RESEARCH LETTER

Transcriptomic microarray analysis of corrinoid responsive genes in *Dehalococcoides ethenogenes* strain 195

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Abstract

Dehalococcoides ethenogenes strain 195 utilizes corrinoid-containing reductive dehalogenases to reduce the environmental pollutants tetrachloroethene and trichloroethene to ethene. Although corrinoids are essential for dehalogenation activity, strain 195 cannot biosynthesize corrinoids *de novo*. To improve our understanding of corrinoid physiology in this bacterium, whole-genome microarrays were applied to characterize the transcriptome during growth with excess and limiting concentrations of the corrinoid cyanocobalamin. Additional studies examined the effects of exposure to spent medium from a methanogenic chloroethene-dehalogenating enrichment culture (designated ANAS). Both excess cyanocobalamin and ANAS spent medium resulted in the downregulation of two genes (DET0125–0126) that are encoded downstream of a putative cobalamin riboswitch. In contrast, only ANAS spent medium resulted in the downregulation of three duplicated genes (DET0657–0659/DET0691–0693) encoded downstream of a second putative cobalamin riboswitch. These latter genes are predicted to be involved in synthesizing the lower ligand base that is attached to cobyric acid. It is also notable that only excess cyanocobalamin resulted in the downregulation of a predicted cobalamin transport system. Together, these results imply that ANAS spent medium contains corrinoid forms different from cyanocobalamin and that strain 195 adjusts its metabolism according to the corrinoid forms available for uptake.

Introduction

Dehalococcoides species are members of the *Chloroflexi* and are unique in their ability to completely dehalogenate the environmental pollutants tetrachloroethene (PCE) and trichloroethene (TCE) to ethene (Maymó-Gatell *et al.*, 1997). *Dehalococcoides* species can also dehalogenate chlorinated ethanes, benzenes, phenols, biphenyls, naphthalenes, and dioxins, and polybrominated diphenyl ethers (Maymó-Gatell *et al.*, 1999; Adrian *et al.*, 2000, 2007; Bunge *et al.*, 2003; Fennell *et al.*, 2004; He *et al.*, 2006). The dehalogenation process is referred to as reductive dehalogenation or, when coupled with growth, as halorespiration (Smidt & de Vos, 2004).

Studies have shown that *Dehalococcoides* growth is strictly dependent on the availability of exogenous corrinoids (Maymó-Gatell *et al.*, 1995, 1997; Fennell *et al.*, 1997; He *et al.*, 2007). For *Dehalococcoides ethenogenes* strain 195, corrinoids are cofactors of the key reductive dehalogenases (RDases) PceA and TceA, which are responsible for the complete dehalogenation of tetrachloroethene to ethene by this strain (Magnuson *et al.*, 1998). Although strictly dependent on corrinoids, strain 195 does not encode a system for *de novo* corrinoid biosynthesis (Seshadri *et al.*, 2005). Instead, strain 195 has predicted duplicated systems for cobalamin transport and uptake (DET0650–0652/DET0684–0686) and for synthesizing the lower ligand base that is attached to cobyric acid (DET0657–0660/DET0691–0694) (Seshadri *et al.*, 2005). The latter system is hypothesized to be important for salvaging and activating cobalamin precursors scavenged from the environment (Seshadri *et al.*, 2005).

Rapid advances have been made toward understanding how corrinoids regulate gene expression in *Bacteria*, most

notably by interacting with cobalamin-binding riboswitches (Winkler & Breaker, 2005). Riboswitches are RNA elements located in the 5′-untranslated regions of transcripts that act to control gene expression after transcription initiation (Winkler & Breaker, 2005). Cobalamin riboswitches are typically composed of two parts, an aptamer domain that binds cobalamin and an expression platform domain that translates cobalamin binding into a conformational change of the transcript. The conformational change typically results in the formation of secondary structures that either prematurely terminate transcription or inhibit translation by blocking the ribosomal-binding site (Winkler & Breaker, 2005). The genes controlled by cobalamin riboswitches are often involved in cobalamin metabolism and transport, but can also regulate genes involved in cobalt transport, cobalamin-independent ribonucleotide reductases, and glutamate and succinate fermentation (Rodionov *et al.*, 2003; Vitreschak *et al.*, 2003; Nahvi *et al.*, 2004; Winkler & Breaker, 2005).

