

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY)		2. REPORT TYPE Technical Report		3. DATES COVERED (From - To) -	
4. TITLE AND SUBTITLE Insights gained from the “Dehalococcoides ethenogenes” strain 195’s transcriptome responding to a wide range of respiration rates and substrate types.			5a. CONTRACT NUMBER W911NF-07-1-0249		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 611102		
6. AUTHORS Cresten Mansfeldt, Annette Rowe, Gretchen Heavner, Stephen Zinder, Ruth Richardson			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Cornell University Office of Sponsored Programs Cornell University Ithaca, NY 14853 -2801				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211				10. SPONSOR/MONITOR'S ACRONYM(S) ARO	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) 52345-LS.5	
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT Bacteria of the group “Dehalococcoides” display the ability to respire recalcitrant chlorinated organic compounds. “Dehalococcoides” strains’ respiratory pathways and function of most genome-encoded enzymes responsible for dechlorination, reductive dehalogenases (RDases), remain incompletely annotated. To further the description of the biological organization of “Dehalococcoides”, this study monitored the transcriptomic response of “Dehalococcoides					
15. SUBJECT TERMS Dehalococcoides; reductive dechlorination; dehalorespiration, PCE; TCE; DCE; chlorophenol; microarrays; transcriptional regulation; transcriptome; continuous feed cultures, batch fed cultures, pseudo-steady state transcript levels, and reductive					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Ruth Richardson
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 607-255-3233

Report Title

Insights gained from the “Dehalococcoides ethenogenes” strain 195’s transcriptome responding to a wide range of respiration rates and substrate types.

ABSTRACT

Bacteria of the group “Dehalococcoides” display the ability to respire recalcitrant chlorinated organic compounds. “Dehalococcoides” strains’ respiratory pathways and function of most genome-encoded enzymes responsible for dechlorination, reductive dehalogenases (RDases), remain incompletely annotated. To further the description of the biological organization of “Dehalococcoides”, this study monitored the transcriptomic response of “Dehalococcoides ethenogenes” strain 195 using two-color microarrays. This study analyzed the transcriptome of 23 varied continuous feed (or pseudo-steady state (PSS)) conditions and two distinct batch fed conditions. The continuous feed experiments were comprised of 57 cultures with varying electron acceptor feed rates (0-504 $\mu\text{eq}/(\text{L}\cdot\text{hr})$), electron acceptor types (tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), 2,3-dichlorophenol (DCP), and no electron acceptor), electron donor to acceptor ratios (0.7 to 17 on an electron equivalence (eeq) basis), and electron donor type (butyrate, lactate, yeast extract, fermented yeast, pure hydrogen, or endogenous biomass decay). When similarly respiring ($\sim 120 \mu\text{eq PCE}/(\text{L}\cdot\text{hr})$) batch and PSS cultures were contrasted, the RDases DET1545 and DET0180 were up-regulated in the PSS cultures indicating activity at lower overall electron acceptor concentration. For all continuous fed chloroethene cultures, members of the RDase family and electron transport chain displayed unique clusters of transcripts responding either positively, negatively, or indifferently to the respiration rate. An RDase within the indifferent group, DET1171, was highly (31 ± 15 fold) up regulated in response to DCP being fed as the electron acceptor. DET1171 could potentially play a role with DET1559 (27 ± 4.1 fold up-regulated during DCP growth) and DET0318 pceA in the dechlorination of chlorophenols.

