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PCB dechlorination hotspots and reductive dehalogenase genes in sediments from a contaminated wastewater lagoon

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Abstract

Polychlorinated biphenyls (PCBs) are a class of persistent organic pollutants that are distributed worldwide. Although industrial PCB production has stopped, legacy contamination can be traced to several different commercial mixtures (e.g., Aroclors in the USA). Despite their persistence, PCBs are subject to naturally occurring biodegradation processes, although the microbes and enzymes involved are poorly understood. The biodegradation potential of PCB-contaminated sediments in a wastewater lagoon located in Virginia (USA) was studied. Total PCB concentrations in sediments ranged from 6.34 to 12,700 mg/kg. PCB congener profiles in sediment sample were similar to Aroclor 1248; however, PCB congener profiles at several locations showed evidence of dechlorination. The sediment microbial community structure varied among samples but was dominated by Proteobacteria and Firmicutes. The relative abundance of putative dechlorinating Chloroflexi (including *Dehalococcoides* sp.) was 0.01–0.19% among the sediment samples, with *Dehalococcoides* sp. representing 0.6–14.8% of this group. Other possible PCB dechlorinators present included the Clostridia and the *Geobacteraceae*. A PCR survey for potential PCB reductive dehalogenase genes (RDases) yielded 11 sequences related to RDase genes in PCB-respiring *Dehalococcoides mccartyi* strain CG5 and PCB-dechlorinating *D. mccartyi* strain CBDB1. This is the first study to retrieve potential PCB RDase genes from unenriched PCB-contaminated sediments.

Keywords

PCBs; *Dehalococcoides mccartyi*; Reductive dehalogenase genes; *Geobacteraceae*; Chloroflexi; *Clostridium*; Reductive dechlorination; Wastewater lagoon sediments

Introduction

Forty years after the halt of polychlorinated biphenyl (PCB) production in the USA, these persistent organic pollutants remain a threat to the environment and human health. More than 19% of USEPA National Priority List sites name PCBs among the contaminants of

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concern. Because of their persistence and toxicity, the Stockholm Convention banned PCBs globally in 2001. Although there are 209 possible PCB congeners, a significant amount of environmental PCB contamination in the USA can often be traced to commercial Aroclor mixtures (Erickson 1997) which collectively contain 140–150 different congeners (Frame et al. 1996). Aquatic sediments represent a large sink for spilled and improperly disposed Aroclor mixtures (Beyer and Biziuk 2009). PCBs are hydrophobic and therefore adsorb strongly to aquatic sediments or form an oil-water sludge at the bottom of contaminated water bodies (Wiegel and Wu 2000). An equilibrium is established between PCBs adsorbed to particles or sediments and those desorbed into water or biota (Beyer and Biziuk 2009). The bioavailable PCBs may then bioaccumulate in aquatic organisms and biomagnify in the food chain (Borja et al. 2005).

Despite the documented persistence of PCBs in the environment, biodegradation has been observed under both aerobic and anaerobic conditions (Borja et al. 2005; Flanagan and May 1993; Quensen et al. 1988; Wiegel and Wu 2000). Anaerobic reductive dechlorination processes, which remove chlorines from halogenated organics and replace them with hydrogen atoms, display distinct congener specificities. Removal of the *meta*- and *para*-substituted chlorines appears to be a primary PCB dechlorination mechanism in aquatic sediments resulting in an enrichment of *ortho*-substituted congeners (Bedard 2003). PCB reductive dechlorination yields congeners more susceptible to mineralization by aerobic biotransformations (Quensen et al. 1988).

Several bacterial genera within the phylum Chloroflexi, including *Dehalococcoides*, *Dehalogenimonas*, and *Dehalobium*, are known to dechlorinate specific PCB congeners as well as Aroclor mixtures either cometabolically or by organohalide-respiration (Bedard et al. 2007; Wang et al. 2014; Wang and He 2013a, b; Zhen et al. 2014). Additionally, 16S ribosomal RNA (rRNA) gene sequence analysis of *meta*- and *para*-PCB dechlorinating sediment microcosms implicated several *Clostridium* spp. (Firmicutes) in Aroclor 1242 dechlorination (Hou and Dutta 2000). Recent evidence indicates *Geobacteraceae* (δ -Proteobacteria) may play a role in PCB dechlorination (Praveckova et al. 2016).

