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# Comparative Genomics of "*Dehalococcoides ethenogenes*" 195 and an Enrichment Culture Containing Unsequenced "*Dehalococcoides*" Strains▽†

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**Tetrachloroethene (PCE) and trichloroethene (TCE) are prevalent groundwater contaminants that can be completely reductively dehalogenated by some "*Dehalococcoides*" organisms. A *Dehalococcoides*-organism-containing microbial consortium (referred to as ANAS) with the ability to degrade TCE to ethene, an innocuous end product, was previously enriched from contaminated soil. A whole-genome photolithographic microarray was developed based on the genome of "*Dehalococcoides ethenogenes*" 195. This microarray contains probes designed to hybridize to >99% of the predicted protein-coding sequences in the strain 195 genome. DNA from ANAS was hybridized to the microarray to characterize the genomic content of the ANAS enrichment. The microarray results revealed that the genes associated with central metabolism, including an apparently incomplete carbon fixation pathway, cobalamin-salvaging system, nitrogen fixation pathway, and five hydrogenase complexes, are present in both strain 195 and ANAS. Although the gene encoding the TCE reductase, *tceA*, was detected, 13 of the 19 reductive dehalogenase genes present in strain 195 were not detected in ANAS. Additionally, 88% of the genes in predicted integrated genetic elements in strain 195 were not detected in ANAS, consistent with these elements being genetically mobile. Sections of the tryptophan operon and an operon encoding an ABC transporter in strain 195 were also not detected in ANAS. These insights into the diversity of *Dehalococcoides* genomes will improve our understanding of the physiology and evolution of these bacteria, which is essential in developing effective strategies for the bioremediation of PCE and TCE in the environment.**

Chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are prevalent groundwater contaminants. Microorganisms from various genera, including *Dehalobacter* (17), *Desulfitobacterium* (11, 12), *Desulfomonile* (8), *Desulfuromonas* (22), *Enterobacter* (34), and *Sulfurospirillum* (26, 32), are able to dechlorinate PCE and TCE to predominantly dichloroethene (DCE). To date, the only microorganisms known to completely dechlorinate PCE and TCE to the innocuous end product ethene are "*Dehalococcoides*" bacteria (28).

The sequencing and annotation of the genome of "*Dehalococcoides ethenogenes*" 195, the first *Dehalococcoides* bacterium sequenced, provided insights into its unique physiology (33). The subsequent sequencing of the genome of *Dehalococcoides* sp. strain CBDB1, which has different dechlorination abilities than strain 195 (1, 4), allowed a genetic comparison of two functionally different *Dehalococcoides* organisms (23). That study revealed a high degree of gene sequence conservation and synteny within central metabolism and information-processing systems ("housekeeping" genes). However, these genomes appear to be punctuated by regions of high plasticity, many of which contained putative reductive dehalogenase (RD) genes and integrated genetic elements (IEs), suggesting a high degree of evolutionary variability (23). Many coding regions found in strain 195 are absent in strain CBDB1, including all nine predicted IEs in strain 195 and six putative RD genes, along with their predicted regulatory systems (23). Other strains of *Dehalococcoides*, specifically *Dehalococcoides* sp. strain BAV1 (15) (U.S. Department of Energy Joint Genome Institute [DOE JGI], completed genome), *Dehalococcoides* sp. strain VS (6) (DOE JGI, draft), and *Dehalococcoides* sp. strain GT (35) (DOE JGI, draft), have been isolated and selected for genome sequencing to further improve our understanding of the physiology and evolution of this unique group of microorganisms.

In addition to pure-culture studies, *Dehalococcoides*-organism-containing enrichment cultures have also received significant attention (7, 21, 37). *Dehalococcoides* strains were found to be dominant members in a number of anaerobic TCE-dehalogenating enrichment cultures, including ANAS, an anaerobic microbial consortium enrichment culture derived from contaminated sediments taken from Alameda Navel Air Station in California that dehalogenates TCE to ethene (9, 18,

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31). ANAS contains two different strains of *Dehalococcoides*, one that dechlorinates TCE and DCE to vinyl chloride (VC) and another that dechlorinates TCE, DCE, and VC to ethene (18). The *Dehalococcoides* strains in ANAS are functionally distinct from strain 195 in that they lack the ability to degrade PCE (31). Because enrichment cultures can reflect the communities and microbial interactions occurring in the environment more closely than pure cultures, the study of enrichment cultures capable of complete reductive dechlorination, such as ANAS, may help to elucidate the natural microbial interactions required to support the degradation of chlorinated solvents in the environment. However, the complex nature of mixed microbial communities impedes the application of typical genetic and genomic-scale studies that might reveal crucial physiological processes.

Microarrays have been successfully applied to analyze intraspecies genome mutations in yeasts and bacterial pathogens (5, 13, 16, 36, 38) but have not been widely used for genomic comparisons of microorganisms involved in environmental biodegradation. These studies revealed fundamental strain-specific differences. For example, genome rearrangements and deletions in strains of *Yersinia pestis* and *Yersinia pseudotuberculosis* are often a result of the recombination of insertion sequences and the acquisition of phage-related sequences (16). The strain 195 genome, likewise, contains several IEs and phage-related genes (33), making strain 195 an interesting candidate for comparative genomics studies. With an analogous framework, this study applies a microarray designed to query the whole genome of strain 195 to characterize the genomic content of the *Dehalococcoides*-organism-containing ANAS enrichment. The results provide further insights into the diversity and evolution of this environmentally important microorganism.

