymerization at 72°C for 30 sec, and ending with a 10 min extension period at 72°C. Separate PCRs without polymerase, template DNA, or primers were performed as detection of contamination controls. Plasmid libraries for each sample were generated by ligating purified PCR fragments (Wizard® PCR Preps DNA Purification System, Promega, USA) into the pGEM®-T Easy Vector Systems (Promega) in accordance with the manufacturer's instructions.

Library screening

From each of the DNA samples amplified by PCR with eubacterial or archaeal primers, two hundred clones were randomly chosen. The clones were grown overnight in LB with ampicillin. The partial 16S rRNA gene fragments were purified by PCR amplification directly from 2 μl of an overnight grown LB broth culture by pre-treatment of the cell suspension at 94°C for 10 min followed by addition of 48 μl of PCR mixture. PCR consisted of 40 cycles as described for sediment DNA amplification, except that annealing time was reduced to 30 sec. The resulting PCR products were digested separately with the restriction endonucleases *Hae*III and *Hha*I (Promega). The restriction digests were electro-

phoresed in a 3% NuSieve 3:1 agarose gel (Sigma- Aldrich, USA) along with PCR marker DNA. Clones were categorized according to their distinct restriction banding pattern.

Sequencing and phylogenetic analysis

The partial 16S rRNA gene fragment from a representative clone from selected RFLP groups was sequenced on an ABI PRISM 377XL automated DNA sequencer (PE-Biosystems) following DyeDeoxy Terminator cycle sequencing. Both the forward and reverse strands were sequenced using the PCR primers as sequencing primers. Sequences were analyzed with the BLASTN (v. 2.0) program of the National Center for Biotechnology and Information, and the Sequence Match and Chimera Check (SimRank v. 2.7) programs of the Ribosomal Database Project (Maidak *et al*., 2001). Nucleotide sequence data for RFLP groups listed in Table 1 were submitted to GenBank and assigned the following accession numbers: from AF276441 to AF276445 for archaeal bacteria and from AF276446 to AF276462 for eubacteria (Table 1). Partial 16S rDNA sequences (850 bp) used to construct a consensus tree were aligned using the PileUp program from the SeqWeb interface (v. 1.1) to the Genetics Computer Group Wisconsin Package (v. 10.0, Genetics Computer Group, USA). All tree-construction programs used were part of the PHYLIP software package (Phylogeny Inference Package v. 3.57c, University of Washington, USA). The SEQBOOT program was used to generate 100 bootstrap data sets. Evolutionary distances for each bootstrap data set were calculated using the corrections of Jukes and Cantor with the DNADIST program. The FITCH program was used to generate trees from the calculated evolutionary distances for each data set and the CONSENSE program was then used to construct a single consensus tree.

Dot-blot hybridization

DNA was extracted from sediment samples collected at various time points during the course of PCB dechlorination using the protocol from the UltraClean soil DNA kit (Mo Bio Laboratories, USA) with no modifications. Aliquots of extracted DNA were applied to a nylon membrane (Hybond™ -N^+ nylon, Amersham Biosciences, USA) using a dot-blot manifold (Bio-Dot® Microfiltration Apparatus, Bio-Rad, USA). The 16S rRNA gene oligonucleotide sequences for five of the RFLP clone groups (17E, 18E, 28E, 34E, and 92E) were used as probes. Probes were labeled using a 3'-oligonucleotide fluorescent labeling kit (Amersham Biosciences), which used fluorescein 11-dUTP as the label. Signal amplification was performed using the ECF detection module from Amersham Biosciences. A FluroImager (Molecular Dynamics) was used to detect fluorescent hybridization signals. Hybridization signal intensities were determined using Scion Image software (Scion Corporation, USA).