To improve our physiological understanding of corrinoids in *Dehalococcoides* species, we first used Rfam (Griffiths-Jones *et al.*, 2003) to identify putative cobalamin-binding riboswitches in *Dehalococcoides* genomes. We then applied whole-genome microarrays to characterize the transcriptome of strain 195 during growth with excess or limiting concentrations of the corrinoid cyanocobalamin. Finally, because methanogenic *Archaea* are known to produce exceptionally large amounts of corrinoids (Mazumder *et al.*, 1987; DiMarco *et al.*, 1990; Ryzhkova, 2002), we examined whether the addition of cell-free spent medium from a methanogenic *Dehalococcoides*-containing enrichment culture could induce differential expression of corrinoid-related genes.

Materials and methods

Bacterial strain and growth conditions

Dehalococcoides ethenogenes strain 195 was grown in 100-mL batch cultures with a defined mineral salts medium (Dhc medium) as described elsewhere (Lee *et al.*, 2006; He *et al.*, 2007). Liquid trichloroethene was supplied as the terminal electron acceptor and corrinoid was supplied as cyanocobalamin. All experimental cultures were inoculated with 1 mL of a stock culture that contained 1 μg L^{-1} cyanocobalamin and that had completely dehalogenated one 7-μL dose of liquid trichloroethene to vinyl chloride (VC) and ethene. The masses of chlorinated ethenes were monitored by GC (Lee *et al.*, 2006), and cell densities of strain 195 were quantified by quantitative PCR (qPCR) using protocols described elsewhere (Holmes *et al.*, 2006; Johnson *et al.*, 2008).

Validation of corrinoid-limited dehalogenation activity

Six parallel bottles containing Dhc medium were inoculated with strain 195 and amended with two sequential 7-μL doses of trichloroethene (each of *c.* 74 μmol trichloroethene). After the rates of trichloroethene dehalogenation slowed (occurring after the dehalogenation of *c.* 130 μmol), three bottles were amended with additional cyanocobalamin to a concentration of 100 μg L^{-1}. The other three bottles were maintained with 1 μg L^{-1} cyanocobalamin to serve as controls. Chlorinated ethenes were monitored by GC (Lee *et al.*, 2006), and cell densities were quantified by qPCR (Holmes *et al.*, 2006; Johnson *et al.*, 2008) as described elsewhere.

Cyanocobalamin experiments

Seventy-two parallel bottles containing Dhc medium were inoculated with strain 195 and amended with one 3-μL dose of trichloroethene. Of these bottles, 36 contained 1 μg L^{-1} cyanocobalamin while the other 36 contained 100 μg L^{-1} cyanocobalamin. After complete conversion of trichloroethene to VC and ethene, an additional 3-μL dose of trichloroethene was added to each bottle, and the bottles were incubated for an additional 24 h to ensure that cultures were actively growing and dehalogenating. Bottles were then sacrificed and cells were collected by filtration as described elsewhere (Johnson *et al.*, 2008).

ANAS spent medium experiment

The effect of exposure to cell-free spent medium from a methanogenic trichloroethene-dehalogenating culture (designated ANAS) on global gene expression in strain 195 was also examined in this study. The ANAS culture was originally inoculated from chlorinated ethene-contaminated soil from the Alameda Naval Air Station in California; it completely dehalogenates trichloroethene to ethene, and is composed of *c.* 30% *Dehalococcoides* bacteria (Richardson *et al.*, 2002). The ANAS culture was grown in a 1.5-L (400 mL liquid volume) semi-batch reactor with a mineral salts medium (ANAS medium), with lactate as the electron donor, trichloroethene as the electron acceptor, and 1 μg L^{-1} cyanocobalamin (Richardson *et al.*, 2002; Lee *et al.*, 2006). Spent medium was periodically removed from the reactor and stored anaerobically under an N_2–CO_2 (90 : 10, v/v) headspace at room temperature. For this investigation, 400 mL of this spent medium was centrifuged (12 000 ***g***), and the supernatant was filter sterilized using hydrophilic Durapore membrane filters (47 mm diameter, 0.22 μm pore size) (Millipore, Billerica, MA) in an anaerobic chamber.