## MATERIALS AND METHODS

**Bacterial cultures.** *Dehalococcoides ethenogenes* 195 and *Dehalobacter restrictus* PER-K23 (DSMZ strain number 9455) were grown in 100-ml-batch pure cultures with a defined mineral salts medium containing 5 mM acetate as the carbon source, $H_2$ as the electron donor (added as $H_2$:$CO_2$ gas [80:20, vol/vol]), and 78 μmol TCE as the electron acceptor, as previously described (14, 24).

ANAS was enriched from contaminated soil obtained from Alameda Naval Air Station and has been functionally stable for over seven years, with the ability to reductively dechlorinate TCE completely to ethene. Previous clone library studies have shown that *Dehalococcoides* bacteria comprise approximately 30% of the ANAS culture (31). Lactate (25 mM final concentration) is supplied as both the carbon source and electron donor, while TCE (0.1 mM final concentration) is supplied as the terminal electron acceptor. The semibatch growth and maintenance procedures have been previously described (24, 31).

**DNA extraction.** A 50-ml sample of the ANAS culture was collected after one 100 μM dose of TCE was degraded to VC and ethene. Cells were collected by centrifugation (12,000 × *g* for 3 min at 4°C), the supernatants were discarded, and the cell pellets were stored at −80°C until processing. ANAS genomic DNA (gDNA) was isolated from frozen cell pellets by using an UltraClean Mega Prep soil DNA kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. DNA was stored at −80°C prior to further use.

*D. restrictus* and strain 195 cells were harvested from 10 100-ml batches per culture to ensure that a sufficient quantity of DNA could be extracted for application to microarrays. Cells were collected by vacuum filtration with hydrophilic Durapore membrane filters (0.22-μm pore size, 47-mm diameter; Millipores, Billerica, MA), and the filters were stored in 2-ml microcentrifuge tubes at −80°C until further processing. gDNA was isolated from the filters by using a phenol extraction method as follows. The 2-ml microcentrifuge tubes containing frozen filters were amended with 250 μl lysis buffer (200 mM Tris [pH 8.0], 50 mM EDTA, and 200 mM NaCl), 100 μl 10% sodium dodecyl sulfate, 1 g 100-μm-diameter zirconia-silica beads (Biospec Products, Bartlesville, OK), and 1.0 ml buffer-equilibrated phenol (pH 8.0) (Sigma-Aldrich, St. Louis, MO). The cells were lysed by heating the tubes to 65°C for 2 min, bead beating with a mini bead beater (Biospec Products) for 2 min, incubating for 8 min at 65°C, and bead beating again for an additional 2 min. Cellular debris was collected by centrifugation (12,000 × *g* for 5 min at 4°C), and the aqueous lysate was transferred to a new, DNase-free microcentrifuge tube. The aqueous lysate was extracted twice with 1 volume of phenol (pH 8.0)-chloroform-isoamylalcohol (125:24:1, vol/vol) and once with 1 volume of chloroform-isoamylalcohol (24:1, vol/vol) (Sigma-Aldrich). DNA was precipitated by adding 0.1 volume of 3 M ammonium acetate (pH 5.2) and 1 volume of 100% isopropanol. The precipitate was collected by centrifugation, washed once with 80% ethanol, and resuspended in 40 μl of nuclease-free water. Contaminating RNA was removed by RNase digestion with DNase-free RNase according to the manufacturer's instructions (Roche Applied Science, Indianapolis, IN). The purified DNA was stored at −80°C prior to further use.

The mass of DNA per volume was quantified by using a fluorometer (model TD-700; Turner Designs, Sunnyvale, CA) and a Quant-iT PicoGreen dsDNA assay kit (Invitrogen Molecular Probes, Carlsbad, CA) according to the manufacturer's instructions.

**Microarray design.** The microarrays targeting genes within the genome of *Dehalococcoides ethenogenes* 195 were produced by Affymetrix (Santa Clara, CA) as prokaryotic midi format (format 100) photolithographic microarray chips. Each target sequence is represented on the array as a collection of 11 probe pairs (22 total probes) consisting of 25-mer oligonucleotide probes which are distributed along the length of the respective gene and are collectively referred to as a probe set. Each chip contains 1,624 probe sets. To control for nonspecific hybridization, each probe pair consists of a perfect-match probe and a corresponding single-mismatch probe in which the 13th nucleotide consists of a base that would mismatch the target. While the perfect-match probe provides measurable fluorescence when sample binds to it, the paired mismatch probe is used to detect and eliminate any false or contaminating fluorescence within that measurement resulting from nonspecific hybridization (2). Probe sets were designed to target 1,560 nonredundant genes from the genome of strain 195, including 48 of 51 structural-RNA-coding sequences and 1,512 of 1,514 of the predicted protein-coding sequences identified by Seshadri et al. (33). The number of probe sets on the array is less than the total number of genes in the strain 195 genome because the genome contains a 33-gene duplication and a 22-gene triplication. In addition, 45 probe sets, 24 as positive controls and 21 as negative controls, were designed on the array to facilitate calibration and to resolve background signals. The positive-control probe sets targeted *Bacillus subtilis* genes *lys*, *phe*, *thr*, and *dap*. To generate control DNA, plasmids containing *lys*, *phe*, *thr*, and *dap* were purified from strains ATCC 87482, ATCC 87483, ATCC 87484, and ATCC 87486, respectively. The negative-control probe sets were designed to hybridize with specific *Escherichia coli* and bacteriophage genes that were not expected to be present in the experimental cultures.

