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Global Gene Expression of *Dehalococcoides* Within a Robust Dynamic TCE-Dechlorinating Community Under Conditions of Periodic Substrate Supply

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ABSTRACT: A microarray targeting four sequenced strains in the *Dehalococcoides* (Dhc) genus was used to analyze gene expression in a robust long-term trichloroethene (TCE)-degrading microbial community (designated ANAS) during feeding cycles that involve conditions of periodic substrate supply. The Dhc transcriptome was examined at three time-points throughout a batch feeding cycle: T_1 (27 h) when TCE, dichloroethene (DCE), and vinyl chloride (VC) were present; T_2 (54 h) when only VC remained; and T_3 (13 days) when Dhc had been starved of substrate for 9 days. Ninety percent of the Dhc open reading frames (ORFs) that were detected in the ANAS DNA were found to be expressed as RNA sometime during the time course, demonstrating extraordinary utilization of the streamlined genome. Ninety-seven percent of these transcripts were differentially expressed during the time course indicating efficiency of transcription through regulation in Dhc. Most Dhc genes were significantly down-regulated at T_3, responding to a lack of substrate as would be expected. The *tceA* and *vcrA* genes, which code for proteins with known chlorinated ethene reduction functions, were highly expressed at both T_1 and T_2, whereas two other putative reductive dehalogenase genes (DET0173 and DET1545) were most highly expressed at T_2, likely in response to the presence of VC. Hydrogenases were most highly expressed at T_1, reflecting their important role in accumulating electrons used to initiate reductive dechlorination and other biosynthesis pathways. Cobalamin transport genes were preferentially expressed at T_2, reflecting an increase in corrinoid transport as chloroethenes were degraded and a decrease in activity of the transport system after dehalogenation was complete. This is the first application of a microarray targeting a known genus, including both core genomes and identified strain-specific genes, to improve our understanding of transcriptional dynamics within an undefined microbial community.

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KEYWORDS: differential expression; microarray; *Dehalococcoides*; bioremediation; chlorinated solvents

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Introduction

The remediation of trichloroethene (TCE), a wide-spread environmental contaminant (US EPA, 2001; US HHS, 2007) and human carcinogen (US EPA, 2011), has been an ongoing challenge since TCE first received regulatory attention in the 1980s. In groundwater environments, TCE biodegradation to dichloroethene (DCE) can be catalyzed by a variety of subsurface bacteria via anaerobic

reductive dechlorination (Freedman and Gossett, 1989; Gossett and Zinder, 1997). However, dechlorination beyond DCE to vinyl chloride (VC) and non-toxic ethene has only been found to be catalyzed by members of the genus *Dehalococcoides* (Dhc; Fennell et al., 2001; Hendrickson et al., 2002; Maymó-Gatell et al., 1997). Although Dhc are promising bacteria for use in bioremediation strategies, they grow relatively slowly in isolation, with doubling times of 1–2 days (He et al., 2007), making the growth of Dhc isolates a challenge to grow rapidly and robustly for bioaugmentation. Further, identified Dhc have fastidious nutritional requirements, requiring acetate as a carbon source, hydrogen as electron donor (Maymó-Gatell et al., 1997), and a source of exogenous corrinoids to serve as cofactors for their reductive dehalogenases (RDases; Magnuson et al., 1998). Dhc exhibit significantly higher dechlorination rates and specific cell densities when grown in mixed communities than when grown in isolation (He et al., 2007; Maymó-Gatell et al., 1997), likely because other members of the community provide supporting compounds that Dhc is not capable of generating de novo. Consequently, studying the behavior of Dhc in a diverse microbial community will improve our understanding of the microbial interactions that more closely resemble those in the environment.