1 Title:
2 Insights gained from the “*Dehalococcoides ethenogenes*” strain 195’s transcriptome responding
3 to a wide range of respiration rates and substrate types.
4 Authors:
5 Cresten Mansfeldt, Annette Rowe, Gretchen Heavner, Stephen Zinder and Ruth Richardson.
6
7
8
9 Keywords: *Dehalococcoides*; reductive dechlorination; dehalorespiration, PCE; TCE; DCE;
10 chlorophenol; microarrays; transcriptional regulation; transcriptome; continuous feed cultures,
11 batch fed cultures, pseudo-steady state transcript levels, and reductive dehalogenases.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

1 **Abstract:**

2 Bacteria of the group “*Dehalococcoides*” display the ability to respire recalcitrant chlorinated
3 organic compounds. “*Dehalococcoides*” strains’ respiratory pathways and function of most
4 genome-encoded enzymes responsible for dechlorination, reductive dehalogenases (RDases),
5 remain incompletely annotated. To further the description of the biological organization of
6 “*Dehalococcoides*”, this study monitored the transcriptomic response of “*Dehalococcoides*
7 *ethenogenes*” strain 195 using two-color microarrays. This study analyzed the transcriptome of 23
8 varied continuous feed (or pseudo-steady state (PSS)) conditions and two distinct batch fed
9 conditions. The continuous feed experiments were comprised of 57 cultures with varying electron
10 acceptor feed rates (0-504 $\mu\text{eq}/(\text{L}\cdot\text{hr})$), electron acceptor types (tetrachloroethene (PCE),
11 trichloroethene (TCE), dichloroethene (DCE), 2,3-dichlorophenol (DCP), and no electron
12 acceptor), electron donor to acceptor ratios (0.7 to 17 on an electron equivalence (eeq) basis), and
13 electron donor type (butyrate, lactate, yeast extract, fermented yeast, pure hydrogen, or
14 endogenous biomass decay). When similarly respiring ($\sim 120 \mu\text{eq PCE}/(\text{L}\cdot\text{hr})$) batch and PSS
15 cultures were contrasted, the RDases DET1545 and DET0180 were up-regulated in the PSS
16 cultures indicating activity at lower overall electron acceptor concentration. For all continuous-
17 fed chloroethene cultures, members of the RDase family and electron transport chain displayed
18 unique clusters of transcripts responding either positively, negatively, or indifferently to the
19 respiration rate. An RDase within the indifferent group, DET1171, was highly (31 ± 15 fold) up-
20 regulated in response to DCP being fed as the electron acceptor. DET1171 could potentially play
21 a role with DET1559 (27 ± 4.1 fold up-regulated during DCP growth) and DET0318 *pceA* in the
22 dechlorination of chlorophenols.

23

24

25

26

1 **Introduction:**

2 Recalcitrant halogenated organics contaminate a majority of active hazardous waste cleanup sites
3 (55). Site managers and engineers have turned toward utilizing populations of the bacteria
4 “*Dehalococcoides*” (DHC) in bioremediation efforts (47). DHC strains are capable of
5 anaerobically reductively dehalogenating halogenated compounds including chlorinated-ethenes
6 (48,50,54), -ethanes (18,25,54), -phenols (2,21), -benzenes (1,3,21), -dioxins (11,13,22), -
7 dibenzodioxins (21), -dibenzofurans (46), -naphthalene (21), and -biphenyls (8,21,80) allowing
8 for flexibility in the bioremediation application of this bacteria. Additionally, DHC is often found
9 and active at chlorinated organic contaminated sites (7,8,10,12,14,24,31,35,44,81-82) and need
10 only to be biostimulated to begin remediating the chlorinated organic compounds. Several case
11 studies focusing on bioaugmentation of DHC at PCE and/or TCE contaminated sites have also
12 showed success (19,45,52,67).