For this experiment, 30 parallel bottles containing 80 mL Dhc medium and 1 μg L^{-1} cyanocobalamin were inoculated with strain 195 and amended with one 7-μL dose of

trichloroethene. After complete conversion of trichloroethene to VC and ethene, a second 7-μL dose of trichloroethene was added to each bottle and the bottles were incubated for an additional 24 h to ensure that cultures were actively dehalogenating. Thereafter, the bottles were amended with 20 mL of spent ANAS medium or 20 mL of fresh ANAS medium to serve as controls. After amendment, the bottles were incubated for a further 24 h and GC was used to verify that trichloroethene was still present in the bottles. The cultures were then sacrificed and cells were collected by filtration as described elsewhere (Johnson *et al.*, 2008).

RNA and DNA extraction

RNA and DNA were extracted from filters using phenol-based methods described elsewhere (Johnson *et al.*, 2008). RNA was purified from contaminating DNA by DNAse I treatment using the DNA-*free* kit (Ambion, Austin, TX) according to the manufacturer's instructions. Purified RNA and DNA were stored at $-80\,^{\circ}C$ before further use.

Microarray sample preparation, hybridization, and scanning

The Affymetrix GeneChip microarray applied in this study targets $>99\%$ of the genes within the genome of strain 195 (Affymetrix, Santa Clara, CA) and has been described in detail elsewhere, including an assessment of its dynamic range (West *et al.*, 2008). Five micrograms of total RNA was used as the starting material for each microarray analysis, where each 5-μg pool was collected from multiple independent 100-mL cultures. Three replicate microarray analyses were performed for each condition. cDNA was synthesized, fragmented to sizes between 50 and 200 bases, labeled, and hybridized to arrays according to the protocols outlined in section 3 of the Affymetrix GeneChip Expression Analysis Technical Manual. Hybridized arrays were stained and washed according to standard Affymetrix protocols.

Microarray data analysis

All microarray data analyses were performed with packages available from BIOCONDUCTOR version 1.9 (http://www.bioconductor.org) (Gentleman *et al.*, 2004). Probe-set hybridization signal intensities were calculated using the function 'mas5' from the package 'affy' with global scaling to a value of 2500 (Affymetrix, 2001; Gautier *et al.*, 2004). Using this procedure, the mean and median coefficients of variation among replicate measurements of individual probe-set signal intensities were 8.4% and 5.5%, respectively. To test the hypothesis that a gene was differentially expressed between two conditions, the Benjamini and Hochberg procedure (Benjamini & Hochberg, 1995) was applied using the function 'rawp2adjp' from the package 'MULTTEST' (Gentleman *et al.*, 2004). The procedure was applied to control the false discovery rate (FDR) $<1\%$. Differentially expressed genes were further restricted by the requirement of the absolute hybridization signal intensity to be >200 for at least one condition and a fold change >2 between two conditions.

Bioinformatics

Gene annotations and orthologue detection were obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and the Department of Energy (DOE) Integrated Microbial Genomes (IMG) (http://img.jgi.doe.gov) databases. Operon predictions were obtained from the Microbes Online Database (http://www.microbesonline.org) (Alm *et al.*, 2005; Price *et al.*, 2005). Putative cobalamin riboswitches were identified using Rfam (http://www.sanger.ac.uk/Software/Rfam/) (Griffiths-Jones *et al.*, 2003). All databases (NCBI, DOE IMG, Microbes Online, and Rfam) were accessed in May 2008.

Results

Corrinoid-limited trichloroethene dehalogenation

A recent study showed that cyanocobalamin deficiency could limit the rate of trichloroethene dehalogenation by strain 195 (He *et al.*, 2007). To explore whether cyanocobalamin deficiency could also limit the total amount of trichloroethene that can be dechlorinated by these cultures, strain 195 was inoculated into a medium containing 7 μL of trichloroethene and $1\,\mu g\,L^{-1}$ of cyanocobalamin. Over the first 27 days, the quantity of 16S rRNA genes increased exponentially by *c.* 60-fold (a growth rate of $0.17\,day^{-1}$), and 74 μmol of trichloroethene was dehalogenated without an observable lag phase (Fig. 1). Between days 27 and 37, cultures continued to dehalogenate at a rapid rate but without any apparent increase in cellular density (Fig. 1). This indicates that net growth had become uncoupled from dehalogenation, similar to the behavior reported previously for strain 195 (Maymó-Gatell *et al.*, 1997; Johnson *et al.*, 2008) and for the ANAS enrichment culture (Johnson *et al.*, 2005). After day 37, substantial growth and trichloroethene dehalogenation activity were no longer observed.