Table 1. Phylogenetic relationship of eubacterial and archaeal RFLP groups from St. Lawrence River PCB-dechlorinating enrichment cultures based on 16S rDNA sequences (GenBank accession numbers for closest relatives are given in parentheses)

RFLP group	Nearest phylogenetic relative	Similarity (%)
2E	*Bacteriodes merdae* (X83954)	90
3E	*Escherichia coli* (J01859)	97
8E	Uncultured eubacterium WCHA1-24 (AF050602)	91
10E	*Desulfocapsa thiozymogenes* (X95181)	94
13E	*Bacteriodes fragilis* (X83939)	89
14E	*Escherichia coli* (J01859)	95
17E	*Ruminococcus bromii* (X85099)	92
18E	*Clostridium pascui* (X96736)	92
20E	*Bacteriodes fragilis* (X83939)	70
21E	*Atopobium minutum* (X67148)	92
28E	*Atopobium minutum* (X67148)	94
31E	*Lactosphaera pasteurii* (X87150)	99
34E	*Dehalobacter restrictus* (U84497)	92
39E	*Cytophaga* sp. Sva1038 (AJ240979)	92
40E	*Bacteriodes merdae* (X83954)	90
50E	*Clostridium thermocellum* (L09173)	90
51E	Uncultured eubacterium WCHB1-29 (AF050544)	95
62E	*Clostridium hydroxybenzoicum* (L11305)	93
92E	Uncultured eubacterium WCHA1-01 (AF050541)	98
102E	*Lactosphaera pasteurii* (X87150)	97
1A	*Methanosarcina barkeri* (AF028692)	99
15A	Unidentified crenarchaeote (U59986)	97
43A	*Methanosaeta concilii* (X16932)	95
214A	*Methanosarcina barkeri* (AF028692)	98
308A	*Methanosarcina barkeri* (AF028692)	99

Results and Discussion

Dechlorination of Aroclor 1248 and growth of anaerobic bacteria

Dechlorination of Aroclor 1248 occurred mostly before 6 weeks in cultures without added inhibitors (Fig. 1). A lag period of two weeks was apparent, followed by a two-week exponential phase. After 6 weeks of incubation, dechlorination reached a plateau with no further changes out to 14 weeks of incubation. A congener-specific PCB analysis at the plateau level showed that, on average, the sediment microorganisms dechlorinated 38% of the total chlorines, removing both *meta*- and *para*-chlorines. On the other hand, dechlorination was less extensive in cultures amended with BES, removing only about 17% of total chlorines. The addition of molybdate had no effect on extent of Aroclor 1248 dechlorination (Fig. 1). The less dechlorination in the samples amended with BES was caused by the absence of *meta*-rich congeners such as 2,5,2'-, 2,5,3'-, and 2,5,2',5'-chlorobiphenyls (data not shown). After 14 weeks of incubation, the extent of *meta*- and *para*-dechlorination in BES-amended sediments were 14% and 36%, respectively, compared to 65% and 38% in BES-free sediments.

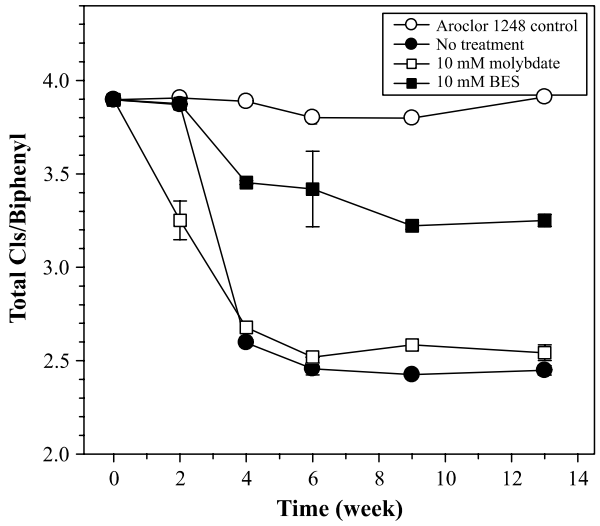

Fig. 1. Time course of Aroclor 1248 dechlorination expressed as the number of Cl per biphenyl in sediments added with molybdate or 2-bromoethanesulfonate (BES). Each point represents the Mean (±SD) of triplicate samples.