Reductive dehalogenases (RDases) are the key enzymes of PCB dechlorination processes, and nearly all known reductive dechlorinators possess multiple reductive dehalogenase genes (Hug et al. 2013). RDases involved in PCB dechlorination have been elusive until recently when three PCB RDase genes, known as *pcbA1*, *pcbA4*, and *pcbA5*, were identified in *Dehalococcoides mccartyi* strains CG1, CG4, and CG5, respectively (Wang et al. 2014). These RDases are similar to RDases found in *D. mccartyi* strains 195, GT, and JNA, although *D. mccartyi* strain CBDB1 shares no *pcbA1*, *pcbA4*, and *pcbA5* orthologs (Bedard 2014).

Despite numerous microcosm and sediment-free culture studies (Krumins et al. 2009; Matturro et al. 2016a; Wang et al. 2014; Wang and He 2013a), the diversity of PCB dechlorinators and relevant RDases in the environment remains unclear. The purpose of this study was to search for evidence of PCB reductive dechlorination processes in unenriched sediments from a PCB-contaminated wastewater lagoon (up to 12,700 mg PCB/kg of sediment), characterize the microbial community structure in the sediments with respect to

known and potential PCB dechlorinating bacteria, and conduct a survey for putative PCB RDase genes.

Materials and methods

Site description and sample collection

The PCB-contaminated site is an emergency wastewater overflow lagoon located in Altavista, Virginia. This site became contaminated with PCBs at some point prior to 1977. No other contaminants are currently known to be present at the site. Sediment samples (27) were collected by hand auger on a boat in September 2015 according to a grid pattern (Fig. 1). The sediment samples, which had a sludge-like consistency, were subsequently homogenized by hand, placed into pre-cleaned glass containers, and stored at 4 °C prior to further analysis.

PCB extraction and congener analysis

Preparation, extraction, and cleanup procedures for measuring PCB sediment concentrations were performed as described previously (Martinez and Hornbuckle 2011). Briefly, ~ 0.5 g of wet sediment was mixed with combusted diatomaceous earth, spiked with 50 ng of surrogate standard [PCB14 (3,5-dichlorobiphenyl), PCB65-d5 (2,3,5,6-tetrachlorobiphenyl-d5, deuterated) and PCB166 (2,3,4,4',5,6-hexachlorobiphenyl)] and extracted by pressurized fluid extraction (ASE, Dionex ASE-300). The extracts were concentrated and cleaned through a Pasteur pipette filled with 0.1 g of combusted silica gel and 1 g of acidified silica gel and eluted with hexane. The elute was concentrated to ~ 0.5 mL and spiked with 25 ng of internal standard [[PCB30-d5 (2,4,6-trichlorobiphenyl-2',3',4',5',6'-d5, deuterated) and PCB204 (2,2',3,4,4',5,6,6'-octachlorobiphenyl)].

PCB identification and quantification were conducted employing a modified US EPA method 1668C (Herkert et al. 2016; Martinez et al. 2017). Tandem mass spectrometry GC/MS/MS (Agilent 7000) in multiple reaction monitoring mode was utilized to quantify all 209 congeners in 171 individual or coeluting congeners peaks. The GC was equipped with a Supelco SBP-Octyl capillary column (30 m × 0.25 mm ID, 0.25 mm film thicknesses) with helium as carrier gas.

QA/QC

Surrogate standards and laboratory blanks were used to evaluate our QA/QC. Percentage recoveries of surrogate standards were 79 ± 19 and 76 ± 16 for PCB14 and PCB65-d5, respectively. Surrogate PCB166 yielded high values ($260 \pm 600\%$) due to presence of PCB128 in the samples. PCB128 coelutes with PCB166 but typically is not present in the environment. However, due to the high values of PCBs in these sediment samples, the presence of PCB128 affected PCB166 recoveries. Thus, PCB congener masses were corrected using the percentage recovery of congeners PCB14 (congeners 1–39) and PCB65-d5 (congeners 40–209). Total PCB mass in field and laboratory blanks was on average less than 1% of the total PCB mass detected in the samples. The limit of quantification (LOQ) for each PCB congener was obtained from the upper 95% confidence interval from the mass of the laboratory blanks ($n = 5$). Congener-specific LOQs ranged from 0.0001 to 1.8 ng per

g, with a Σ PCB mass of 24 ng per g. Any congener mass value below the LOQ was substituted with zero.