**Sample preparation for microarray.** gDNA was prepared for application to the microarrays according to the protocols outlined in section 3 of the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA), with minimal modifications. Briefly, purified gDNA (1 μg per array) was mixed with the positive-control plasmids (7.6 nM *lys*, 15.2 nM *phe*, 30.4 nM *thr*, and 114.0 nM *dap*) described above and fragmented to an average size of 50 to 200 bp by enzymatic digestion with amplification grade DNase I (Invitrogen Life Technologies, Carlsbad, CA). Each 50-μl fragmentation reaction consisted of gDNA and 0.02 units DNase I per μg DNA diluted in 1× One-Phor-All buffer (Amersham Biosciences). The mixture was incubated for 20 min at 25°C, followed by 10 min at 98°C to inactivate the DNase I. The fragmentation products were visualized on a 4% MetaPhor agarose (Cambrex Bio Science Rockland, Inc., Rockland, ME) electrophoresis gel run at 70 V for 2 h. A 25-μl amount of the fragmentation product was then used for further processing. The fragmentation products were biotin end labeled by adding 25 μl of fragmentation product to 10 μl of 5× reaction buffer (Affymetrix, Santa Clara, CA), 2 μl 7.5 mM GeneChip DNA-labeling reagent (Affymetrix), 2 μl terminal deoxynucleotidyl transferase (Promega), and 11 μl nuclease-free water, incubating at 37°C for 60 min, and adding 2 μl 0.5 M EDTA (pH 8.0) (Invitrogen Life Technologies, Carlsbad, CA) to stop the reaction. Microarray chips were prehybridized with 1× hybridization buffer (100 mM morpholineethanesulfonic acid [MES; Sigma-Aldrich], 1 M $Na^+$ [Sigma-Aldrich], 20 mM EDTA [Sigma-Aldrich], 0.01% Surfact-Amps Tween 20 [Pierce Chemical]) in a chip-rotisserie oven for at least 20 min at 60 rpm and 45°C. The samples were denatured by being heated to 99°C for 5 min. Hybridization buffer was removed from each microarray chip and replaced with an equal volume of sample hybridization mixture (1× MES hybridization buffer, 7.8% dimethyl sulfoxide [Sigma-Aldrich], 0.1 mg/ml herring sperm DNA [Promega Corporation], 0.5 mg/ml bovine serum albumin [Invitrogen Life Tech-

nologies, Carlsbad, CA], 50 pM B2 control oligonucleotide [Affymetrix], and 1 μg fragmented, labeled DNA). The hybridization was carried out by incubation in a chip-rotisserie oven (Affymetrix) for 16 h at 45°C and 60 rpm. Following hybridization, the hybridization mixture was removed, and the microarrays were processed and scanned according to standard Affymetrix protocols.

**Data analysis.** gDNA from each culture was divided into replicate samples which were independently fragmented, labeled, and hybridized to the arrays. Two microarrays were processed for the positive control (strain 195), two for the negative control (*D. restrictus*), and five for ANAS (two analyses from one biological sample followed 1 year later by three analyses of a second biological sample).

The hybridization signal intensity for perfect match and mismatches in each probe set from each scan was computed by using Affymetrix GeneChip software and the MAS5 algorithm (3). The data set of each microarray was normalized by scaling the signal intensities of the added positive controls to a target signal intensity of 2,500, thus allowing for comparisons between microarray chips. The GeneChip MAS5 software was used to implement the Wilcoxon signed-rank-based algorithm to evaluate the "presence" or "absence" of individual target sequences and return a statistical *P* value (3, 25). A gene was considered "present" in a sample if each replicate probe set had a signal intensity greater than the highest signal intensity measured for the negative controls and a *P* value of less than 0.05. Due to the stringency of the *P* value criterion, only 8 genes total were designated absent due solely to the signal intensity criterion.

**PCR confirmation of microarray results.** PCR primer sets were designed to target several selected protein-coding genes of strain 195. These primer sets were then used to test for the presence of these genes in the ANAS enrichment. PCR amplification was performed in reaction mixtures containing 20 to 100 ng of gDNA and (as final concentrations) 1× PCR buffer II (Perkin Elmer), 2.5 mM $MgCl_2$, a 200 μM concentration of each deoxynucleoside triphosphate, a 300 nM concentration of each forward and reverse primer, and 0.025 U of AmpliTaq Gold (Perkin Elmer) per μl of reaction mix. The reaction mixtures were incubated in an Eppendorf Mastercycler gradient thermocycler (Eppendorf, Westbury, NY) at 94°C for 12 min (for initial denaturation and activation of AmpliTaq Gold), followed by 30 cycles at 94°C for 1 min, 50°C for 45 s, and 72°C for 2 min and then by a final extension period of 12 min at 72°C. Amplified DNA was processed by using an Agilent 2100 bioanalyzer. Visual detection of a band corresponding to a DNA fragment of expected size indicated the presence of a gene. The absence of a band of the expected fragment size indicated the absence of the gene. The PCR results were compared with the microarray probe hybridization results to support determinations of presence/absence.

**Microarray data accession number.** The microarray data analyzed in this study have been deposited in the NCBI Gene Expression Omnibus database under accession number GSE9612 (http://www.ncbi.nlm.nih.gov/projects/geo/index.cgi).