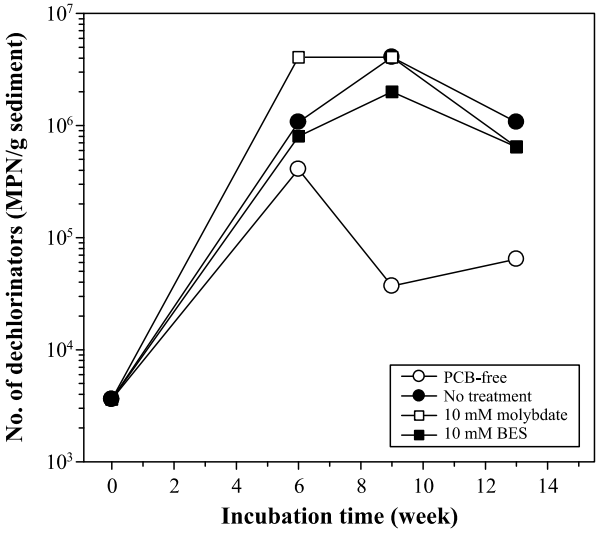

Fig. 2. The growth of PCB-dechlorinators in sediments added with molybdate or 2-bromoethanesulfonate (BES).

In sediments without added inhibitors, the number of dechlorinating microorganisms increased from an initial size of 3.6×10^3 to 4.1×10^6 MPNs/g sediment by 9 weeks with little changes out to 13 weeks (Fig. 2). At the time of sampling for DNA extraction for ARDRA, the number of dechlorinators in these samples was 1×10^6 MPNs/g sediment. In PCB- and inhibitor-free sediments, the number of PCB-dechlorinating microorganisms increased from 3.6×10^3 to a maximum of 4×10^5 MPNs/g sediment, and then decreased to 6×10^4 MPNs/g sediment by 13 weeks of incubation (Fig. 2). In BES-amended sediment, dechlorinator population size increased to 4.1×10^6 MPNs/g sediment after 6 weeks, then reached a plateau at 6.5×10^5 MPNs/g sediment by 13 weeks. An addition of molybdate did not have any effect on the growth of PCB dechlorinators; the dechlorinating populations increased to 4.5×10^6 MPNs/g sediment after 6 weeks.

MPN estimates for methanogens and SRB were calculated from the same MPN vials used to enumerate PCB-dechlorinators. At the time of sampling for DNA extraction, the estimated number of methanogens in both PCB-spiked and PCB-free cultures without inhibitors were similar at 2.3×10^7 and 2.7×10^7 MPNs/g sediment, respectively. The number of SRB in PCB-free and PCB-spiked cultures was not significantly different at 8.7×10^7 and 6.9×10^7 MPNs/g sediment, respectively. Estimated number of methanogens and SRB were at least one order of magnitude greater than the number of PCB dechlorinators throughout the incubation time course (data not shown).

Characterization of eubacterial populations

Analysis of 200 clones from each library by RFLP revealed 23 eubacterial groups. Eubacterial RFLP group 2E was the most predominant group accounting for 31% of the clones in PCB-free sediments and 20% in PCB-spiked sediments (Fig. 3A). Sequence analysis identified RFLP group 2E as *Bacteriodes merdae* (Table 1 and Fig. 4). A total of 11 eubacterial RFLP groups were enriched in the PCB-spiked sediment library and six (14E, 20E, 28E, 31E, 34E, and 50E) were found exclusively in the PCB-spiked sediment library (Fig. 3A). The nearest relatives of the remaining clones found exclusively in the eubacterial PCB-spiked sediment clone library were *Escherichia coli* (14E), *Lactosphaera pasteurii* (31E), *Dehalobacter restrictus* (34E), and *Clostridium thermocellum* (50E) (Table 1 and Fig. 4). The RFLP groups 2E, 10E, 22E, and 113E were more frequent in the PCB-free library than in the PCB-spiked library. No RFLP group was found exclusively in the PCB-free sediment library. RFLP group 1E and 17E were found in equal proportion in both clone libraries.