Although microbial communities containing Dhc have received significant attention (Cupples et al., 2004; Duhamel et al., 2004; Fennell and Gossett, 1998; Freeborn et al., 2005; Krajmalnik-Brown et al., 2007; Richardson et al., 2002), the effects of community structure on the gene expression of Dhc are largely unknown. Many molecular techniques used to measure gene expression in microbial communities do not provide a comprehensive transcriptomic profile. A number of studies have investigated functional gene expression of Dhc in communities using reverse transcription—quantitative PCR (RT-qPCR) to target specific genes (Behrens et al., 2008; Futamata et al., 2009; Holmes et al., 2006; Lee et al., 2006; Popat et al., 2012; Rahm et al., 2006; Rowe et al., 2008; Tas et al., 2009; Waller et al., 2005). Transcripts of Dhc 16S ribosomal RNA genes are commonly measured to track their growth (Behrens et al., 2008; Futamata et al., 2009; Tas et al., 2009), and RDases are commonly monitored as biomarkers of degradation activity using either specific primers for known functional genes (Behrens et al., 2008; Holmes et al., 2006; Lee et al., 2006, 2008; Popat et al., 2012; Rahm et al., 2006; Tas et al., 2009) or degenerate primers to detect putative RDase genes in the community (Futamata et al., 2009; Waller et al., 2005). Hydrogenases and other highly expressed genes have also been targeted to represent Dhc activity in communities (Morris et al., 2006; Rahm et al., 2006). Although these specific gene targeting methods are highly advantageous for quantification, methods that simultaneously analyze the expression profile of all open reading frames (ORFs) in a genome can provide more comprehensive information of complex bacterial behavior. Microarrays are an ideal platform for such a method. A microarray that targets the genome of *Dehalococcoides mccartyi* 195 has previously been applied to provide comprehensive gene expression data for this strain grown under a variety of conditions (Johnson et al., 2008, 2009; Lee et al., 2009). This microarray was also applied to provide comparative genomics data for Dhc in a mixed microbial community (West et al., 2008). Subsequently, two more comprehensive microarrays were independently designed to target the complete genomes of the four published *Dehalococcoides* strains (195, CBDB1, BAV1, and VS) as of June 2010. One of these genus-wide arrays also incorporates genes found in the KB-1 Dhc-containing community and was validated with an in silico examination of Dhc strain GT (Hug et al., 2011). The other genus-wide microarray does not consider KB-1, but additionally targets all known RDases from Dhc and other genera as well as functional genes common to reductive dehalogenating microbial communities (Lee et al., 2011). This genus-wide microarray has been successfully applied to characterize the genomic content of several Dhc isolates and mixed cultures (Lee et al., 2011).

This study focuses on an enrichment culture, denoted ANAS, that dehalogenates TCE to ethene and has been functionally stable for over a decade. This community contains organisms from over 1,000 Bacterial and Archaeal taxa including Dhc (Brisson et al., 2012), and has been extensively characterized (Johnson et al., 2005b; Lee et al., 2006; Richardson et al., 2002; West et al., 2008). A recent study by Lee et al. (2011) revealed that ANAS includes two functionally distinct Dhc strains whose genomes are 97.7% similar to each other by gene content based on comparative genome hybridization, and are 83.1% and 81.3% similar to strain 195, respectively. Since ANAS is maintained as a semi-batch culture with periodic feeding and wasting cycles, it exhibits a dynamic community structure in which microbial members respond to the constantly changing conditions within a cycle. Many environmental bacteria exist in a starvation state with only occasional exposure to utilizable growth substrates, especially in biostimulation applications in which nutrients are periodically added to the system. Thus, it is essential to understand how periodic starvation affects activity and viability. This study utilizes the ANAS reactor to study the dynamic responses of the Dhc transcriptome within ANAS during a feast and famine feeding cycle. Additionally, this study represents the first application of a Dhc genus-wide microarray to improve our understanding of gene expression by these unique bacteria within an active microbial community.

Materials and Methods

Culture Growth, Maintenance, and Sampling

The Dhc-containing microbial culture, ANAS, was enriched from chloroethene-contaminated soil obtained from Alameda Naval Air Station. ANAS is maintained in a semi-batch mode, enriched with lactate (25 mM or 2.8 g/L) and TCE (0.1 mM or 13 μg/L) every 14 days producing

cyclic feast and famine stages for many years prior to the experiments in this study as previously described (Lee et al., 2006; Richardson et al., 2002). The addition of TCE and lactate are denoted as T_0. 30 mL samples were extracted from ANAS at 27 h, 54 h, and 13 days after the initial TCE and lactate amendments for a total of 90 mL of sample taken during a cycle. Fourteen days after the TCE and lactate were added, the reactor headspace was purged with N_2/CO_2 (90/10) and typically 100 mL of the culture liquid would be removed and replaced with fresh medium; however, to account for the loss of volume due to sampling in this experiment, no additional volume was removed from the reactor and 90 mL of medium was added to replenish lost nutrients and liquid. Because there is only one ANAS reactor and it runs in a semi-batch configuration, three sets of samples were collected from three different 14-day cycles of the culture to achieve biological triplication of data.

Chemical Analyses and Molecular Methods

Details of the methods for gas chromatography, high-performance liquid chromatography, qPCR analysis, DNA and RNA extraction, and microarray sample preparation and application are supplied in the Supplementary Information and draw on previously published methods (Freeborn et al., 2005; Johnson et al., 2005a; Lee et al., 2006; West et al., 2008).

Affymetrix GeneChip Characteristics

The Affymetrix (Santa Clara, CA) GeneChip microarray applied in this study contains 4,744 probe sets that represent more than 6,000 (>98%) ORFs from four published *Dehalococcoides* genomes (strain 195, VS, BAV1, and CDBD1) and 348 functional genes in other microorganisms common to reductive dehalogenating microbial communities.