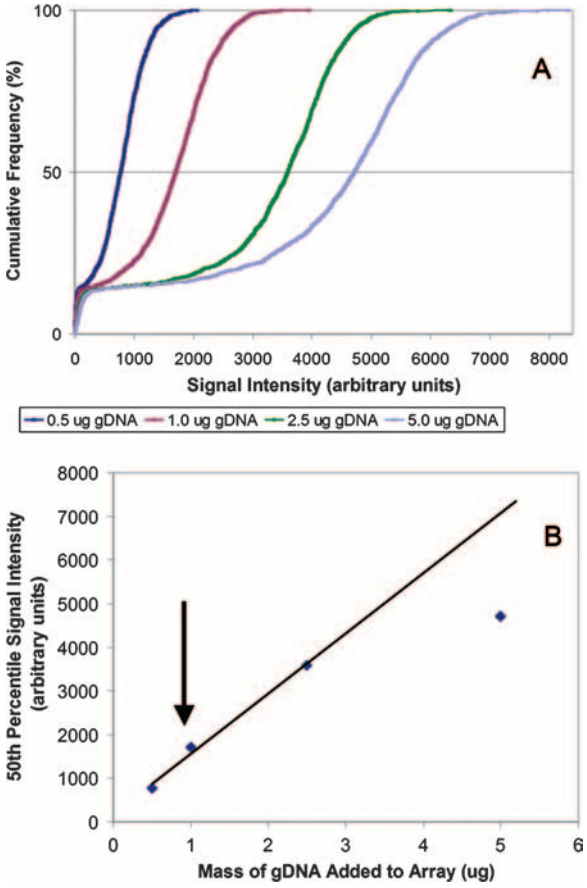


FIG. 1. Dynamic range of ANAS gDNA applied to whole-genome microarrays based on strain 195. (A) A frequency distribution of signal intensities on the microarray is presented for each mass of gDNA applied to the arrays. (B) The average signal intensities of the arrays are plotted against the masses of gDNA applied to the arrays. The arrow indicates the mass of gDNA chosen as the mass to add to subsequent DNA arrays.

## RESULTS

**Validation of the whole-genome microarray.** To evaluate the hybridization efficiency of the microarray probe sets and to detect probe sets that might yield false negatives, gDNA isolated from strain 195 was applied to the microarrays. All (1,579 of 1,579) of the probe sets on the array were positively detected. Conversely, the specificity of the microarray was tested by applying gDNA from *D. restrictus*, a dehalogenating bacterium which shares little sequence homology with strain 195. This hybridization resulted in 1,575 probe sets (99.7%) for which the hybridization results did not meet our criteria for classification as present and were thus deemed absent. The four probe sets that were deemed present with gDNA from *D. restrictus* were all tRNA-encoding genes. Cross-species hybridization with tRNAs is not surprising since these sequences tend to be highly conserved among bacteria. No significant hybridization was found with the *D. restrictus* 16S rRNA gene, which shares approximately 75% identity over 1,511 bases with that from strain 195.

The dynamic range of the microarray was examined in a titration experiment in which different masses of gDNA (0.5 μg, 1 μg, 2.5 μg, and 5 μg) from ANAS were applied to the arrays. The results showed that average signal intensities increased in a nonlinear manner corresponding to increasing masses of gDNA applied to the arrays, exhibiting a dynamic response over the mass range of applied DNA (Fig. 1A and 1B). The coefficients of variation (CVs) across the disparate probe sets within each microarray data set are 58%, 53%, 47%, and 48% for 0.5 μg, 1 μg, 2.5 μg, and 5 μg of gDNA applied to the arrays, respectively. The similarity of the CVs between data sets suggests that the mass of DNA had little effect on the variation of hybridization efficiencies. Each array gave a range of signal intensities above the background noise, indicating that even the lowest mass of DNA tested was sufficient to allow the differentiation of signal intensities between probe sets and to produce detectable signals. The data demonstrate a predictable trend of higher masses of applied DNA yielding greater signal intensity with slightly less variation between probe sets. A mass of 1 μg of gDNA was chosen for subsequent application to microarrays in this study because this mass falls within the observed linear range of correspondence between signal intensity and gDNA mass (Fig. 1B).

The microarray data were highly consistent for replicate arrays containing DNA from a single culture. The hybridiza-

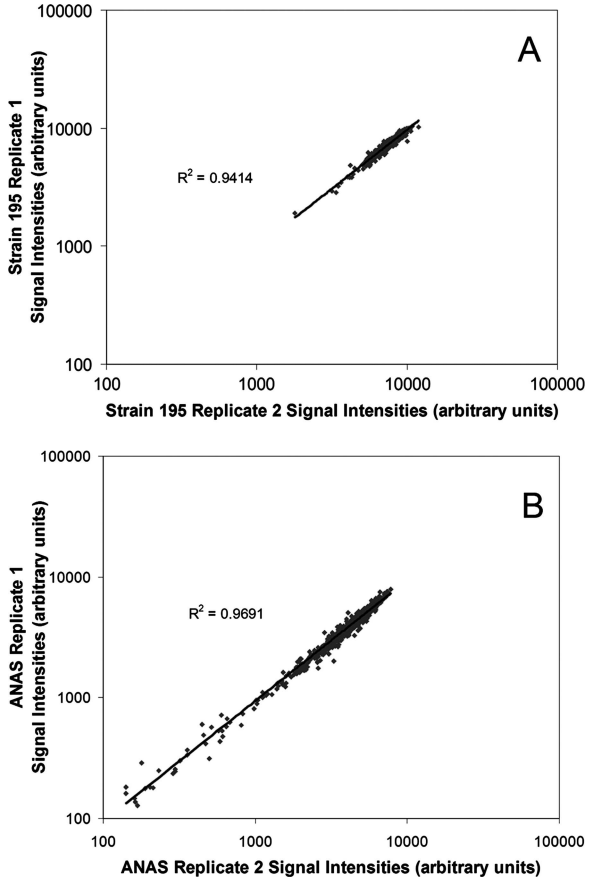


FIG. 2. Analytical reproducibility of replicate microarrays. Each symbol represents a unique protein-coding gene probe set to which gDNA hybridized. A linear regression has been performed on each data set, and the *R*-squared values are shown on the graphs. (A) One microgram of gDNA from *Dehalococcoides ethenogenes* 195 was applied to duplicate microarrays. (B) One microgram of gDNA from the *Dehalococcoides*-containing ANAS enrichment culture was applied to duplicate microarrays.

tion signal intensity profiles for replicate arrays of DNA extracted from strain 195 and ANAS demonstrate a high level of analytical reproducibility (Fig. 2). The CV was calculated for each probe set to which ANAS gDNA hybridized across the five replicate ANAS microarrays. The average CV for a probe set across replicates is 10.1%, demonstrating that the data are highly reproducible among both biological and analytical replicates.