To test whether cyanocobalamin was limiting the amount of trichloroethene that could be dehalogenated, extra cyanocobalamin was added to three bottles to a concentration of $100\,\mu g\,L^{-1}$ on day 85, while the other three bottles were maintained with $1\,\mu g\,L^{-1}$ cyanocobalamin as controls. An additional 7 μL of trichloroethene was then added to all six bottles. Within 30 days, the cultures receiving extra cyanocobalamin degraded 100% of the trichloroethene present in

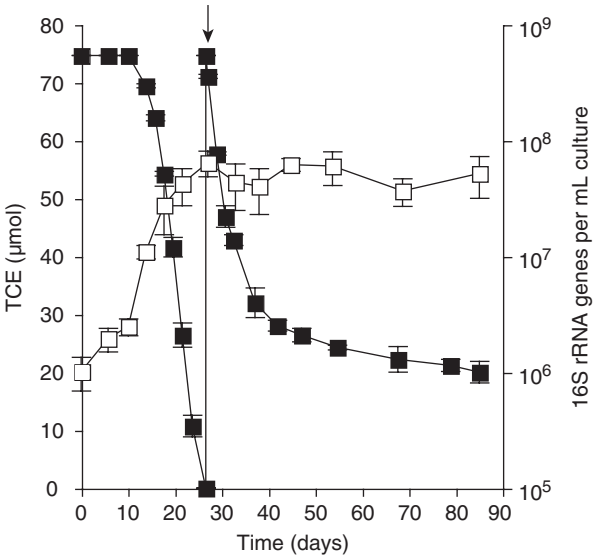

Fig. 1. The amount of trichloroethene (■) and the quantity of *Dehalococcoides*-specific 16S rRNA genes (□) in batch cultures of strain 195 grown with 1 μg L^{-1} cyanocobalamin. Three cultures were amended with two successive 74-μmol doses of trichloroethene on days 0 and 27. The vertical arrow indicates as to when the second amendment of trichloroethene was added. All measurements are averages from three biological cultures and error bars are 1 SD.

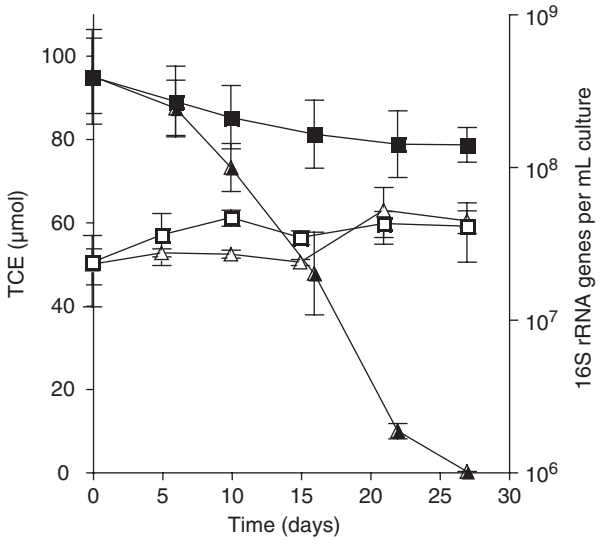

Fig. 2. The effect of corrinoid addition on trichloroethene dehalogenation activity in stationary-phase cultures of strain 195. Three cultures were amended with extra cyanocobalamin to a concentration of 100 μg L^{-1} (△, 16S rRNA genes; ▲, trichloroethene) while three additional cultures were maintained with 1 μg L^{-1} cyanocobalamin (□, 16S rRNA genes; ■, trichloroethene). The *x*-axis is the elapsed time after the addition of extra cyanocobalamin. All measurements are averages from three biological cultures and error bars are 1 SD.

the bottles while the cultures not receiving extra cyanocobalamin degraded only 17% of the trichloroethene (Fig. 2), indicating that cyanocobalamin can indeed limit the total amount of trichloroethene that can be dehalogenated. Based on these results, 100 and 1 μg L^{-1} of cyanocobalamin are referred to as excess and limiting cyanocobalamin concentrations, respectively.