A complete description of the microarray has been reported elsewhere, including an assessment of the positive and negative controls used to validate its hybridization efficiency and quantitative response (Lee et al., 2011).

Microarray Data Analysis

Replicate samples were independently collected, and simultaneously but individually extracted, fragmented, labeled, and hybridized to arrays. Three DNA samples (one from each of the three replicate cycles) and nine RNA samples (one from each of the three time-points in each of the three replicate cycles) were prepared for microarray analysis. Statistical analysis methods and criteria are described in the Supplementary Information and draw on previously published methods (Affymetrix, 2001; Benjamini and Hochberg, 1995; Culhane et al., 2005; Eisen et al., 1998; Gentleman et al., 2004; Liu et al., 2002). The microarray data analyzed in this study and information regarding the microarray platform were deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under the following accession numbers: complete RNA under dataset GSE42635, DNA as samples GSM585180, GSM585181, and GSM585182 under data set GES23707, and platform under GPL10838.

Results

Growth and Dechlorination

The goal of this study was to examine the gene expression of Dhc within a dynamic microbial community under conditions of substrate supply and deprivation. To accomplish this objective, the ANAS community was monitored for levels of chlorinated ethenes (Fig. 1A),

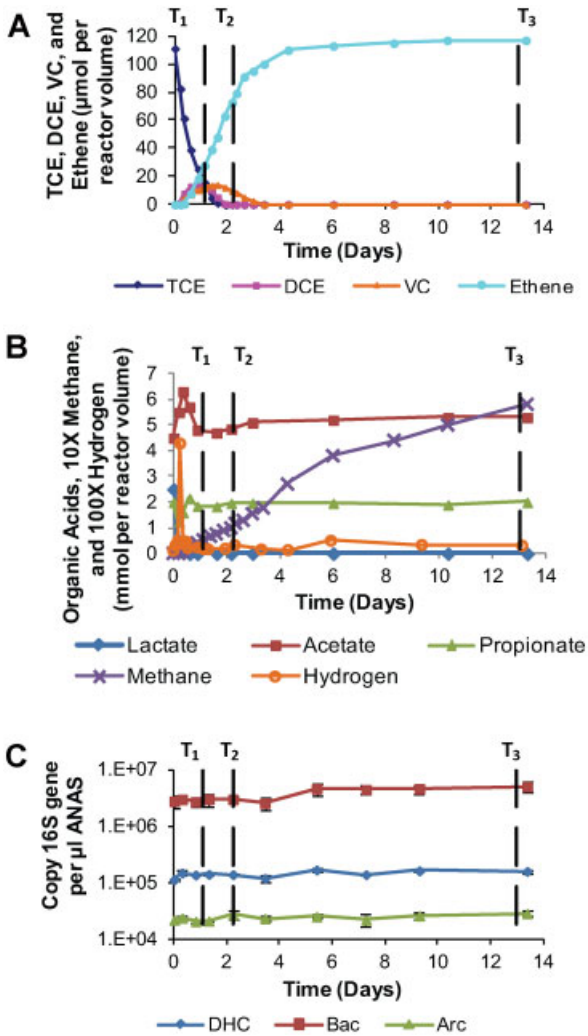

Figure 1. Temporal changes in a representative cycle of ANAS showing chlorinated ethene dehalogenation (**A**), organic acids and hydrogen concentrations (**B**), and cell densities of *Dehalococcoides*, total Bacteria, and total Archaea (**C**). Dashed vertical lines indicate the three time-points at which samples were taken for microarray analyses.

hydrogen (Fig. 1B), and several organic acids (Fig. 1B) throughout its 14-day feeding cycle. Lactate was depleted within 1 day, TCE was degraded within 2 days, and complete reductive dechlorination to ethene was achieved within 4 days. Cell density measurements of total Bacteria, total Archaea, and Dhc (Fig. 1C) show minimal cell growth throughout the cycle approximately equal to the rate of the semi-batch dilution of the reactor (ca. 1/3 volume), as would be expected. Microbial cell samples were taken for DNA and RNA microarray analyses from ANAS at three distinct time-points in the cycle: 27 h (designated T_1), 54 h (T_2), and 13 days (T_3) after the cycle was initiated by amendment with TCE and lactate. At T_1, the dwindling presence of TCE and the production of reductive dechlorination daughter products (DCE, VC, and ethene) demonstrate that dechlorinating bacteria within the consortia were active, and the production of methane demonstrates the activity of methanogens. At T_2, TCE and DCE were fully depleted, and VC was the only chloroethene remaining in the culture, while ethene and methane production were ongoing. At T_3, approximately 9 days had passed with no chloroethenes to support Dhc growth, but methane production continued. The final 10 days of the feeding cycle represent a famine period in which ANAS Dhc modify their gene expression to persist without substrate. Each of these three time-points represents a unique phase of the microbial community.