**Strain 195 genes identified in ANAS.** Of the 1,579 probe sets on the strain 195 microarray, ANAS gDNA hybridized to 1,369 (86.7%), representing genes that are conserved between strain 195 and the ANAS enrichment (Fig. 3).

*Dehalococcoides* strains within the ANAS enrichment show extensive gene similarity with the putative housekeeping genes of strain 195. Key conserved genes included *gyrA* (DET1630), *gyrB* (DET0004), *ftsZ-1* (DET0343), *ftsZ-2* (DET0636), *dnaG* (DET 0552), *frr* (DET0374), *infC* (DET0752), *nusA* (DET0985), *pgk* (DET0744), *pyrG* (DET1410), *rpoD* (DET0551), and *smpB* (DET1506). To verify the microarray results, *Dehalococcoides*-specific PCR primers were designed for three of these housekeeping genes, the ANAS gDNA was amplified, and the corresponding genes were confirmed to be present in ANAS by visual detection of the amplified fragment (Table 1).

Metabolically important genes, such as hydrogenase genes and genes involved in nitrogen fixation, carbon metabolism, cobalamin processing, and amino acid biosynthesis, have been putatively identified in strain 195 (33). ANAS DNA hybridized to probes for nearly all of these metabolic genes. All of strain 195's five putative hydrogenase complexes (26 genes), nine putative nitrogen fixation genes, 13 genes associated with the Wood-Ljungdahl carbon fixation pathway, and 20 genes implicated in acetate assimilation, gluconeogenesis, and the citric acid cycle were found in the ANAS culture. Genes thought to be involved in cobalamin transport and processing (including DET0245 and -0246, DET0650 to -0652, DET0654, DET0658, DET0684 to -0686, DET0688, DET0692, DET1138, and DT1139) were also detected in the ANAS culture. PCR was performed to corroborate the presence or absence of 22 *Dehalococcoides*-specific genes in the ANAS culture (Table 1).

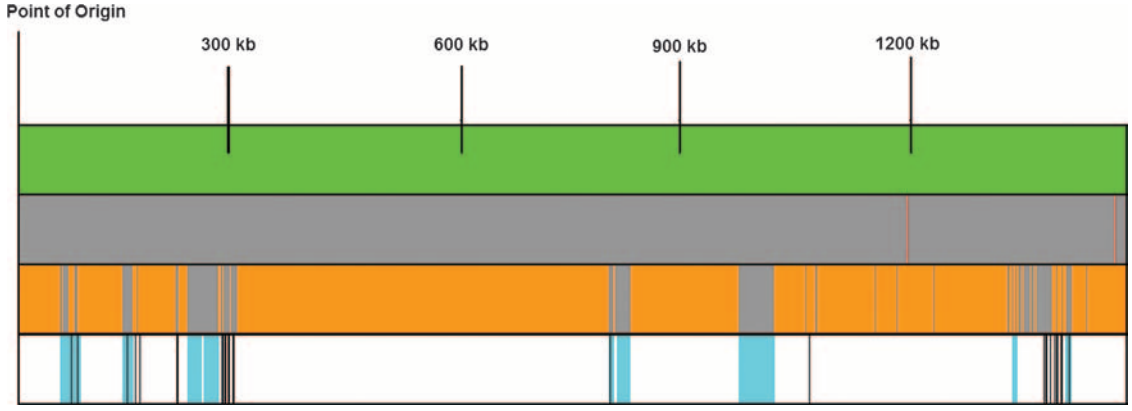


FIG. 3. Linear representation of the *Dehalococcoides ethenogenes* 195 genome. Gray sections represent genes that are not found in the corresponding cultures, and colored sections represent strain 195 genes identified in the corresponding cultures. The top and second rows depict genes detected by microarray analysis of strain 195 (the positive control) in green and of *Dehalobacter restrictus* (the negative control) in red, respectively. The third row depicts the genes detected in the *Dehalococcoides*-containing ANAS enrichment culture in orange mapped in parallel to the strain 195 genome. The bottom row depicts RDs and IEs, identified by Seshadri et al. (33), in black and blue, respectively.

TABLE 1. PCR analysis of microarray results for the *Dehalococcoides*-containing ANAS enrichment culture[a]

| Locus tag | Description | Presence/absence in ANAS by PCR[b] |
|---|---|---|
| DET0079 | Trichloroethene RD | + |
| DET0119 | Oxaloacetate decarboxylase, alpha subunit | + |
| DET0185 | Putative formate dehydrogenase accessory protein FdhE | + |
| DET0186 | Putative formate dehydrogenase, membrane subunit | + |
| DET0318 | *pceA* | − |
| DET0343 | Cell division protein FtsZ | + |
| DET0551 | RNA polymerase sigma factor RpoD | + |
| DET0666 | Carbon monoxide dehydrogenase, alpha subunit | + |
| DET0667 | Corrinoid/iron-sulfur protein, small subunit | + |
| DET0668 | Methylenetetrahydrofolate dehydrogenase and methenyltetrahydrofolate cyclohydrolase | + |
| DET0669 | Carbon monoxide dehydrogenase nickel-insertion accessory protein | + |
| DET0670 | Iron-sulfur cluster binding protein | + |
| DET0671 | Formate-tetrahydrofolate ligase | + |
| DET1158 | Nitrogenase iron protein | + |
| DET1481 | Anthranilate synthase component I | +[c] |
| DET1483 | Anthranilate phosphoribosyltransferase | − |
| DET1484 | Indole-3-glycerol phosphate synthase | − |
| DET1488 | Tryptophan synthase, alpha subunit | − |
| DET1494 | Peptide ABC transporter, periplasmic peptide-binding protein | − |
| DET1559 | Putative RD | − |
| DET1574 | Putative hydrogenase, HycD subunit | + |
| DET1630 | DNA gyrase, A subunit | + |