PCB congener profiles obtained from sediment samples were compared to the Aroclor 1248 congener profile, previously analyzed and quantified with the analytical method described above (Koh et al. 2015), according to cosine theta analysis (DeCaprio et al. 2005; Martinez and Hornbuckle 2011). The relative abundance (%) of *meta*-, *para*-, and *ortho*-chlorines in PCB congeners, the relative abundance (%) of PCB congeners with 1–3, 4–6, and 7–10 chlorines, and the molar dechlorination product ratio (MDPR) of PCB congener profiles was determined as shown below (Liang et al. 2014).

$$MDPR = \frac{\sum_{i=1,4,8,10,19,54} \frac{\text{mol}}{\text{g total PCB}}}{\sum_{j=1}^{209} \frac{\text{mol}}{\text{g total PCB}}}$$

Organic carbon analysis and nucleic acid extraction from sediment samples

For leachable organic carbon analysis, 5–10 g of sediment was transferred into a 40-mL vial, and 20 mL of deionized water was added. The vials were capped, briefly mixed, then held overnight at 4 °C. The supernatant was decanted to a clean vial and acidified to pH < 2 with sulfuric acid. Total organic carbon measurements of the supernatant were made on a Shimadzu TOC-V analyzer operating in non-purgable organic carbon mode. DNA extractions were performed in replicate for each sediment sample (~ 0.25 g) with the DNeasy PowerSoil Kit (Qiagen, Germantown, MD) according to the kit provided protocol. DNA extracts (with concentrations ranging from 7.4 to 51 ng/μl except for A1 (< 0.5 ng/μl)) were stored at – 80 °C prior to further analysis.

High-throughput 16S sequencing and analysis

Replicate DNA extracts from the 27 sediment samples collected in 2015 were subjected to partial 16S rRNA gene PCR amplification using a bar code containing primer set 515f/806r (Bates et al. 2011) according to a previously described thermocycling protocol (Caporaso et al. 2012). Amplicon sequencing was performed on an Illumina MiSeq benchtop sequencer at the Argonne National Laboratory Next Generation Sequencing Core (Argonne, IL).

A MiSeq standard operating procedure (Kozich et al. 2013) for Mothur version 1.37.6 (Schloss et al. 2009) was used to analyze both archaeal and bacterial sequence data. Paired-end reads were assembled, and sequencing errors and detected chimeras were removed. For bacteria, 6,321,776 sequences remained after the quality check, generating 270,980 operational taxonomic units (OTUs) at a 97% sequence similarity cutoff value. After removing singletons (sequences that only appeared once), 6,105,824 sequences (55,028 OTUs) remained. After rarefaction, 48,545 sequences from each sample were used for subsequent analysis (except for sample A1) (Fig. S1). The number of sequences analyzed for Sample A1 were 8017 (replicate A1a) and 38,460 (replicate A1b). For archaea, 189,924 sequences remained after the quality check and 6878 OTUs were generated (97% sequence similarity cutoff value). After singleton removal, 185,176 sequences and 2130 OTUs

remained. After rarefaction, 249 sequences from each sample were used for subsequent analysis (Fig. S1). Sample A1a was removed from the archaea analysis because of low total archaeal sequence abundance. Bacterial and archaeal OTU taxonomic assignments were made with the Greengenes database (DeSantis et al. 2006) at an 80% cutoff value.

Real-time qPCR

The abundances of total bacterial 16S rRNA genes, putative dechlorinating Chloroflexi 16S rRNA genes, *Dehalococcoides*-like 16S rRNA genes, and *bphA* were estimated as described previously (Liang et al. 2014) using the primers described in Table S1. RDase gene RD14 from *D. mccartyi* strain CG5 was estimated with the qPCR primers previously described (Wang et al. 2014) (Table S1). Each 20 μ L qPCR contained 10 μ L Power SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA) and variable amounts of primers and templates (Table S2). Possible PCR inhibition was alleviated with bovine serum albumin (BSA; 0.5 μ g). Thermocycler conditions for all primer sets were as follows: 10 min at 95 $^{\circ}$ C, followed by 40 cycles of 95 $^{\circ}$ C (15 s), and 60 $^{\circ}$ C (1 min), concluding with a PCR product melt-curve procedure. All qPCR was performed with an ABI QuantStudio Flex 7 Real-Time PCR System (Applied Biosystems, Grand Island, NY) and fluorescence data was analyzed by ABI QuantStudio Real-Time PCR Software (Applied Biosystems, Grand Island, NY). For each primer set, the target gene was either not detected in no template controls or if detected, the Ct value was > 35. Melt-curve analysis of qPCR products revealed primarily single peaks with melting temperatures > 77 $^{\circ}$ C, indicating that primer dimer formation and non-specific amplification was minimized. Additional qPCR information, including primer concentrations, template concentrations, qPCR linear range, qPCR efficiency, and *Y*-intercepts range of the standard curves are listed in Table S2, in accordance with MIQE guidelines (Bustin et al. 2009).