Global Gene Expression

When ANAS community DNA and RNA were hybridized to the microarrays, 89.6% of the ORFs that were statistically detected by DNA analysis were also detected in at least one time-point in expressed RNA. To correlate gene expression with physiological characteristics of ANAS, the Dhc transcriptome was analyzed at each time-point. Of the Dhc ORFs that were expressed in ANAS (1,296 total), 98% (1,265) were detected at T_1, 99% (1,280) at T_2, and 50% (654) at T_3. Although all expressed ORFs meet the statistical criteria as present in RNA samples, signal intensities varied greatly, indicating a wide range of expression levels. Throughout the growth cycle of ANAS, hypothetical proteins and tRNAs represent a large portion of the most highly expressed ORFs (Fig. 2). The ORFs that were most highly expressed at T_1 were dominated by genes involved in translation, ribosomal biogenesis, and energy conservation,

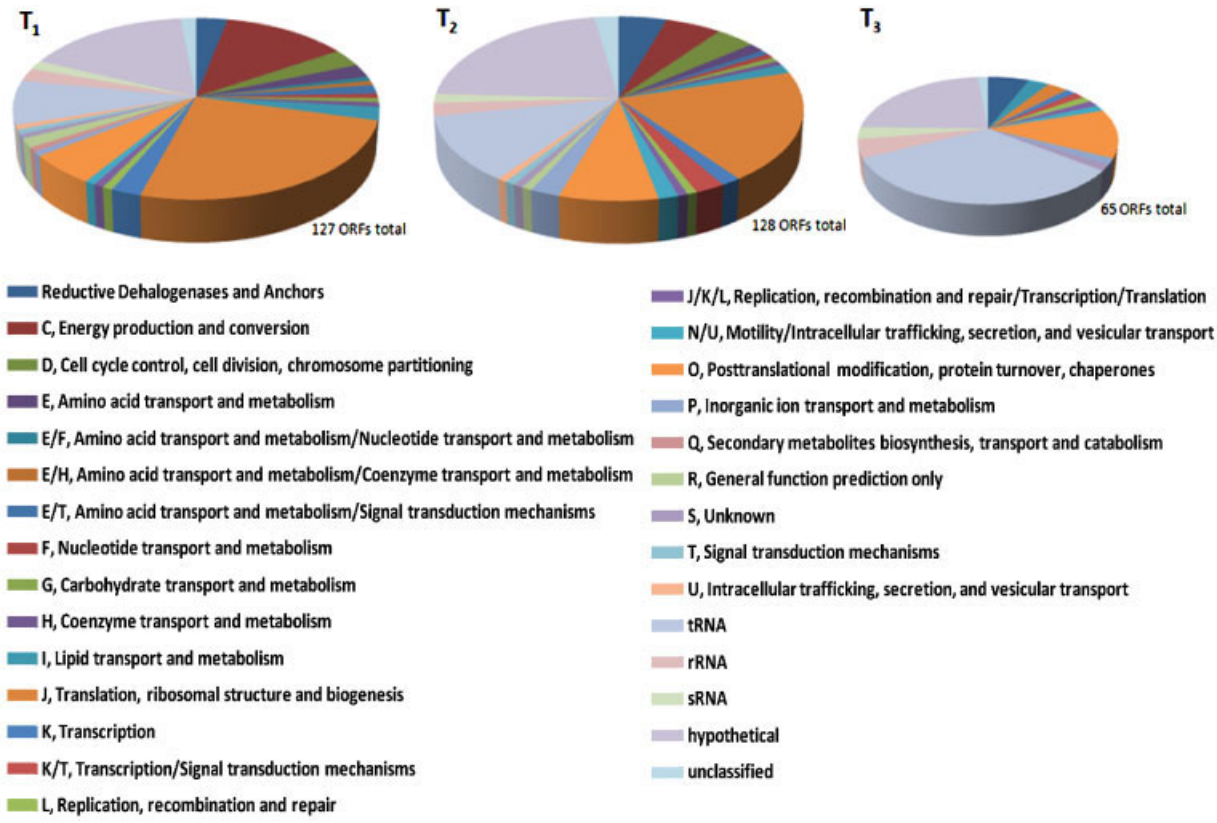

Figure 2. Functional classification of ANAS *Dehalococcoides* ORFs with the 10% highest signals at each time-point at which samples were taken for microarray analyses. Clusters of orthologous groups (COGs) are each represented by a unique color. Color and order of COGs are consistent between the three graphs. Graph size scales to the number of ORFs that comprise the top 10% at each time-point.

although a wide variety of clusters of orthologous groups (COGs) were represented (Fig. 2). The COGs with highest transcript levels at T_2 were slightly less diverse and dominated by genes involved in translation and ribosomal biogenesis (Fig. 2). At T_3, ORFs involved in post-translational modification, protein turnover, and chaperone functions had the highest transcript levels, and fewer functional categories were represented (Fig. 2).