Identification of putative cobalamin riboswitches

Two unique putative cobalamin-binding riboswitches were identified in the genome of strain 195 when screened using Rfam (Griffiths-Jones *et al.*, 2003) (Table 1). One riboswitch (position 122538–122730; 192 bases) is located upstream of a predicted transcript encoding two proteins; one annotated as a Zn-containing alcohol dehydrogenase (DET0125) and the other as an anthranilate phosphoribosyltransferase (TrpD) involved in aromatic amino acid biosynthesis (DET126). The second riboswitch is present in two identical copies (positions 605397–605595/636408–636606; 198 bases), each of which is located upstream of one set of duplicated and cotranscribed genes (DET0657–0660/DET0691–0694). These genes are annotated as *cobT*, *cobS*, *cobC*, and *cobU*, respectively (Seshadri *et al.*, 2005), and their products are predicted to be involved in synthesizing and attaching the lower ligand base to cobyric acid (Warren *et al.*, 2002; Escalante-Semerena, 2007).

To examine whether these riboswitches are conserved among *Dehalococcoides* isolates, the genomes of *Dehalococcoides* sp. strains BAV1 (NCBI accession number NC_009455), CBDB1 (Kube *et al.*, 2005), and VS [NZ_ABFQ00000000 (unfinished sequence)] were screened for homologous riboswitches using Rfam and downstream gene sets using BLASTN. Indeed, both homologous riboswitches and downstream gene sets are present in each of the strains (Table 1). There are, however, several differences between the strains. First, *cobC* is annotated as a phosphoglycerate mutase-encoding *phoE* homologue in strains BAV1 and VS. Second, the operon containing *cobT*, *cobS*, *cobC*, and *cobU* is duplicated in strain 195 but not in any other sequenced isolate.

Transcriptomic analysis of corrinoid-limited growth

Whole-genome microarrays were applied to exponential-phase cultures growing with excess or limiting concentrations of cyanocobalamin. In total, 20 genes were differentially regulated between the two conditions. Of these, 19 genes were downregulated (Table 2) while only one gene was upregulated by excess cyanocobalamin (Supporting Information, Table S1).

Table 1. Summary of putative cobalamin riboswitches identified in *Dehalococcoides* isolates

Strains	Genomic location of cobalamin riboswitch*	Downstream predicted transcription unit (locus tags)†	Annotation of genes in transcription unit‡
195	122538–122730	DET0125	Alcohol dehydrogenase
		DET0126	TrpD
195	605397–605595/636408–636606§	DET0657/DET0691§	CobT
		DET0658/DET0692§	CobS
		DET0659/DET0693§	CobC
		DET0660/DET0694§	CobU
BAV1	251798–251990	DehaBAV1_0246	Alcohol dehydrogenase
		DehaBAV1_0245	TrpD
BAV1	614337–614530	DehaBAV1_0626	CobT
		DehaBAV1_0627	CobS
		DehaBAV1_0628	PhoE
		DehaBAV1_0629	CobU
CBDB1	137269–137461	cbdbA145	Alcohol dehydrogenase
		cbdbA146	TrpD
CBDB1	527045–527238	cbdbA641	CobT
		cbdbA642	CobS
		cbdbA643	CobC
		cbdbA644	CobU
VS	Unfinished sequence	DeVSDRAFT_1672	Alcohol dehydrogenase
		DeVSDRAFT_1673	TrpD
VS	Unfinished sequence	DeVSDRAFT_1539	CobT
		DeVSDRAFT_1540	CobS
		DeVSDRAFT_1541	PhoE
		DeVSDRAFT_1542	CobU

*Location of riboswitches was determined by Rfam (Griffiths-Jones *et al.*, 2003).

†Operon predictions were obtained from Microbes Online (Alm *et al.*, 2005; Price *et al.*, 2005).

‡Annotations were obtained from NCBI.

§This putative riboswitch and downstream genes are present in two identical copies.