13 The first strain of DHC identified, “*Dehalococcoides ethenogenes* strain 195” (DET), was
14 isolated in 1997 (53) and its genome sequenced, assembled, and annotated in 2005 (69). Genomes
15 of additional DHC members, such as CBDB1 (42,74), VS (58,75,49), GT (71), and BAV1
16 (27,40,49), have been sequenced and annotated since, with other members being isolated and/or
17 characterized (e.g. strain FL2(28), KB1(17) metagenome available through the Joint Genome
18 Institute). However, much of the biological organization of DHC remains unknown. DHC is an
19 obligate hydrogenotroph and dehalorespirer (32). The identified (69) putative enzymes and
20 regulatory elements comprising the electron transport chain include a unique ensemble of
21 reductive dehalogenases (RDases), which reduce halogenated organics; hydrogenases, which
22 oxidize DHC’s sole electron donor (hydrogen); and other oxidoreductases of unknown or
23 misannotated function (e.g. Fdh, format dehydrogenase; Nuo, NADH ubiquinone oxidoreductase;
24 Mod, molybdopterin containing oxidoreductase). Every DHC strain has a unique subset of over
25 90 RDases (41) likely imparting capabilities for dehalogenating different chlorinated substrates in
26 unique patterns (33,34,51,79). Of DET’s seventeen functional RDases, DET0079 (TceA),

1 DET0318 (PceA), DET1559, and DET1545 are the most investigated across multiple
2 experiments within a mixed community culture containing DET as the sole dehalogenator (Donna
3 II) (23,36,37,43,51,60-62,75). DET's NiFe hydrogenase Hup is the only hydrogenase predicted to
4 face the periplasm (56,69,73). Although RDases, Hup, and other membrane bound
5 oxidoreductase proteins have been documented in several DHC studies (57,77), the complete
6 organization and interaction between these respiration elements is not yet fully understood (59).
7 Observations of broad expression patterns across many experiments generate hypotheses about
8 which transcripts are working in concert to fulfill specific biological functions.

9 To broaden the understanding of the biological organization of "*Dehalococcoides*", this study
10 employed microarray technology to monitor the transcriptomic response of "*Dehalococcoides*
11 *ethenogenes* strain 195" in a mixed community culture to 19 varied experimental continuous-feed
12 or pseudo steady state (PSS) conditions (a total of 57 total cultures are monitored considering
13 biological replicates). In these studies, PSS conditions refer to a steady mRNA transcript level
14 and not biomass. Previous transcriptomic studies performed on DHC include characterizing
15 unknown species or cultures (16,78) and monitoring both pure and mixed well defined cultures
16 (37,76). The microarray studies investigating the entire transcriptome under pure-culture and
17 batch fed conditions with trichloroethene (TCE) and hydrogen fed (as the electron acceptor and
18 electron donor respectively) (37) revealed key insights into the organism's time-dependent
19 response, carbon metabolism (72), corrinoid response (38), and predicted nitrogen fixation
20 pathway (43).

21 In order to extend the existing microarray datasets and more closely simulate field conditions this
22 study analyzed the transcriptome of cultures grown in conditions with varying electron acceptor
23 feed rates (from 0-504 $\mu\text{eq}/(\text{L}\cdot\text{hr})$), electron acceptor types (between tetrachloroethene (PCE),
24 trichloroethene (TCE), dichloroethene (DCE), 2,3-dichlorophenol (DCP), and no electron
25 acceptor), electron donor to acceptor ratios (from 0.7 to 17 on an electron equivalence basis), and
26 electron donor type (between butyrate, lactate, yeast extract, fermented yeast, pure hydrogen, or

1 endogenous biomass decay only). By continuously feeding substrates over the course of days, a
2 near constant (PSS) pool of mRNA was established. A large enough dataset potentially provides
3 the association of specific genes' perturbations to gradations in experimental conditions rather
4 than responses confounded by non-steady conditions or batch growth. By monitoring the
5 transcriptome, the genome-wide response can be described. Additionally, the growth of DET in a
6 non-pure culture setting allows a fuller insight into the maintained mixed community cultures
7 (Cornell Donna II (53), Pinellas (26), Victoria (30), KB-1 (18) and ANAS cultures (64)) and a
8 better representation of field conditions in which DET will compete with methanogens for
9 hydrogen produced from organisms that ferment organic substrates, when provided (20,65,70).

10 **Materials and Methods:**

11 *Culture Growth Conditions*

12 All subcultures studied in this experiment were 100 mL samples from a maintained 6L mixed
13 community culture (Donna II) previously described (20,60-62,65) The culture contains a DET
14 population (near 5×10^8 cells/mL; 60% of total population on per cell basis) mixed with
15 methanogens and organisms that ferment organic substrates.