Differential Gene Expression

Comparative transcriptomics of the microarray data revealed genes and groups of genes that were differentially expressed at each time-point and potentially indicative of physiological conditions. Overall, 97.4% of the genes that were expressed in at least one time-point throughout the dechlorination cycle were differentially expressed between the three time-points. Hierarchical clustering was applied to sort genes into groups that exhibit similar patterns of transcription among a set of conditions (Eisen et al., 1998). Two main clusters emerged from the analysis (Fig. 3); most differentially expressed genes were significantly down-regulated at T_3 as the culture reached the end of the batch cycle (Group 1), however a small cluster (Group 2) was unexpectedly up-regulated at the final time-point. Three of the four genes that comprise this deviant Group 2 cluster encode hypothetical proteins (Table S1), and the fourth is annotated as an acetyltransferase/FMN reductase gene (DET1321).

The Group 1 cluster of genes that were down-regulated at T_3 can be further split into three groups (Fig. 3; Table S1).

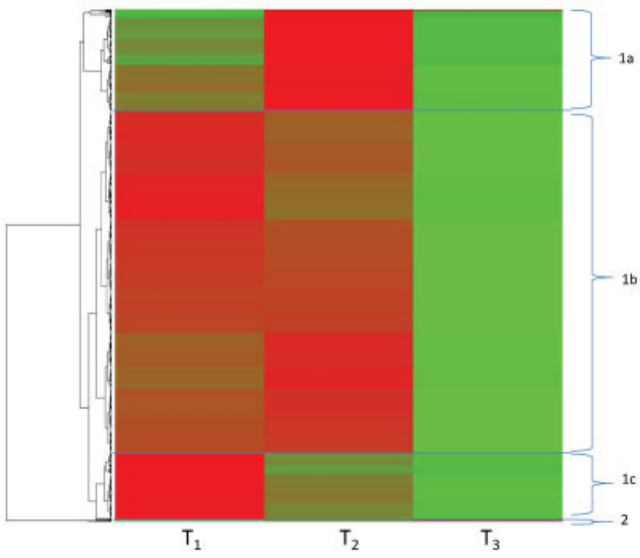

Figure 3. Hierarchical clustering and dendrogram of 1,262 differentially expressed genes (>twofold; false discovery rate, <0.05). The color gradient from green to red represents increasing microarray hybridization intensity.

The group designated 1a fluctuates in expression as these genes were expressed at low levels at T_1 while TCE was prevalent, high levels at T_2 while VC was present, and low levels again at T_3 when Dhc electron acceptors and donors had long been depleted. Two putative RDase genes (DET0173 and DET1545) follow this trend in expression levels, implying that the function of these unknown genes might be more closely linked with the presence of VC than TCE. Group 1a also includes 6 of 18 genes identified as corrinoid biosynthesis genes and one corrinoid transport gene. Group 1b genes were expressed at relatively high levels at T_1 and T_2 and down-regulated at T_3. Most Dhc genes fall into this group, responding to a lack of chlorinated ethenes at T_3 by reducing transcription as could be expected. The *tceA* and *vcrA* RDase genes both follow this trend, being among the most highly expressed genes at T_1 and T_2. Also included in Group 1b are genes that encode the FpoF oxidoreductase and Vhu hydrogenase, 12 of the 18 expressed corrinoid biosynthesis genes, and four of the five genes identified as corrinoid transport genes. Group 1c genes were highly expressed at T_1 when TCE was abundant and down-regulated at both T_2 and T_3. Genes encoding seven oxidoreductases (*mod*, *hup*, *hym*, *fdh*, *por*, *ech*, and *nuo*) were represented in Group 1c.

Differential expression analysis of genes from the first two time-points of active dechlorination (T_1 and T_2) could identify Dhc genes that respond specifically to the presence of TCE, DCE, and VC. Twenty-nine genes were found to be differentially expressed in T_1 and T_2 (Table S2), 11 of which are annotated as hypothetical proteins. Only three of these genes were down-regulated from T_1 to T_2: one encoding a chorismate mutase implicated in phenylalanine, tyrosine, and tryptophan biosynthesis (DET0461); one encoding an amidotransferase used in glutamate synthesis (DET1130); and a transcription elongation factor gene (DET0770). Additionally, the *tceA* gene was statistically down-regulated, however, the signal intensity decreased by less than twofold, excluding it from being identified as differentially expressed. All 26 other differentially expressed genes were up-regulated from T_1 to T_2, suggesting that, in general, transcription increased during this transition. Four of the 29 differentially expressed genes fall within the top 10% most highly expressed genes, each with a higher signal at T_2 than at T_1. Of note, genes encoding a protein chaperone (DET0954), an oxidoreductase (DET0955), and a superoxide dismutase (DET0956) were differentially expressed, had high signal intensities, and are located contiguously in the Dhc genome. Several other ORFs identified as differentially expressed between T_1 and T_2 are also co-located in the genome, however, they do not compose operons with predicted functions.