[a] Genes were selected to represent key functional groups and location in the strain 195 genome.
[b] +, present in ANAS; −, absent in ANAS.
[c] DET1481 was identified as absent by microarray analysis because data did not meet both criteria; however, PCR analysis identified the gene as present in ANAS.

**Strain 195 genes not detected in ANAS.** Two hundred ten probe sets (13.3%) did not meet the criteria to be regarded as present in response to ANAS gDNA (see Table S1 in the supplemental material). The genes from strain 195 that were not identified in the ANAS culture can be organized by their location in the strain 195 genome and by their predicted function. Most of the genes not detected in the ANAS culture are localized in clusters in the strain 195 genome (Fig. 3). Interestingly, 123 of the 210 probe sets that did not hybridize with ANAS gDNA represent genes located within potential IEs identified by Seshadri et al. (33) in strain 195 that include genes predicted to encode mobile genetic elements (MGEs), such as phage proteins, endonucleases, transposases, recombinases, resolvases, and restriction modification system proteins. In fact, 88% of the genes located in IEs in strain 195 were not detected in ANAS, including five of the nine IEs that were missing entirely. Further, no intact IEs from strain 195 were detected in ANAS (Table 2). Other genes not detected in the ANAS culture, such as DET0315 to -0323 and DET1508 to -1530, are localized in clusters and flanked by recombination-related genes but are not located inside identified IEs.

TABLE 2. Summary of the detection of integrated elements from *Dehalococcoides ethenogenes* 195 in the ANAS enrichment culture

| IE | Locus tags | Presence of gene in ANAS as a percentage of IE |
|---|---|---|
| I | DET0063 to -0091 | 48.3 |
| II | DET0155 to -0169 | 0 |
| III | DET0251 to -0272 | 0 |
| IV | DET0273 to -0295 | 0 |
| V | DET0875 to -0883 | 33.3 |
| VI | DET0884 to -0905 | 0 |
| VII | DET1066 to -1118 | 0 |
| VIII | DET1472 to -1478 | 71.4 |
| IX | DET1552 to -1581 | 11.1 |

**Functional analysis of genes not detected in ANAS.** Genes identified in strain 195 that were not detected in ANAS can be sorted by function (Table 3). One hundred twenty-nine (61.4%) of the 210 probe sets to which ANAS gDNA did not hybridize cluster into a group of genes described as unclassified or poorly classified by an analysis of clusters of orthologous

TABLE 3. Distribution of functional categories of strain 195 genes not detected in the ANAS enrichment culture[a]

| COG | No. of probe sets not found in ANAS |
|---|---|
| Unclassified and poorly classified | 129 |
| Transcription | 22 |
| Replication, recombination, and repair | 19 |
| Signal transduction mechanisms | 13 |
| Amino acid transport and metabolism | 12 |
| Energy production and conversion | 12 |
| Inorganic ion transport and metabolism | 4 |
| Cell wall/membrane/envelope biogenesis | 3 |
| Posttranslational modification, protein turnover, chaperones | 3 |
| Cell cycle control, cell division, chromosome partitioning | 2 |
| Coenzyme transport and metabolism | 2 |
| Defense mechanisms | 2 |
| Carbohydrate transport and metabolism | 1 |
| Intracellular trafficking, secretion, and vesicular transport | 1 |

[a] ANAS gDNA did not hybridize to 13.3% of the probe sets on the strain 195 whole-genome microarray. The 252 genes targeted by these probe sets cluster into 14 COGs.

TABLE 4. Detection of strain 195 RDs in the ANAS enrichment culture[a]

| Locus tag | Description | Detected in ANAS | Average signal intensity of replicate microarrays | Highest *P* value of replicate microarrays |
|---|---|---|---|---|
| DET0079 | *tceA* | + | 1,398.0 | 0.0003 |
| DET0088 | Domain protein | + | 5,135.0 | 0.00002 |
| DET0162 | Point mutation | − | 25.5 | 0.9915 |
| DET0173 | Putative RD | + | 5,559.5 | 0.00002 |
| DET0180 | Putative RD | + | 5,309.3 | 0.00002 |
| DET0235 | Putative RD | − | 57.5 | 0.2740 |
| DET0302 | Putative RD | − | 53.4 | 0.2426 |
| DET0306 | Putative RD | − | 39.9 | 0.4677 |
| DET0311 | Putative RD | − | 20.7 | 0.6926 |
| DET0318 | *pceA* | − | 39.2 | 0.5194 |
| DET0876 | Putative RD | − | 27.2 | 0.4291 |
| DET1171 | Putative RD | −[b] | 126.6 | 0.0229 |
| DET1519 | Putative RD | − | 89.4 | 0.2740 |
| DET1522 | Putative RD | − | 49.0 | 0.5194 |
| DET1528 | Putative RD | − | 39.5 | 0.6811 |
| DET1535 | Putative RD | + | 262.7 | 0.0023 |
| DET1538 | Putative RD | −[b] | 96.1 | 0.0037 |
| DET1545 | Putative RD | + | 4,911.7 | 0.00002 |
| DET1559 | Putative RD | − | 26.6 | 0.7150 |

[a] A gene was determined to be present if the probe set targeting the gene returned a signal intensity above the background noise and a *P* value of less than 0.05 in all five replicate microarrays.