In a previous study using RFLP to determine microbial

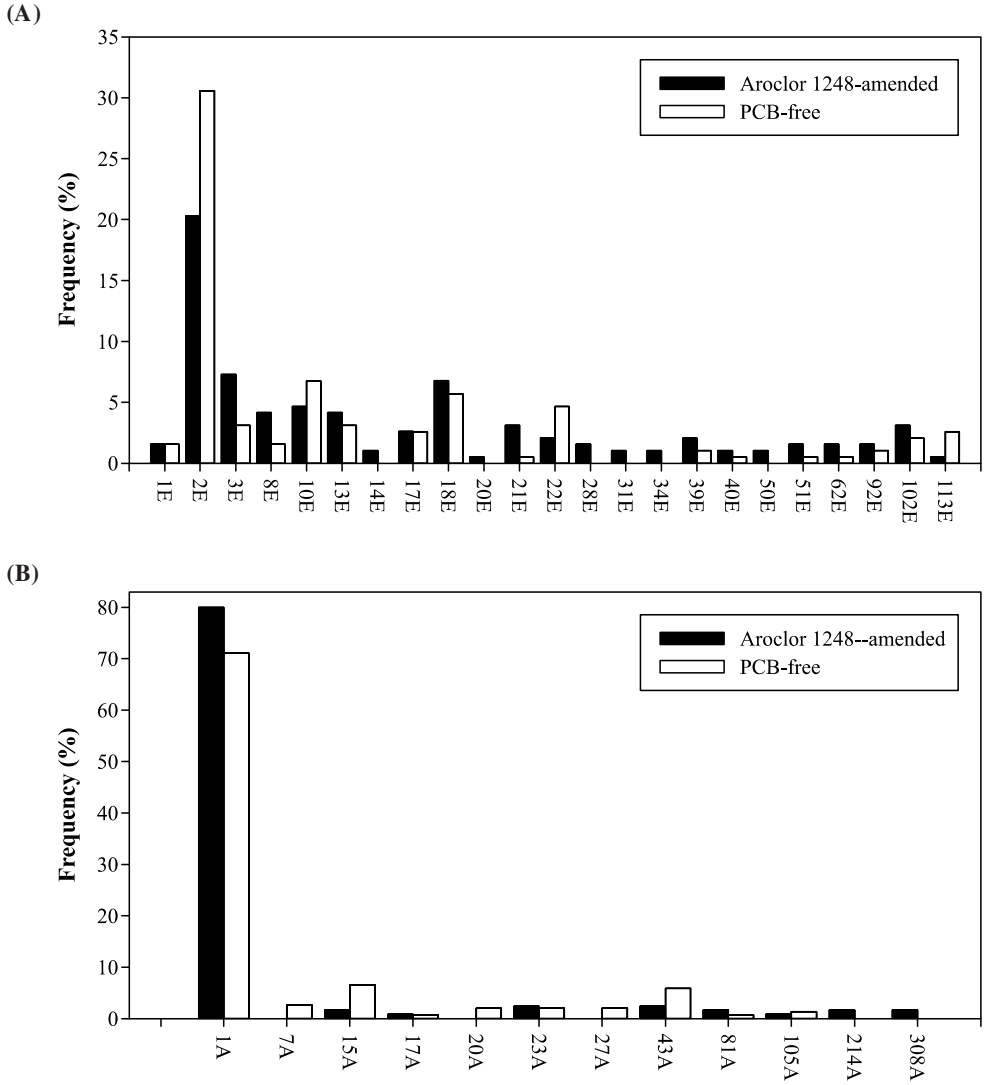

Fig. 3. Distribution and frequency of RFLP groups from 16S rRNA gene clone libraries from PCB-spiked and PCB-free St. Lawrence River sediments. (A) Eubacterial RFLP groups (B) Archaeal RFLP groups.

populations responsible for PCB dechlorination, several RFLP groups belonging to various physiological phylogenetic subgroups specific for PCB-contaminated sediments were identified (Holoman *et al*., 1998). Because the same restriction enzymes used in the previous study were employed in the current study, a direct comparison of the RFLP groups could be performed. The predominant groups observed in the previous study were not the predominant ones in our sediments. For example, the predominant RFLP group in eubacterial clone libraries of PCB-spiked and PCB-free sediments was 2E, which had sequence homology to *Bacteroides merdae*. The predominant eubacteria groups observed in Holoman *et al*. (1998) were *Clostridium litorale* and *Desulfosarcina variabilis* in PCB-free and PCB-amended sediments, respectively. These RFLP groups were observed at much lower frequency in our sediments.

Sediment DNA used for the RFLP analysis by Holoman *et al*. (1998) was from a culture that had been selectively enriched on the PCB congener 2,3,5,6-tetrachlorobiphenyl and demonstrated only *ortho* dechlorination. The original inoculum source of this enrichment culture was Baltimore Harbor sediment. The difference in RFLP groups between two studies may be due to sediment sources (harbor vs. river sediments) contaminated with different PCB mixtures (Aroclor 1260 vs. Aroclor 1248) and exhibiting different PCB-dechlorination competence (*ortho* dechlorination vs. *meta* and *para* dechlorination) that are likely the result of differences in the microbial populations.