PCR products amplified from *Burkholderia xenovorans* strain LB400 with primer set 8F/1492R were used as the standard for bacterial 16S rRNA gene qPCR (Table S1). For *Dehalococcoides*-like 16S rRNA genes, putative dechlorinating Chloroflexi 16S rRNA genes, *bphA*, and *D. mccartyi* strain CG5 RDase genes, standard DNA templates were pCR 2.1-TOPO vectors containing target PCR products amplified with primer set bph463f/674r, chl3487f/dehal884r, and dhc793f/946r, respectively (Table S1).

Amplification, cloning, and sequencing of reductive dehalogenase genes

A real-time PCR method was used to screen selected DNA extracts with 44 degenerate primer sets that target known reductive dehalogenase gene ortholog groups (Hug and Edwards 2013). Reactions (20 μ L) contained Power SYBR Green PCR Master Mix (10 μ L), forward and reverse primers (0.5 μ M each), BSA (0.5 μ g), and DNA template (~ 20 ng). Thermocycling conditions for all primer sets were as follows: initial denaturation at 95 $^{\circ}$ C for 5 min, followed by 3 cycles of 95 $^{\circ}$ C (30 s), annealing at 30 $^{\circ}$ C (30 s), and elongation at 72 $^{\circ}$ C (90 s); 3 cycles of 95 $^{\circ}$ C (30 s), 45 $^{\circ}$ C (30 s), and 72 $^{\circ}$ C (90 s); and 30 cycles of 95 $^{\circ}$ C (30 s), 50 $^{\circ}$ C (30 s), and 72 $^{\circ}$ C (90 s); as well as a final extension of 72 $^{\circ}$ C (10 min) and a dissociation step.

Reactions displaying distinct dissociation peaks at temperatures above 80 °C were subjected to a second PCR amplification (25 µL with 12.5 µL TaqMan Universal PCR Master Mix, forward and reverse primers (0.5 µM), and 1 µL of PCR product from the initial reaction) with the same thermocycling parameters described above. PCR product size was determined using gel electrophoresis. Discrete bands of the appropriate size were excised and purified with the QIAquick Gel Extraction Kit and cloned into 2.1-TOPO vectors (TOPO® TA Cloning® Kit). Positive clones were Sanger-sequenced at the Iowa Institute of Human Genetics of the (University of Iowa).

GenBank submission

The high-throughput partial 16S rRNA gene sequencing data generated was deposited in the GenBank Sequence Read Archive under BioProject number PRJNA382682. RDase genes retrieved from sediment samples are deposited under GenBank accession numbers MF421731-MF421741.

Results

PCB congener profile analyses reveals potential PCB dechlorination hotspots in lagoon sediments

Total PCB concentrations in sediment samples displayed spatial variability, ranging from 6.34 to 12,700 mg/kg dry weight (Fig. 1). PCB congener profiles at each sample location were also compared with PCB congener profiles of known Aroclor mixtures (e.g., 1248, 1242) to determine the possible source Aroclor. This revealed that the average PCB congener profile among all 27 samples was similar to Aroclor 1248 (Fig. 2). However, a cosine theta ($\cos \theta$) analysis of the Aroclor 1248 profile with PCB congener profiles at individual sampling locations revealed that although 18 samples showed $\cos \theta$ to Aroclor 1248 of 0.94 and higher, 9 samples showed $\cos \theta$ to 1248 lower than 0.89, including three sample locations with correlations ranging from 0.37 to 0.77 (Fig. 3a).

The spatial differences in PCB congener profiles could have resulted from PCB reductive dechlorination processes. Examining the relative abundance of congeners with varying numbers of chlorines reveals a pattern of decreasing abundance of congeners with four to six chlorines with a concomitant increase in abundance of congeners with one to three chlorines (Fig. 4a). The relative abundance of congeners with 7–10 chlorines varied little among the 27 samples, ranging between 0.19% (sample D2) and 0.47% (sample B4). In comparison to the other samples, D2 displayed extensive evidence of dechlorination of tetra- and penta-chlorinated congeners with relatively high percent accumulation of mono- (5.3%), di- (27.8%), and tri- (41.8%) chlorinated congeners (Fig. 4a).

Analysis of the relative abundance of chlorines located in the *meta*-, *para*-, and *ortho*-positions in PCB congener samples revealed that decreases in percentage of meta-chlorines corresponded to increases in percentage of *ortho*-chlorines in several samples (Fig. 4b). The exception was sample A4, where a small decrease in *para*-chlorines appears to correspond with an increase in *ortho*-PCBs. The MDPR metric describes accumulation of certain *ortho*-PCBs in sediment samples. Of the 27 sediment samples, 14 (51.8%) had MDPR values

ranging from 0.009 to 0.022, which is similar to the MDPR of Aroclor 1248 (0.006–0.017) (Fig. 3b). However, the remaining 13 samples had MDPR values ranging from 0.03 to 0.31 (i.e., 1.8- to 18.2-fold greater than the Aroclor 1248 MDPR). This confirms that *ortho*-chlorinated PCB congeners have accumulated in nearly 50% of the samples, with samples C3, D2, and E2 displaying the most extensive dechlorination (MDPRs of 0.13–0.31) (Fig. 3b).