Trends in Functionally Important Genes

The ANAS culture contains seven putative RDase genes detected by array analysis, five that were identified in strain

195 (DET0079 (*tceA*), DET0088, DET0173, DET0180, DET1545; Seshadri et al., 2005) and two that were identified in strain VS (DhcVS_1305, DhcVS_1291 (*vcrA*); McMurdie et al., 2009). Of these seven RDase genes, four (*tceA*, DET0173, DET1545, and *vcrA*) were expressed in ANAS throughout the time course (Fig. 4). *TceA* and *vcrA* were both highly expressed at T_1 and T_2, when TCE and VC were present, respectively, and were down-regulated at T_3. DET0173 and DET1545 follow a different expression profile, with both putative RDase genes exhibiting the highest levels of transcription at T_2, when VC was the sole chlorinated ethene available.

In an attempt to elucidate the roles of Dhc oxidoreductases in ANAS, the expression of five putative hydrogenase genes (*hyc*, *ech*, *hup*, *hym*, *vhu*) and several other oxidoreductase genes (*fdh*, *mod*, *nuo*, *fpoF*, and *por*) were examined in this study (Fig. 4). The putative periplasmic formate dehydrogenase gene (*fdh*) was highly expressed, especially at T_1. Other putative respiration-associated genes that were highly expressed in this study encode a potential molybdopterin oxidoreductase (Mod), a membrane-bound Hym [Fe]-hydrogenase, a cytoplasmic Vhu hydrogenase, and a potential pyruvate ferredoxin oxidoreductase (Por). The genes that encode an NADH dehydrogenase (Nuo) and the membrane-bound periplasmic Hup hydrogenase were only moderately expressed. Genes encoding Ech and FpoF were expressed at low levels throughout the time course. The gene that encodes the membrane-bound Hyc hydrogenase was detected in ANAS by DNA analysis, but not in transcript arrays.

Because genome annotation of currently sequenced strains revealed that Dhc must rely on exogenous corrinoids for RDase activity, genes associated with corrinoid salvaging and transport were examined in this study (Fig. 4). The ABC-type cobalamin transport system (DET0650–0652/DET0684–0686) was the most highly expressed set of corrinoid-associated genes in this time course. This set of genes was up-regulated from T_1 to T_2 and severely down-regulated from T_2 to T_3, implying an increase in corrinoid transport when TCE has been degraded to VC and minimal activity of the transport system after complete dehalogenation. Although not as highly expressed as the cobalamin transport system, several corrinoid biosynthesis genes were also expressed at relatively high levels at T_1 and T_2 including genes that encode CobB (DET0128), CbiZ (DET0653/0687), CobC (DET0659/0693), CobQ (DET0936), and CobA (DET1224). The products of these genes act in various stages of cobalamin biosynthesis; CobB and CobQ are