Two clone groups, which were found exclusively in the PCB-spiked sediment library, showed homology to *Clostridum thermocellum* (50E) and *Dehalobacter restrictus* (34E). The genus *Dehalobacter* is closely related to the genus *Clostridium*, and a strain identified as *Dehalobacter restrictus* is known to reductively dechlorinate tetrachloroethene and trichloroethene (Wild *et al*., 1996). The genus *Clostridium* was also represented by two of the 11 RFLP groups (18E and 62E) present

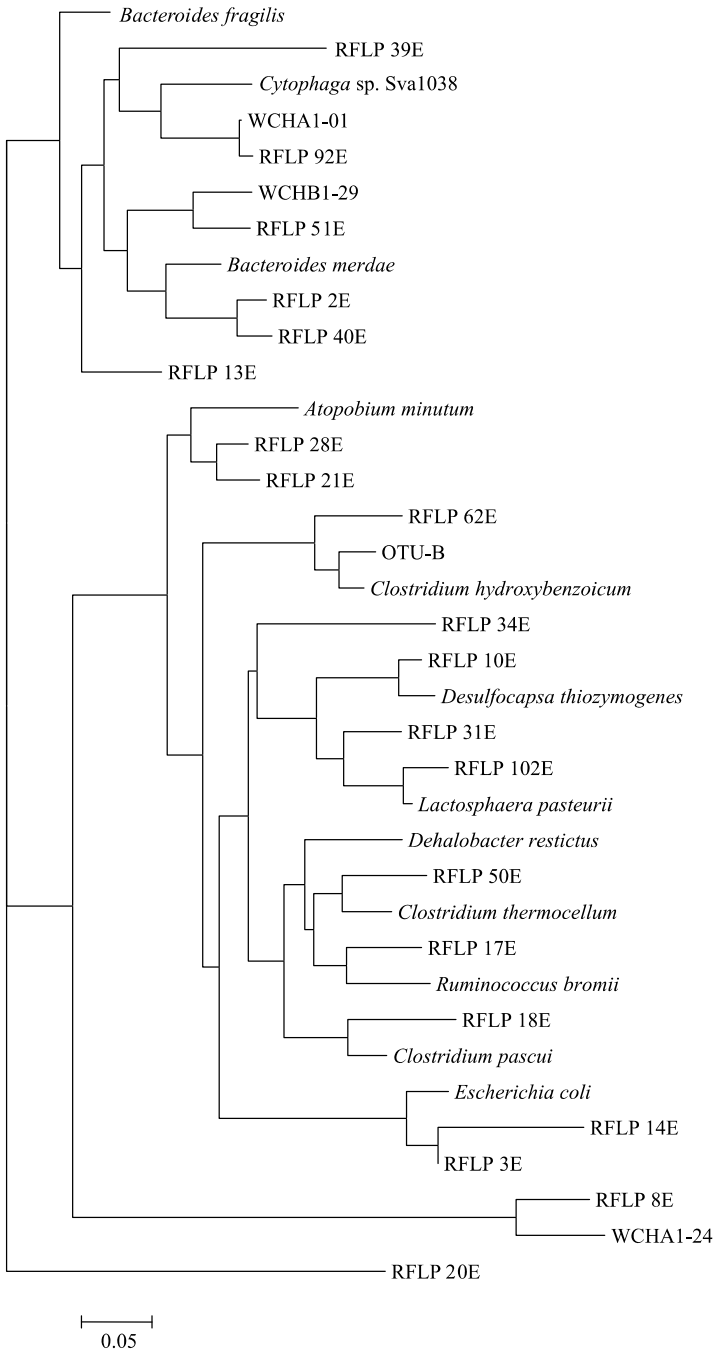

Fig. 4. Consensus dendrogram of eubacterial partial 16S rRNA gene sequences.

at greater frequencies in the eubacterial PCB-spiked sediment library than in the PCB-free sediment library. In total, RFLP groups with sequence homology to species within the genera *Clostridium* and *Dehalobacter* represented approximately 10% of the total clones in the PCB-spiked sediment library, compared to about 6% in the PCB-free sediment library. All the RFLP groups with sequence homology to species in the genus *Clostridium* were found either exclusively or at greater frequency in the PCB-spiked sediment library than in the PCB-free sediment library.