Because PCB dechlorination is a terminal electron accepting process, electron donors in the sediment must be available to drive this process. We broadly characterized electron donor availability by estimating organic carbon concentrations in sediment samples. Our approach entailed washing each sediment sample (5–10 g) in 20 mL deionized water and measuring the organic carbon concentration of the resulting supernatant. This indicated that leachable organic carbon concentrations ranged from 62 to 2400 mg/kg dry sediment among the samples (Fig. S3).

Spatial variations in microbial community structure in sediment samples

Hypothesizing that microbial processes could explain the changes in PCB congeners profiles, we first used high-throughput 16S rRNA gene amplicon sequencing to determine overall microbial structure among the sediment samples. Microbial communities in sediment samples were composed primarily of bacteria. The bacterial community composition varied among the samples but was dominated by Proteobacteria (13.3–78.3%) and Firmicutes (5.5–53.5%) in all samples (Fig. 5; Table S3). The next most abundant phylum was the Chloroflexi, which ranged in relative abundance from 2.0 to 10.1%.

Relative archaeal 16S rRNA gene sequence abundance was 3.0% of the total 16S rRNA gene sequences on average, ranging from 0.2 to 9.4%. Sample B4 displayed the highest relative abundance of Archaea (~ 10%) among the samples. Within the archaeal community, the Euryarchaeota was dominant with relative abundance ranging from 73.3 to 94.6% of the total archaea (Fig. S2; Table S4).

Bacteria associated with aerobic and anaerobic PCB biodegradation processes are present in sediment samples

Among the 30 most abundant OTUs observed across all sediment samples, 7 OTUs were classified as members of the Class Clostridia (Phylum Firmicutes). Other highly represented classes in the top 30 OTUs included β -Proteobacteria (6 OTUs), δ -Proteobacteria (5 OTUs), and γ -Proteobacteria (4 OTUs). Only one OTU among the top 30 was classified as a member of the Chloroflexi. A heatmap depicts the relative abundance of the top 30 OTUs across all samples (Fig. S4).

Based on previous observations of dehalogenating activity, microbial groups considered potential PCB dechlorinators include the putative dechlorinating Chloroflexi (i.e., the *Dehalococcoideaceae*), the Clostridia, and the *Geobacteraceae* (δ -Proteobacteria). Estimating the relative abundance of these three bacterial groups in the high-throughput 16S rRNA amplicon sequencing data revealed that all were present to varying extents in the sediment samples, with the *Clostridium* sp. generally the most abundant (1.1–38.5%) (Fig. 6a), followed by the *Geobacteraceae* (0.01–3.8%) (Fig. 5c), with the *Dehalococcoidaceae*

the least abundant (0.02–0.36%) (Fig. 6c). We also performed qPCR to estimate the abundance of total bacterial 16S rRNA genes (Fig. 6b), putative dechlorinating Chloroflexi 16S rRNA genes (Fig. 6d), and total *Dehalococcoides* 16S rRNA genes (Fig. 6f) as a means of determining the presence and relative abundance of potential dechlorinating Chloroflexi independent of 16S rRNA gene amplicon sequencing analysis. The relative abundance of putative dechlorinating Chloroflexi 16S rRNA genes according to this qPCR approach was 0.01–0.19% among the sediment samples, with *Dehalococcoides* sp. representing 0.6–14.8% of this group (Fig. 6b, d, f).

Identification of reductive dehalogenase and biphenyl dioxygenase genes in sediment samples

We hypothesized that reductive dehalogenase (RDase) genes will co-occur with the presence of putative PCB dechlorinating bacteria in PCB-contaminated sediment samples. To assess the potential diversity of RDase genes at this site, we conducted a broad PCR survey using a previously reported suite of degenerate primer sets targeting RDase genes (Hug and Edwards 2013). Fourteen subsamples were chosen for primer screening based on *Dehalococcoides*-like 16S rRNA gene and putative dechlorinating Chloroflexi 16S rRNA gene abundance, as well as PCB congener profile. After screening each of the 44 primer sets on these 14 selected sediment samples, PCR products of the expected size were noted with primer sets targeting RDase groups 12 (sample location B1) and 14 (sample locations A2, B1, C4, and E4). One group 12 sequence retrieved was related to an RDase gene found in *D. mccartyi* strains DCMB5 and BAV1 (Fig. 7a). The remaining 10 RDase sequences was similar to an RDase (RD14) found in PCB-respiring *D. mccartyi* strains CG5, which were also related to RDases found in *D. mccartyi* strains CBDB1, JNA, and GT (Fig. 7a). The abundance of the RD14 RDase gene from strain CG5 was further estimated by qPCR, which revealed that it was present in all 27 samples at concentrations ranging from 6.3×10^3 (A1) to 3.7×10^7 copies per gram sediment (Fig. 7b).