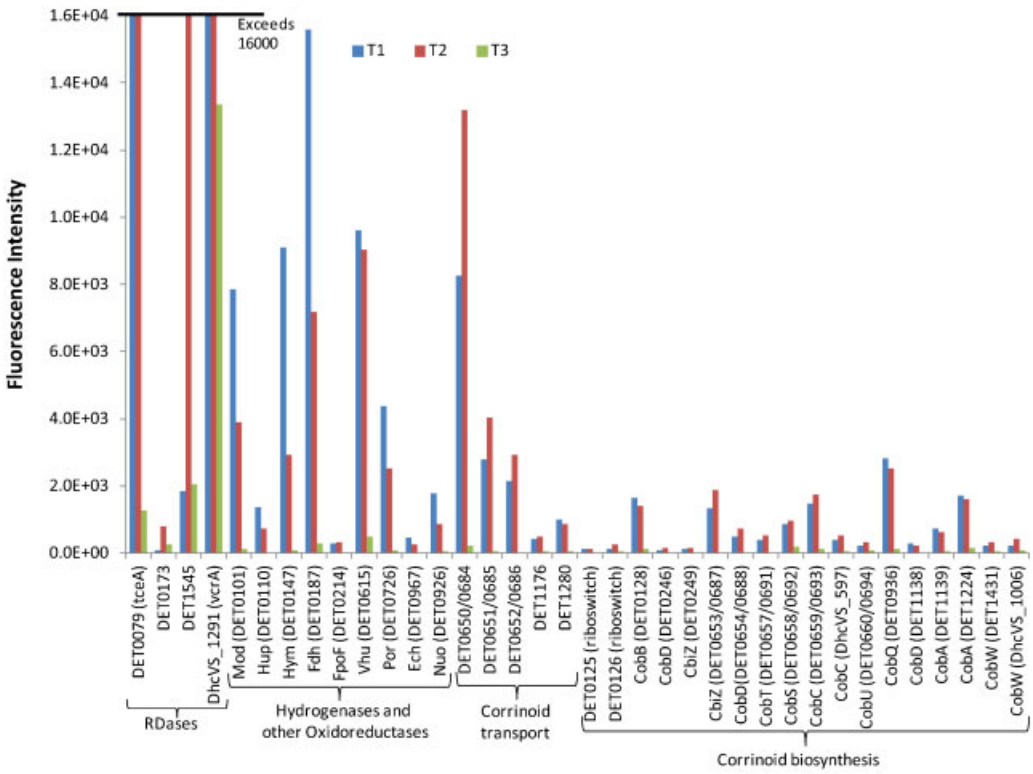

Figure 4. Transcription profiles of selected genes in key functional groups. Raw signal hybridization intensities are shown at each time-point at which samples were taken for microarray analyses. Hybridization fluorescence intensities exceed the values shown on the graph for the following RDase genes and time-points: *tceA* at T_1 reached 75352, *tceA* at T_2 reached 56150, DET1545 at T_2 reached 27184, *vcrA* at T_1 reached 125810, and *vcrA* at T_2 reached 122528.

predicted to amidate the side chains of the corrin ring, CbiZ to ligate the lower ligand to the corrinoid, CobC to remove the phosphoryl group attached to the lower ligand base and CobA is involved in upper ligand modification.

Discussion

The transcriptome of Dhc within the robust ANAS microbial community was examined during a feeding cycle featuring a feast and famine progression as a model of environmental communities which undergo nutrient flux such as biostimulation. As would be expected, most genes were highly expressed while electron donors and acceptors were available to be utilized in growth and dechlorination activity (T_1 and T_2) and exhibited significantly decreased transcript levels when substrates became unavailable (T_3) indicating conservation of cellular activity through down-regulation or a decline in new transcription (Fig. 3). The mRNA that was transcribed during the starvation stage of the cycle was dominated by genes indicative of stress (Fig. 2). Global gene expression was not considerably different between a time-point corresponding to the simultaneous presence of TCE, DCE, and VC (T_1) and a time-point with only VC and ethene (T_2), as indicated by the 29 ORFs that were statistically differentially expressed in the T_1/T_2 analysis relative to the 1,262 ORFs differentially expressed in a comparison of all three time-points. However, the T_1/T_2 analysis revealed the up-regulation of co-localized genes including those encoding a chaperone, an oxidoreductase, and a superoxide dismutase that might suggest an increased stress response from T_1 to T_2. All ORFs differentially expressed between T_1 and T_2 are present in both Dhc isolates within ANAS (Lee et al., 2011), so the differential expression is not attributable to competition between the two Dhc strains.

Expression of RDase genes has been the focus of many studies (Johnson et al., 2005a,b; Lee et al., 2006; Rahm et al., 2006; Waller et al., 2005) because RDases bestow the ability to dechlorinate solvents, the activity that supplies energy for growth of Dhc and affords anthropogenic significance to the bacteria. The expression patterns of *tceA* and *vcrA* observed in this study were consistent with previous findings showing *tceA* and *vcrA* up-regulated in response to TCE, DCE, and VC and transcripts returning to background levels within days of chlorinated ethene depletion (Lee et al., 2006). Because the Dhc strains in ANAS can be differentiated by the RDases they carry, this genus-based array is able to track the activity of individual strains within the mixed culture. The *tceA* gene, which is found in strain ANAS1 within the ANAS culture (Lee et al., 2011) and is associated with metabolism of TCE and DCE and cometabolic degradation of VC (Magnuson et al., 2000), exhibited a slight decrease in transcript levels at T_2 compared to T_1, in congruence with diminished substrate availability. The *vcrA* gene, which is carried by ANAS2 and couples TCE, DCE, and VC reduction to growth (Lee et al., 2011; Müller et al., 2004), was expressed similarly at T_1 and T_2 when various substrates were available. Interestingly, putative RDase genes DET0173 and DET1545, both with undetermined substrate specificity, were more highly expressed in T_2 when only VC remained, suggesting that VC might stimulate transcription of these genes. DET1545 is among the most highly expressed genes in this study with RNA/DNA ratios an order of magnitude higher than those for DET0173, and was highly expressed in other mixed community studies (Rahm et al., 2006; Waller et al., 2005), but its substrate and specific function remain unknown. Little is known about DET0173. Both genes were found to have the same expression pattern in a time course study of strain 195 grown in isolation in which they were characterized by a large increase in expression from the "transition from exponential growth to stationary phase" during which TCE, DCE, and VC were present to the "late stationary phase" in which VC is dominant (Johnson et al., 2008). Johnson et al. also found DET0180 to follow the same expression pattern as DET0173 and DET1545 in the strain 195 time course; in contrast, although DET0180 is present in ANAS by DNA analysis, its transcripts were not detected in this time course study.