Clostridium litorale, the most predominant clone in a PCB-spiked sediment library from a Baltimore Harbor enrichment culture showing *ortho* dechlorination, was ruled out as a possible PCB-dechlorinator (Holoman *et al*., 1998). However, RFLP clone groups with sequence homology to two *Clostridium* species dominant in cultures showing *para* or *meta* dechlorination have been reported (Lamontagne *et al*., 1998; Hou and Dutta, 2000). One of the RFLP groups, OTU-B, is predominant in a *para*-dechlorinating culture and was approximately 88% identical to our RFLP group 62E

(Hou and Dutta, 2000). Both OTU-B and 62E showed sequence homology to *Clostridium hydroxybenzoicum*. Evidence for the presence of *Clostridium* in other non-marine *meta* and/or *para* PCB-dechlorinating populations has also been demonstrated (Ye *et al*., 1992, 1995; Williams, 1994; Bedard and Quensen, 1995).

The majority of clones (5 clone groups out of 11) enriched in the PCB-spiked sediment library were related to species in the genera *Bacteroides* or *Cytophaga*. The only clone group with homology to the genus *Bacteriodes* that was not enriched in the PCB-spiked sediment library was 2E, which was the most predominant clone group in both libraries. Excluding 2E, the total frequency of groups related to the *Bacteroides* or *Cytophaga* was about 9% in the PCB-spiked sediment library compared to about 6% in the PCB-free sediment library. One clone groups related to the genus *Bacteroides* was found in the Baltimore Harbor *ortho*-dechlorinating clone library (Holoman *et al*., 1998). A total of 8 clone groups with sequence homology to the division Cytophagales were detected at low frequency (<1~2%) in a 16S rRNA gene clone library of an aquifer contaminated with hydrocarbons and chlorinated solvents (Dojka *et al*., 1998).

RFLP groups 21E and 102E showed sequence homology to *Atopobium minutum*, and *Lactosphaera pasteurii*, respectively. RFLP group 8E was found to be related to eubacterial clone WCHA1-24, which is a member of a proposed new division, OP11, which currently has no cultivated species (Harris *et al*., 2004). All three of these groups are probable candidates for PCB dechlorinators because clones were detected at greater frequency in the PCB-spiked sediment library than in the PCB-free sediment library. In addition, sequences homologous to all three were detected at low frequency (1~4%) in a eubacterial clone library from a contaminated aquifer (Dojka *et al*., 1998). However, sequences homologous to any of the three were not detected in the clone library from Baltimore Harbor *ortho*-dechlorinating cultures (Holoman *et al*., 1998).

Two eubacterial RFLP groups, 3E and 14E, had high sequence homology to *E. coli*. We did not expect to find sequences homologous to *E. coli*, especially not at the relatively high frequency seen for RFLP group 3E. The PCB-dechlorinating culture used as the DNA source was one that had been successively transferred and incubated at least three times in Aroclor 1248-contaminated sediment prior to DNA extraction. Growth of *E. coli* under the strict anaerobic conditions of the PCB-dechlorinating culture would be unlikely, especially for more than one transfer. It is possible that these RFLPs came out from 16S rDNA of *E. coli* host strain used in cloning because we amplified 200 clones directly by colony PCR method. When we compared the restriction patterns with the two enzymes utilized in this study and 16S rDNA sequence of *E. coli* host cell, they did not match the restriction patterns and sequences observed for RFLP groups 3E and 14E. Therefore, we suspect that although the sequences for 3E and 14E are closely related to *E. coli*, they may be closer to an as yet undetermined eubacterial genus and/or species. This possibility exists because the ribosomal database comprises mostly cultivated organisms of clinical importance.

Although the presence of SRB in our cultures was indicated by blackening of sediments, only one RFLP group was determined to have homology to a known SRB. RFLP group 10E was found to be related to *Desulfocapsa thiozymogenes*, which is a novel genera and species within the Desulfobulbus division (Janssen *et al*., 1996). Our RFLP analysis did not reveal high frequencies of SRB in either the PCB-free or PCB-contaminated sediment libraries, which is in contrast to results obtained by Holoman *et al*. (1998). One explanation for this might be that SRB play a more predominant role in PCB dechlorination in sediments from marine or estuarine environments than in freshwater ones due to a greater concentration of sulfate in these environments.