Biphenyl dioxygenase gene (*bphA*) abundance was also estimated in the sediment samples by qPCR. The average *bphA* abundance was 9.4×10^5 genes per gram sediment and ranged from 3.3×10^1 (sample location A1) to 1.5×10^7 (sample location D4) (Fig. S5)

Discussion

Analysis of PCB congener profiles in 27 sediment samples from a wastewater lagoon suggests that this site was historically contaminated with a single commercial mixture—Aroclor 1248. The extensive spatial variability in total PCB concentrations within the lagoon sediments complicates characterizing to what extent total PCB concentrations are decreasing by natural attenuation processes. Lagoon sediments were sludge-like, which differ from typical lake or river sediment characteristics. Temporal shifting of sediments in response to wind shear and water currents could partially explain this variability. Developing a temporal dataset of PCB concentrations at these sampling locations would provide insight on this possibility.

Comparison of PCB congener profiles in each sediment sample against the Aroclor 1248 PCB congener profile reveals that PCB dechlorination processes had occurred at some point

in the past and that the extent of PCB dechlorination varies spatially within the lagoon. The more highly chlorinated PCB congeners are likely strongly sorbed to sediment organic carbon. Measuring PCB congeners profiles in sediment pore water (Liang et al. 2014; Martinez et al. 2013) would yield improved insights concerning biodegradation of soluble and bio-available PCB congeners.

Evidence for PCB reductive dechlorination processes via congener analysis was first reported in sediments from the upper Hudson River (Brown et al. 1987). The chlorine distribution patterns of lower chlorinated congeners, which dominated the sediments, suggested that reductive dechlorination of chlorines in the *meta*- and *para*-positions was occurring (Quensen et al. 1988; Quensen et al. 1990). Similar patterns in congener profiles (e.g., removal of chlorines in the *meta* position) were seen in Altavista sediments.

Nine different dechlorination processes, M, Q, H, H', P, N, LP, T, and Z, are known (Bedard 2003; He and Bedard 2016). Dechlorination of flanked *meta*- or *para*-chlorines by Processes H, H', N, and P is most often observed at environmentally relevant temperatures. Processes M, Q, and LP mediate removal of unflanked chlorines generated by Processes H, H', N, and P. Comparing the tetra- and tri-chlorinated congener molar % in Aroclor 1248 with those found in sample D2 revealed > 90% loss of PCB 41 (2,2',3,4-CB), PCB 45 (2,2',3,6-CB), and PCB 48 (2,2',4,5-CB) with significant accumulation of PCB 17 (2,2',4-CB) and PCB 19 (2,2',6-CB). PCBs 17 and 19 are thus plausible *meta* dechlorination products of PCBs 41, 45 and 48 by Process N in sample D2. However, conclusive identification of dechlorination processes in these sediments requires a microcosm study of PCB dechlorination patterns over time.

Observed accumulation of certain *ortho*-substituted PCB congeners (i.e., PCBs 1, 4, 8, 10, 19, and 54) and the lack of *ortho* dechlorination activity in Hudson River sediments (Abramowicz et al. 1993, Quensen et al. 1990) formed the basis for the MDPR (USEPA 1997). The MDPR of Aroclor 1248 is 0.006–0.017 (depending on the lot number). This indicates that samples B4 and E4 showed the least amount of dechlorination (MDPR 0.009–0.01), while samples C3, D2, D3, and E2 showed the most dechlorination among the samples with MDPRs > 0.1. An estimate of leachable (i.e., potentially bioavailable) organic carbon concentrations were > 200 mg/L in all but one sample, suggesting that sufficient electron donor is available to drive reductive dechlorination processes. Leachable organic carbon concentrations were negatively correlated to MDPR values (Spearman's rho = -0.454, $p = 0.0197$).

The microbial communities in the 27 samples were diverse with more than 20 phyla observed with > 1% relative abundance in at least one sample, with the most dominant phyla being the Proteobacteria and the Firmicutes. Archaea were present at low levels with the Euryarchaeota (including the methanogens) dominating.