[b] DET1171 and DET1538 were declared absent in ANAS despite acceptable *P* values of microarray data because the signal intensities for probes targeting the genes were lower than those of the negative controls.

groups (COGs) by DOE JGI. The remaining genes targeted by probe sets to which ANAS gDNA did not hybridize represent 13 COGs as described in the Integrated Microbial Genomes (IMG) database (DOE JGI; http://img.jgi.doe.gov/).

Strain 195 contains 19 genes orthologous to the catalytic A subunit genes of RDs, which may be key functional genes for *Dehalococcoides* bacteria because halogenated organics serve as the only identified terminal electron acceptors for this bacterium. ANAS gDNA did not hybridize to 13 of the 19 probe sets targeting putative RD genes in strain 195 (Table 4). Ten of these 13 RD genes have been included in the energy production and conversion COG by JGI, and the remaining three nonhybridized RD genes, including one ortholog containing a point mutation (DET1062), are unclassified. ANAS gDNA did not hybridize to probes targeting the *pceA* gene (DET0318), which codes for a subunit of the enzyme responsible for reducing PCE to TCE in strain 195 (29). The PCR results suggested the absence of two putative RD genes, including *pceA* in ANAS (Table 1), and correspondingly, ANAS has consistently failed to dechlorinate PCE (31). The gene in strain 195 that codes for subunit A of the protein responsible for TCE reduction to ethene, designated *tceA* (DET0079) (27), was detected in ANAS gDNA by the microarray. Congruently, ANAS is able to completely dechlorinate TCE, and the presence of *tceA* in ANAS was confirmed by PCR (Table 1).

The largest functional category of strain 195 genes not detected in the ANAS culture (22 of 210 probe sets) is labeled "transcription" and includes predicted transcriptional regulators. Of these genes, 13 are located in IEs in the strain 195 genome. The nine putative transcriptional genes not located in IEs are all located near RD genes in the strain 195 genome, including DET0300, DET0304, DET0316, DET1515, DET 1520, DET1523 to -1525, and DET1536. Many of these genes (DET0300, DET0304, DET0316, DET1520, and DET1523 to -1525) encode transcriptional regulators predicted to regulate the expression of RD genes (33) that also were not detected in the ANAS culture. DET1536 is the only putative transcriptional regulator gene not detected in ANAS that is associated with a putative RD gene (DET1535) that is present in ANAS.

Replication, recombination, and repair genes comprise the next largest COG of strain 195 genes not detected in ANAS and include putative transposase and recombinase genes. It is not surprising that 17 of the 19 probe sets in this group target genes located in IEs. The two genes not located in IEs are DET0323, predicted to encode a phage-related recombinase, and DET1301, encoding a putative transposase.

Of the 210 probe sets undetected in ANAS gDNA, 13 genes were categorized as participating in signal transduction, with 8 of these also being classified as participating in transcription. Of these 13 predicted signal transduction genes, 11 are identified as members of two-component systems regulating RD genes that also were not detected in ANAS. The two signal transduction genes not detected in ANAS that are not predicted to regulate RD genes are located in IEs.

Amino acid transport and metabolism genes comprise the next largest COG of strain 195 genes not detected in ANAS. Most genes identified in amino acid biosynthetic pathways of strain 195 were found in ANAS, with the exceptions of one gene located in an IE (DET1118) and two operons that neighbor each other, the putative tryptophan operon and an operon encoding a predicted ABC transporter. The strain 195 tryptophan biosynthesis operon is thought to be composed of eight genes, six of which were identified as absent in the ANAS culture. The genes in ANAS not detected by the microarray (DET1481 to -1485 and DET1487 and -1488) are predicted to encode the pathway responsible for transforming chlorismate to tryptophan. Although the *trpE* gene (DET1481) did not meet the criteria for detection in ANAS by microarray analysis (*P* value of 0.0562, just above the 0.05 threshold, in one of the five replicates), it was detected by PCR and is consequently considered present in ANAS. Because the PCR primers target a different region of DET1481 than the microarray probes, it is probable that the nucleotide sequence within ANAS targeted by the DET1481 PCR probes is identical to that in strain 195; however, there is likely a polymorphism in the region of DET1481 that is targeted by the microarray probes. The PCR analysis also suggested the absence of several other tryptophan genes in ANAS (Table 1). One hypothetical protein and five genes predicted to encode subunits of an ABC transporter comprise an operon in close proximity to the tryptophan operon that is implicated in peptide or nickel transport. Of the six genes in this operon, all five putative ABC transporter subunits (DET1490 to -1494) were not detected in ANAS, and the absence of DET1494 was supported by PCR analysis (Table 1).

## DISCUSSION

This study represents the first application of microarrays to query the whole genome of *Dehalococcoides* bacteria, both in a pure and a mixed culture. The technology was validated with the successful detection of every gene targeted by the array

when 1 μg of strain 195 gDNA was applied. The detection of high numbers of genes when 1 μg of gDNA of the multiple-*Dehalococcoides*-organism-containing mixed culture ANAS was applied to the array extends the usefulness of the array to mixed enrichment cultures and, potentially, environmental samples. The results obtained with traditional PCR techniques targeting more than 20 genes supported the microarray data. However, for a comprehensive analysis of the genetic capacity of *Dehalococcoides* organism-containing mixed cultures, PCR-based approaches are inadequate. Conversely, microarray technology provides a high-throughput method, requiring only small quantities of nucleic acids, to simultaneously examine every gene in these unique bacteria.