Characterization of archaeal populations

The RFLP analysis of 200 clones from each library generated with archaeal PCR primers revealed 12 different groups (Fig. 3B). The most predominant archaeal RFLP group was 1A, which accounted for 71% and 80% of the clones in the PCB-free and PCR-spiked sediment libraries, respectively. Of the 12 total archaeal RFLP groups, only three groups were enriched in the PCB-spiked sediment library (1A, 214A, and 308A). The RFLP groups 214A and 308A were found only in the PCB-spiked sediment library (Fig. 3B), and were highly homologous (98 and 99% identity, respectively) to *Methanosarcina barkerii* (Table 1). The two predominant archaeal clone groups, 1A and 43A, were found to have sequence homology to members of the Euryarcheota, specifically, *Methanosarcina barkerii* and *Methanosaete concilli*, respectively (Table 1). Archaeal clone group 15A was found to have sequence homology to an unidentified crenarcheote.

The predominant archaeal clone 1A and the exclusive archaeal groups 214A and 308A are nearly identical to *Methanosarcina barkeri* (98~99% identity). There is evidence that methanogens may play a role in certain *meta* dechlorinations (Chang *et al*., 1994; Kim and Rhee, 1999; Ye *et al*., 1999). A second methanogen group was identified in our RFLP analysis. RFLP group 43A had homology to *Methanosaete concilli*. Archaeal clone groups with sequence homology to *M. concilli* were also found in Baltimore Harbor sediments demonstrating *ortho* dechlorination (Holoman *et al*., 1998).

One reason why more eubacterial RFLP groups were identified than archeal RFLP groups is that there are more eubacterial anaerobic sediment microorganisms than archaeal ones. Sulfate-, nitrate, and iron-reducing bacteria are in the eubacterial domain and make up a large fraction of the total anaerobic sediment microbial community. Methanogens are in the archaeal domain and comprise a relatively smaller fraction of the total sediment microbial community.

Dot-blot hybridization

DNA was extracted from the cultures containing the inhibitors BES and molybdate, and used in hybridization studies with the PCR-amplified 16S rRNA gene from each eubacterial RFLP group as probes. The signal intensity (minus background) of the culture sediment DNA samples hybridized with each RFLP group probe was plotted against the bacterial cell numbers determined for that sample in order to identify probes that correlated to PCB dechlorination activity. Correlations between the cell numbers and signal

intensity were determined by linear regression analysis. Data for BES-treated samples were not used in the regression analysis because dechlorinator MPN was overestimated due to dilution of the inhibitor during MPN sample preparation allowing for growth of methanogens in the MPN vials (as indicated by detection of methane by GC). Eubacterial RFLP groups 17E and 92E showed MPN:hybridization intensity correlations of 0.92 (linear regression, $P=0.008$) and 0.91 ($P=0.01$), respectively, while RFLP groups 28E and 34E had lower correlations of 0.78 ($P<0.05$) and 0.81 ($P<0.05$), respectively. RFLP group 18E had a regression correlation of 0.78 ($P<0.05$) and all other RFLP groups had correlations below 0.20. Except for 17E, all RFLP groups with correlations above 0.60 were higher in frequency in the clone library from PCB-spiked sediments than in clone library from PCB-free sediments.

The results of DNA hybridization experiments indicate that organisms containing partial 16S rRNA gene sequences with restriction patterns like those of RFLP groups 18E, 28E, 34E, and 92E may be PCB dechlorinators. Further work needs to be conducted before concluding that these RFLP groups are related to PCB-dechlorinating populations.

The goal of this work was to characterize the microbial populations involved in PCB dechlorination in St. Lawrence River sediments. 16S rRNA gene probes are useful to identify specific microbial populations within a mixed sediment community and track the populations as the environment changes (Margesin *et al*., 2003). Although the identification of PCB dechlorinators was not determined in this work, it has yielded information about the kinds of microorganisms possibly involved in PCB dechlorination. Methanogens were determined to be the predominant archaeal group while *Clostridium* and *Bacteriodes*/*Cytophaga* were the predominant eubacterial groups with possible roles in reductive dechlorination of PCBs in this sediment. The results of this study can lead to design of PCB-dechlorinator specific DNA probes, which could be used to track the PCB-dechlorinating population as the environment changes in response to bioremediation efforts.

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