Of the 19 genes that were downregulated by excess cyanocobalamin, the most highly downregulated genes were DET0125 (5.5-fold) and DET0126 (7.3-fold) (Table 2), which are cotranscribed and contain an upstream putative cobalamin riboswitch (Table 1). Also downregulated were a number of genes with annotated functions related to corrinoid metabolism and transport, including five genes located within a duplicated set of operons that encode a cobalamin ABC-type transport system and a CobD homologue (DET0650-0654/DET0684-0688) (Table 2). Another corrinoid-related gene that was downregulated was DET0936, which encodes a CobQ homologue (Table 2). In other bacteria, CobQ is responsible for amidating several side-chains of cobyric acid (Warren *et al.*, 2002). The genes annotated to construct and attach the lower ligand base to cobyric acid (DET0657–0660/DET0691–0694) were not downregulated by excess cyanocobalamin, even though these transcripts are predicted to have an upstream cobalamin-binding riboswitch (Table 1).

The other genes that were downregulated by excess cyanocobalamin include many that encode proteins of unknown functions (Table 2). One notable example is a TetR-type response regulator (DET1580). In a previous study, DET1580 was shown to be growth-phase regulated (Johnson *et al.*, 2008). Thus, its downregulation may reflect minor differences in growth rates between the two cyanocobalamin conditions.

Transcriptomic analysis of cultures exposed to ANAS spent medium

To test whether spent medium from the methanogenic ANAS culture could elicit a response from corrinoid-related genes, cultures of strain 195 were exposed to ANAS spent medium for 24 h and the transcriptome was analyzed. In total, 119 genes were differentially expressed, with 16 downregulated (Table 3) and 103 upregulated (Table S1).

As observed with excess cyanocobalamin, DET0125 and DET0126 were among the most highly downregulated genes after exposure to ANAS spent medium (2.6- and 11.6-fold, respectively) (Table 3). In contrast to excess cyanocobalamin, three of the four genes encoding the predicted system for constructing and attaching the lower ligand base to cobyric acid (DET0657–0659/DET0691–0693) were also downregulated by ANAS spent medium (2.4–5.8-fold) (Table 3). Moreover, the genes encoding the cobalamin ABC-

Table 2. Summary of genes that were significantly downregulated by excess cyanocobalamin (FDR < 1%, > 2-fold)

Locus tag*	Fold downregulation by excess cyanocobalamin (100 μg L^{-1})	Annotation*	Predicted operon unit	Detected riboswitch
DET0014	2.2	Hypothetical protein	DET0014	N
DET0125	5.5	Alcohol dehydrogenase, zinc-containing	DET0125–0126	Y
DET0126	7.3	Anthranilate phosphoribosyltransferase (TrpD)	DET0125–0126	Y
DET0297	2.9	Conserved hypothetical protein	DET0297	N
DET0298	5.0	Hypothetical protein	DET0298	N
DET0650/DET0684	2.3	ABC-type cobalamin Fe^{3+}-siderophores transport systems, periplasmic-binding protein	DET0650–0655/DET0684–0689	N
DET0651/DET0685	2.8	ABC-type cobalamin Fe^{3+}-siderophores transport systems, permease component	DET0650–0655/DET0684–0689	N
DET0652/DET0686	2.2	ABC-type cobalamin Fe^{3+}-siderophores transport system, ATP-binding protein	DET0650–0655/DET0684–0689	N
DET0653/DET0687	2.6	Conserved hypothetical protein	DET0650–0655/DET0684–0689	N
DET0654/DET0688	2.6	Cobalamin biosynthesis protein (CobD)	DET0650–0655/DET0684–0689	N
DET0936	2.7	Cobyric acid synthase (CobQ)	DET0936–0937	N
DET1049	3.3	HD domain protein	DET1049	N
DET1186	2.8	Conserved domain protein	DET1186	N
DET1303	2.7	Fasciclin domain protein	DET1303–1304	N
DET1307	4.8	Conserved hypothetical protein	DET1307	N
DET1324	2.5	Conserved hypothetical protein	DET1324	N
DET1379	2.3	Auxin-responsive GH3 protein homolog, putative	DET1378–1379	N
DET1515	2.0	Conserved hypothetical protein	DET1515	N
DET1580	2.0	Transcription regulator, TetR family	DET1580	N

*Annotations and locus tags were obtained from Seshadri *et al*. (2005).

type transport system and the cotranscribed CobD homologue (DET0650–0654/DET0684–0688) were not downregulated by ANAS spent medium, nor was the gene encoding the CobQ (DET0936) homologue.