The challenges associated with applying DNA from microbial communities to microarrays targeting a single strain must be considered when analyzing the data in this study. For example, it is not possible to determine whether the probe signal intensities are the result of genes carried by individual or multiple strains within the community. In addition, lower overall signal intensities were observed for ANAS DNA than for strain 195 DNA. This result is the expected reflection of both the small portion of total DNA that is derived from *Dehalococcoides* bacteria in ANAS (approximately 10% of total microbial cells) and nucleotide polymorphisms that cause imperfect hybridization to the probes (13, 16, 38). These phenomena likely account for the large range of signal intensities observed for ANAS DNA in comparison to the range observed for strain 195. Also, when using strain-specific microarrays to query complex microbial communities, it is important to note that although genes present in both the isolate and the community can be detected, and genes present in the isolate that are not found in the community can be identified, genes that are present in the community but not in the isolate (such as *vcrA*) (18) will not be detected by this method.

The high degree of hybridization of gDNA from the ANAS enrichment culture to microarrays targeting the whole genome of strain 195 demonstrates the close evolutionary relationship between at least one of the strains within the ANAS enrichment culture and strain 195. Both cultures are able to dechlorinate TCE to ethene, with the distinction that VC and ethene are approximately equal end products for strain 195 while ANAS generates primarily ethene. The cultures share genetic sequences for most housekeeping and metabolic genes other than RD genes, as similarly found in a study comparing the genomes of strain 195 and strain CBDB1 (23). The conservation of many of the sequences of various genes throughout different *Dehalococcoides* strains facilitates the creation of *Dehalococcoides*-specific probes. Such probes can be utilized in both laboratory and field applications to investigate the presence of *Dehalococcoides* organisms but should not be used to infer the dehalogenation ability of *Dehalococcoides*-organism-containing microbial communities, since different strains are known to have diverse RD contents (19, 23, 33). The detection of genes encoding nitrogenase in ANAS is interesting since these genes are not present in the genomes of strains CBDB1 (23) or BAV1, both of which are more genetically distant from strain 195 than are the *Dehalococcoides* strains in ANAS. It is unclear whether the ancestor of these strains possessed nitrogenase and the branch with CBDB1 and BAV1 lost it or whether these genes were transferred to an ancestor of strain 195 and the *Dehalococcoides* organisms in ANAS.

The results of this study identify significant genetic differences between the *Dehalococcoides* strains in ANAS and strain 195 that may be responsible for functional differences in dehalogenation, such as the inability of ANAS to degrade PCE and the dissimilar degradation end products of the two cultures. Similar to comparisons with the genomes of strains CBDB1 (23) and BAV1, the prominent genetic differences between strain 195 and *Dehalococcoides* organisms in ANAS involve RD genes and associated transcriptional regulators, most of which exist in "islands" near the proposed origin of replication, and the absence of several MGEs identified as transposons or bacteriophages. Only 6 of the 19 genes in the strain 195 genome annotated to potentially encode A subunits of RDs are present in ANAS. As previously described (20), *tceA* (DET0079) was detected. Interestingly, DET0088, a gene located near the *tceA* gene that encodes only the C-terminal end of an RD and is missing a cognate B subunit anchoring gene and, therefore, is presumed to be nonfunctional, was detected in ANAS. This gene is not expressed in TCE-grown cells of strain 195 (10) nor found in its proteome (29), and its presence suggests that the truncated RD gene may occur in some *Dehalococcoides* genomes because of its proximity to the highly functional *tceA* gene. The *pceA* gene, the product of which reduces PCE to TCE, is not useful for organisms in the TCE-grown ANAS culture and was not detected. Interestingly, 52% of strain 195 IE I is absent from the ANAS culture, yet two of the six RD genes detected in ANAS are located in this IE, suggesting that RD genes may be transferred in or out of a genome independently of IE carriers. RD genes and RD regulator genes, both with and without proximal MGEs, comprise a group of genes present in strain 195 that are not found in the *Dehalococcoides* strains in ANAS. The high degree of conservation of housekeeping genes and the lack of conservation of RD genes and regulators between strain 195 and ANAS strains is consistent with the hypothesis that the RDs in *Dehalococcoides* strains are commonly exchanged (19, 23), thus transferring the dechlorination abilities of the strains within an evolutionarily short time frame.

An unexpected result of the genomic comparison of strain 195 and the ANAS enrichment culture is found in amino acid transport and metabolism genes. Several genes of the tryptophan operon were not detected in the *Dehalococcoides* strains in ANAS, although these genes were identified among the genes that are most highly conserved between strain 195 and strain CBDB1 (23). These genes, however, are in a genetically active region of the genome proximal to RD genes and IE region VIII of Seshadri et al. (33) and within atypical region F of Regeard et al. (30), and a genetic rearrangement may have facilitated their loss. Indeed, several genes from this region are missing in the ANAS strains (Fig. 3). It is possible that other microorganisms in ANAS provide tryptophan, obviating the need for the *Dehalococcoides* strains to biosynthesize this amino acid and facilitating the evolutionary loss of these genes. The absence of genes in the tryptophan operon has not been described for any other *Dehalococcoides* strains to date. Further investigation of potential tryptophan pathways in ANAS is needed for clarification.

This study also extends the use of whole-genome microar-

rays in environmental microbiology, demonstrating a tool to compare the genomes of isolates with enrichment cultures containing closely related strains. The ability to query complex microbial communities against a known genome to comprehensively identify homology between strains without a priori selection of individual sequences is a valuable tool. This technology has promise to provide insights into the physiology of environmental bacteria that are essential to the degradation of environmental contaminants in natural and engineered bioremediation systems.

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