The variability in overall microbial community structure among the samples was not strongly influenced by total PCB concentration or the extent of dechlorination (MDPR). This is perhaps expected as the relative abundance of putative dechlorinating Chloroflexi 16S rRNA genes is 0.36% or less. It should also be noted that only a subset of the

organohalide-respiring Chloroflexi can reduce PCBs and that these microbes are commonly found in uncontaminated environments (Hug and Edwards 2013; Krzmarzick et al. 2012). However, additional putative (possibly cometabolic) PCB-dechlorinating bacteria, such as certain members of the *Geobacteraceae* and *Clostridia*, represented up to 4 and 30% of the microbial community, respectively. Cometabolic PCB dechlorination can also be mediated by *D. mccartyi* strains (Zhen et al. 2014), provided that there is some other, possibly naturally occurring halogenated compound available to support their growth (Krzmarzick et al. 2012). The presence of halogenated compounds other than PCBs in the Altavista lagoon that might support the growth of organohalide-respiring Chloroflexi was not investigated in this study.

Statistically significant relationships (Spearman's correlation) between the extent of PCB dechlorination (as estimated by the MDPR metric) and the possible PCB-dechlorinating groups were not evident. If cometabolic PCB dechlorinators were responsible for the MDPR changes, no correlation would be expected between their abundance and PCB concentrations or the extent of dechlorination as they derive no metabolic benefit from the process.

We did observe statistically significant (Spearman's $\rho = -0.474$, $p = 0.0144$) negative correlations between the abundance of *Dehalococcoides*-like 16S rRNA genes and the abundance of chlorines in the *para*-position in all congeners. This suggests that members of the *Dehalococcoidaceae* participate in respiratory *para*-dechlorination of PCBs in Altavista sediments. Indeed, PCB-respiring *Dehalococcoides* strains CG1, CG4, and CG5 (Wang et al. 2014) as well as cometabolic PCB-degrading *Dehalococcoides* strains (Adrian et al. 2009, Zhen et al. 2014) are known to mediate *para*-dechlorination of specific PCB congeners and Aroclor mixtures.

We also noted a negative correlation (Spearman's $\rho = -0.555$, $p = 0.00326$) between the relative abundance of *Geobacteraceae* 16S rRNA genes and the abundance of chlorines in the *para*-position in all PCB congeners. A recent report implicated *Geobacteraceae* in PCB dechlorination processes in sediment-free microcosms in part because of a negative correlation between this group and the abundance of penta-chlorinated PCBs (penta-CBs) (Praveckova et al. 2016). It is currently unclear whether *Geobacteraceae* directly participate in PCB dechlorination processes or enhance dechlorination in some way such as contributing essential cobalamin cofactors to relevant *Dehalococcoides* sp. (Yan et al. 2012).

Although estimating the abundance of 16S rRNA genes from known PCB-dechlorinating taxa as well as putative dechlorinators can yield insights into PCB-dechlorinating communities (Kjellerup et al. 2012; Matturro et al. 2016a, b), the specific reductive dehalogenase (RDase) genes involved in PCB dechlorination remain poorly understood. Three RDase genes (*pcbA1*, *pcbA4*, and *pcbA5*) were implicated in PCB dechlorination by gene expression analysis and protein assays (Wang et al. 2014). All three RDases were capable of reducing tetrachloroethene (PCE) as well as certain Aroclor 1260 congeners, which show a relatively broad range of substrate specificity. *PcbA5*'s dechlorination activity most closely resembles that of dechlorination Process N, removing doubly flanked and flanked meta-chlorines from specific congeners. *PcbA1* removed doubly flanked *meta*-

chlorines from some but not all of the same congeners as pcbA5. PcbA4 removes primarily doubly flanked chlorines in the same fashion as the parent culture.

Eight of the inferred partial RDase gene products retrieved from Altavista sediment samples were 89.3–100% identical to RD14 in *Dehalococcoides* strain CG5. Although this RDase was not specifically implicated as a PCB reductive dehalogenase, the RD14 expression level was three times higher when strain CG5 was grown on Aroclor 1260 than when grown on PCE (Wang et al. 2014). Although partial RDase sequences 99% identical to the RD14 sequences (Table S5) were initially retrieved by PCR amplification from samples A2, C4, and E4, subsequent qPCR analysis of this gene revealed that it was present in all samples (Fig. 7). The potential role for CG5 RD14 in PCB dechlorination warrants further study.