The genes that were upregulated by ANAS spent medium are not colocalized within operons or within metabolic pathways to any significant extent (Table S1). Of the 103 genes that were upregulated, nearly 50% were assigned to the poorly classified clusters of orthologous group categories (R, S) or were unclassified. One particularly interesting upregulated gene was DET1138, because it encodes a second CobD homologue (Table S1). This second CobD homologue is divergent from the CobD homologue (DET0654/DET0688) that was downregulated by excess cyanocobalamin (Table 2).

Discussion

The primary objective of this study was to identify genes whose expression levels respond to corrinoid availability in strain 195. The most striking finding was that DET0125–0126 were among the most highly downregulated genes when cells were grown with excess cyanocobalamin or exposed to ANAS spent medium (Tables 2 and 3). The annotations of DET0125–0126, however, are confusing. Although these genes have an upstream putative cobalamin-binding riboswitch (Table 1), neither gene encodes functions with obvious roles in corrinoid metabolism or transport (Seshadri *et al*., 2005). DET0125 encodes a member of a Zn-containing alcohol dehydrogenase family but has no close relationship to genes with known functions. DET0126 is annotated as an anthranilate phosphoribosyltransferase (TrpD), which is involved in aromatic amino acid synthesis (Seshadri *et al*., 2005). Although confusing, these genes and their associated putative riboswitch are highly conserved among *Dehalococcoides* isolates (Table 1), suggesting that they are biologically important.

Further analysis of DET0126 suggests that its product likely performs a function different from TrpD. First, strain 195 contains a second divergent copy of *trpD* (DET1483) that is part of a putative eight-gene operon containing other *trp* genes (Seshadri *et al*., 2005), none of which were differentially regulated under any of the conditions tested in this study. Second, DET1483 shows close homology with proteins from other members of the *Chloroflexi*, while DET0126 does not. Instead, DET0126 shows close homology with proteins from *Desulfotomaculum reducens* of the *Firmicutes*. Finally, analyzing DET0126 with the Orthologue Neighborhood Viewer (http://img.jgi.doe.gov) indicates that nearly all homologues have an upstream gene predicted to encode an alcohol dehydrogenase. Additional experiments are now needed to elucidate the functions of

Table 3. Summary of genes that were significantly downregulated by ANAS spent medium (FDR < 1%, > 2-fold)

Locus tag*	Fold downregulation by exposure to ANAS spent medium	Annotation*	Predicted operon unit	Detected riboswitch
DET0125	2.6	Alcohol dehydrogenase, zinc-containing	DET0125–0126	Y
DET0126	11.6	Anthranilate phosphoribosyltransferase (TrpD)	DET0125–0126	Y
DET0467	2.2	3-Dehydroquinate synthase	DET0460–0468	N
DET0468	2.6	Phospho-2-dehydro-3-deoxyheptonate aldolase	DET0460–0468	N
DET0656	4.9	Hypothetical protein	DET0656	N
DET0657/ DET0691	5.8	Nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase (CobT)	DET0657–0660/ DET0691–0694	Y
DET0658/ DET0692	2.5	Cobalamin-5-phosphate synthase (CobS)	DET0657–0660/ DET0691–0694	Y
DET0659/ DET0693	2.4	α-Ribazole-5-phosphate phosphatase (CobC)	DET0657–0660/ DET0691–0694	Y
DET0748	2.1	Hypothetical protein	DET0748	N
DET0908	2.4	Arsenical pump membrane protein, putative	DET0908	N
DET0909	2.5	Membrane protein, putative	DET0909	N
DET1078	2.4	Tail tape measure protein, TP901 family	DET1077–1094	N
DET1088	2.0	Terminase, large subunit, putative	DET1077–1094	N
DET1094	2.0	HNH endonuclease domain protein	DET1077–1094	N
DET1125	2.4	Ammonium transporter	DET1122–1125	N
DET1296	2.0	Conserved hypothetical protein	DET1296	N

*Annotations and locus tags were obtained from Seshadri *et al.* (2005).