Many strains of Dhc have multiple complexes of hydrogenases and other respiration-associated oxidoreductases that may play important roles in electron transport (Kube et al., 2005; Maymó-Gatell et al., 1997; McMurdie et al., 2009). In general, these genes reached their maximum expression at T_1, supporting the theory that Dhc devotes energy early in a feeding cycle to gathering a pool of electrons to be used for reductive dechlorination (Rahm et al., 2006). As seen in other mixed- and pure-culture Dhc studies (Johnson et al., 2008; Morris et al., 2006; Rahm et al., 2006), the *fdh* dehydrogenase gene was highly expressed in ANAS at both T_1 and T_2, which is interesting given that the Dhc strains in ANAS most closely resemble strain 195, which is not capable of using formate as an electron donor (Maymó-Gatell et al., 1997). The specific trigger for expression of the *fdh* gene remains unknown. The *hup* hydrogenase gene was only moderately expressed in this study, in contrast to the findings of previous studies conducted with mixed and pure Dhc cultures that found *hup* to be highly expressed (Johnson et al., 2008; Morris et al., 2006). The *mod* oxidoreductase gene was highly expressed at T_1 and T_2 in ANAS showing a correlation between Mod activity and Dhc mixed culture growth that was also seen in a previous study comparing Dhc-mixed and pure cultures (Morris et al., 2006). Curiously, data from this and previous studies show that the *ech* hydrogenase gene is consistently more highly expressed in pure culture than in mixed communities (Johnson et al., 2008; Morris et al., 2006), suggesting that the community provides something to Dhc that limits Ech activity or renders it less necessary. *Ech* and *hyc* are genes encoding [Ni,Fe]-hydrogenases that can use proton motive force to develop low-potential electrons for biosynthetic reactions (Johnson et al., 2008; Morris et al., 2006). The minimal expression levels found for the *ech* and *hyc* genes throughout this study are consistent with similar

studies in which *ech* and *hyc* had the lowest expression levels of the hydrogenases observed during time course studies of mixed and pure Dhc cultures (Morris et al., 2006, Rahm et al., 2006), suggesting that these hydrogenases may not be essential in Dhc for typical growth and dechlorination.

Corrinoids are essential for dehalogenation activity as they are cofactors of the RDases (He et al., 2007; Maymó-Gatell et al., 1997), and no currently sequenced Dhc genome contains a complete pathway to biosynthesize them de novo. The corrinoid ABC-type transporter gene of Dhc (DET0650–0652/DET0684–0686) was highly expressed while active dechlorination occurred within the ANAS community, which is consistent with previous pure culture experiments in which these genes were continuously expressed during dechlorination when spent medium from ANAS was amended to the isolate (Johnson et al., 2009). Expression levels suggested that the transport system was minimally transcribed after dechlorination was complete. This active regulation suggest that the uptake of corrinoids by Dhc from the ANAS community peaks during active dechlorination and growth, but might be costly to the cells as it is down-regulated when cobalamin is no longer being utilized. The expression of several cobalamin synthesis genes with diverse functions revealed that various types of corrinoid modifications transpire to produce the cobalamin form necessary for RDase activity, suggesting that different forms of cobalamin precursors are present in the ANAS community.

This study reveals the high genetic efficiency of Dhc within a mixed community, as evidenced by two results. First, 89.6% of the ORFs that were detected in the ANAS Dhc DNA were found to be expressed during the time course, implying substantial utilization of the streamlined genome. Similarly, a previous experiment examining the transcriptome of strain 195 grown in isolation demonstrated that 89.1% of its ORFs were expressed during some growth stage (Johnson et al., 2008). These expression values for Dhc are notably higher than global gene expression values reported for model bacteria such as *E. coli* that range from 75% to 77% (Mohanty and Kushner, 1999; Tao et al., 1999). Second, 97% of the transcripts were differentially expressed during the time course indicating strong regulation of the genes. The 34 genes that were expressed without statistical variance were dominated by 28 tRNA genes, which would be expected to have stable transcriptional profiles, and five hypothetical proteins.

Finally, this is the first application of a microarray targeting a known genus, including both core genome and identified strain-specific genes, to an undefined microbial community to improve our understanding of transcriptional dynamics of specific functional cells. It should be noted that novel genes potentially present in Dhc strains will not be detected in the microarray analysis given that only targeted genes are detected with this high-throughput method. Dhc often contain regions of high plasticity and insertion elements where strain-specific genes commonly reside (McMurdie et al., 2009). With this understanding, the genus-wide microarray has proven to be a powerful tool to simultaneously analyze thousands of genes within complex biological communities.

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