The “ortholog group 14” RDase genes (Hug and Edwards 2013) cloned from Altavista sediment were also related to RDases in *D. mccartyi* strains CBDB1 and JNA. *D. mccartyi* strain CBDB1 is known to respire chlorobenzenes and can cometabolically reduce PCB congeners in Aroclor 1260 (Adrian et al. 2009). The structural similarity between chlorobenzenes and PCBs could account for this overlap in substrate specificity. *D. mccartyi* strain JNA is a pure culture that not only was most recently shown to reduce chlorophenols (Fricker et al. 2014) but has also been shown to respire by dechlorinating Aroclor 1260 congeners, primarily by flanked *meta* dechlorination (Process N) (LaRoe et al. 2014). Unlike strains CG1, CG4, and CG5, specific RDases in strain CBDB1 have not been implicated in dechlorination of PCB congeners.

The presence of *bphA* genes in each of the 27 samples indicates that the lagoon sediments also hold the potential for aerobic PCB oxidation and that complete mineralization of PCBs could occur. The average *bphA* abundance at this site is about one order of magnitude lower than *bphA* abundance in PCB-contaminated sediments from 0 to 1.83 m depth at Indiana Harbor and Ship Canal (Liang et al. 2014). The upper biphenyl pathway, initiated by BphA, is considered ubiquitous among aerobic PCB degraders, and incomplete aerobic PCB metabolism could lead to accumulation of hydroxylated PCB metabolites such as 2,3-dihydroxybiphenyl (Pieper 2005). Biodegradation of hydroxylated PCBs in the environment is also poorly understood, although there is one report of dechlorination by *Desulfitobacterium dehalogenans* (Wiegel et al. 1999).

Although 16S rRNA gene of putative PCB-dechlorinating phylotypes has been enumerated and sequenced directly from soil (Kjellerup et al. 2012), this appears to be the first report of amplification and sequencing of putative PCB reductive dehalogenase genes directly from environmental samples without enrichment. The key was to evaluate 44 previously described degenerate primer sets in an approach that was as unbiased as possible. Enrichment of PCB-dechlorinating microorganisms from these sediments along with concurrent application of molecular diagnostic tools is expected to reveal which RDase genes participate in PCB congener reduction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Plan view of the wastewater lagoon illustrating 27 approximate sediment sample locations and total PCB concentrations (mg/kg) measured in each of these samples

Fig. 2. PCB congener profiles of **a** Aroclor 1248 and **b** average of 27 sediment samples taken from the PCB-contaminated lagoon at Altavista, VA. For the average profile in **b**, the bars represent the average of all analyses and the error bars are the standard deviation ($n = 27$)

Fig. 3. Analysis of PCB congener profiles from 27 sampling locations utilizing **a** cosine theta and **b** MDPR. The cosine theta (1.0) and MDPR of Aroclor 1248 (0.017) are shown for comparison

Fig. 4. Representation of PCB congener profiles from 27 sampling locations according to **a** % congeners containing 1–6 chlorines and **b** % chlorines located in the *ortho*-, *meta*-, and *para*-positions. Note that these samples contained 0.19–0.46% PCB congeners containing 7–10 chlorines—these are not shown on **a** for clarity. Aroclor 1248 is shown in both panels for comparison

Fig. 5.

Overall microbial community composition in sediment samples in terms of relative abundance (%) of phyla that were > 1% of the total rarefied sequences. Percentages for each phylum, rare members (phyla containing < 1% of total sequences in all samples), and unclassified bacteria are shown in Table S3. Percentages were based on the mean value of sequencing results from replicate DNA extracts

Fig. 6. Relative abundance of **a** *Clostridium* sp., **c** *Geobacteraceae*, and **e** *Dehalococcoideaceae* in sediment samples. Column heights shown are the average of duplicate 16S rRNA gene amplicon sequencing reactions. Gene abundance estimates for **b** total 16S rRNA genes, **d** putative dechlorinating Chloroflexi 16S rRNA genes (chl), and **f** *Dehalococcoides* 16S rRNA genes (dhc) were acquired by qPCR. Column heights in **b**, **d**, and **f** shown are the average of duplicate DNA extraction and duplicate qPCR measurements

Fig. 7.
a Amino acid phylogenetic tree of RDase genes found in PCB-contaminated sediment samples A2, B1, C4, and E4. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). The tree with the highest log maximum likelihood (– 810.3446), supported by a bootstrap analysis (500 replicates), is shown. All positions containing gaps and missing data were eliminated leaving 52 positions in the final dataset. **b** qPCR analysis of the *D. mccartyi* strain CG5 group 14 RDase gene homolog shown in **a**. Column heights are the average of duplicate DNA extraction and duplicate qPCR measurements (except sample A1)