DET0125–0126 and to examine whether they have a role in corrinoid metabolism.

The other set of genes that contains an upstream putative cobalamin riboswitch [DET0657–0660/DET0691–0694 (*cobT*, *cobS*, *cobC*, and *cobU*)] does have a clear function related to corrinoid metabolism. The products of these genes are predicted to construct the lower ligand base that is attached to cobyric acid (Warren *et al.*, 2002; Escalante-Semerena, 2007). Typically, this is 5,6-dimethylbenzimidazole in bacteria (including cyanocobalamin) and 5-hydroxybenzimidazole in methanogenic *Archaea* (Daas *et al.*, 1995; Escalante-Semerena, 2007). When cells were grown with excess or limiting cyanocobalamin, all four of these genes were expressed at stable (< 2-fold) but very high levels, having hybridization intensities among the top 7% within the entire nonredundant protein-coding genome (Table S2). The high expression of these genes independent of the cyanocobalamin concentration suggests two possibilities for their functional role. First, *cobT*, *cobS*, *cobC*, and *cobU* might be used to modify the lower 5,6-dimethylbenzimidazole ligand present in cyanocobalamin. Alternatively, these genes might function in the reverse direction to cleave the lower ligand base from cyanocobalamin. In contrast with cyanocobalamin, exposure to ANAS spent medium resulted in the downregulation of *cobT*, *cobS*, and *cobC* (Table 3). *cobU* was also downregulated (1.8-fold), but did not meet our stringent criteria for differential expression (FDR < 1%; > 2-fold) (Table S2). Although their exact function in strain 195 is unclear, the differential expression of *cobT*, *cobS*, *cobC*, and *cobU* by ANAS spent medium but not by cyanocobalamin, along with the detection of an upstream putative cobalamin riboswitch, provides preliminary evidence that the corrinoids taken up from ANAS spent medium likely have different lower ligand bases than 5,6-dimethylbenzimidazole.

Inconsistent expression profiles were also observed for the cobalamin ABC-type transport system (DET0650–0652/DET0684–0686). This transport system is cotranscribed with a *cobD* homologue (DET0654/DET0688) that also shows homology with *cbiB*. In many *Bacteria*, CobD and CbiB are involved in constructing the aminopropanol linkage between cobyric acid and the lower ligand base (Warren *et al.*, 2002). Growth with excess cyanocobalamin effectively repressed the ABC transport system and the cotranscribed *cobD* (Table 2). The coregulation of these genes, therefore, suggests a coupling of corrinoid transport with lower ligand base attachment. In contrast, spent medium from the ANAS community did not modulate the expression of any of these genes. Instead, a separate and divergent *cobD* homologue (DET1138) was upregulated by the ANAS spent medium (Table S1). This provides further support that corrinoids in ANAS spent medium likely have different lower ligand bases than in cyanocobalamin.

Finally, a *cobQ* gene (DET0936) was downregulated by excess cyanocobalamin but not by ANAS spent medium (Tables 2 and 3). In many *Bacteria*, CobQ is involved in amidating several side-chains of cobyric acid (Warren *et al.*, 2002). Most *Bacteria* and methanogenic *Archaea* use

cobalamin forms that have identical side chain amidations (Warren *et al*., 2002; Escalante-Semerena, 2007). Thus, it is unclear why DET0936 was downregulated by cyanocobalamin but not by ANAS spent medium. One possibility is that corrinoids are present in ANAS spent medium that have different side-chain amidations than cyanocobalamin.

Together, the results presented here indicate that corrinoid-related genes respond differently to excess cyanocobalamin and to ANAS spent medium. This suggests that ANAS spent medium contains corrinoid forms different from cyanocobalamin and that strain 195 adjusts its metabolism according to the corrinoid forms available for uptake. It is noteworthy that only corrinoid-related processes were clearly identified as being differentially regulated in strain 195 after exposure to ANAS spent medium. No other metabolic systems were clearly identified (Table 3 and Table S1), suggesting that corrinoid transfer is likely among the key interspecies interactions controlling the behavior of strain 195 within complex communities.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Genes significantly upregulated by excess cyanocobalamin or ANAS spent medium (FDR $< 1\%$, > 2-fold).
Table S2. Hybridization signal intensities of all genes.

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