16 *Continuous Feed or Pseudo-Steady State Setup (PSS):*

17 The continuous feed or pseudo-steady state (PSS) culture setup has been previously described
18 (60). In brief, 160 mL glass serum bottles were filled with 100 mL of 3-day starved mixed
19 community culture sampled from the main reactor (Donna II). Varying combination of Pressure
20 Lok syringe volumes (10 mL, 5 mL, 2.5 mL, 500 μ L, and 100 μ L; VICI Precision Sampling) and
21 concentrations of electron acceptor and donor in media were loaded on a Cole Palmer 74900
22 Syringe Pump to deliver an expected continuous rate.

23 *Gas Chromatography Monitoring*

24 Chlorinated-ethenes, ethene, and methane were monitored utilizing a gas chromatography method
25 previously described (62). Chlorinated phenols were monitored utilizing a modified method
26 previously outlined (39). The chlorinated phenol samples were comprised of 1 mL liquid samples

1 withdrawn from the subcultures and frozen. The thawed samples were transferred to a 1 mL glass
2 vial, 100 μ L of hexane was added to the sample, and the vial was shaken at 300 rpm for six hours.
3 The FID gas chromatography method selected has an injection temperature of 200° C, a constant
4 column temperature of 160° C, and the FID detector temperature of 200° C on a Supelcowax 10
5 column (30 m $\times$ 0.53 mm with 1.00 μ m film, Supelco). The gas mixture was 200 kPa nitrogen as
6 the carrying gas, 200 kPa air, and 250 kPa hydrogen. 5 μ L of the sample in hexane was injected.
7 Chemical standard curves were made in both hexane and media from pure chlorinated phenols
8 (ACROS Organics).

9 *Calculation of respiration rates*

10 Respiration is considered to occur for the higher chlorinated ethenes to VC or ethene (60) with 6,
11 4, and 2 electron equivalents (eeqs) for PCE, TCE, and DCE respectively with the specific
12 calculation previously published (61). Extending this analysis to the dichlorophenol DCP
13 experiments, monochlorophenol (MCP) is the terminal product (21). Therefore, DET will receive
14 2 eeqs per mol DCP. The respiration rate equation for DCP, r_{DCP} , in μ eeq/L/hr is:

$$r_{DCP} = 2 \frac{d(MCP)}{dt}$$

15 *Microarray Design:*

16 The microarray designed for this experiment was an Agilent Technologies© two-color, 15k, 60
17 mer, 8 plex array. The specific designs of the probes utilized a modified method provided by the
18 eArray © software suite (5). The first probe set utilized for the batch versus PSS experiment
19 employed a base-composition (BC) technique for designing and scoring the best probe for each
20 transcript (5). The second probe set redesigned for the remainder of the experiments was
21 expanded to include community members, non-protein encoding RNA transcripts (rRNAs,
22 tRNAs), and a luciferase control. A modified temperature matching (T_m) method developed the
23 probe set for redesigned array. The T_m method searched for an optimal design with a consistent
24 melting point temperature (80° C) while sacrificing the overall quality of the individual probe (5).

1 However, if a BC score of 3,4, or poor was reported for a transcript, multiple probes around the
2 melting temperature of 80° C were designed. The probe with the best base composition score
3 nearest to the 80° C temperature was selected. The designs were searched using the Basic Local
4 Alignment Search Tool (BLAST) (15) against both the National Center for Biotechnology
5 Information (NCBI) nucleotide collection and the assembled mixed community metagenome
6 (metagenome data not reported, currently being compiled by the Joint Genome Institute) to
7 confirm the specificity of all probe sets. Both microarray platform designs are uploaded and
8 freely available at the NCBI Gene Expression Omnibus (GEO) database
9 (<http://www.ncbi.nlm.nih.gov/geo/>).

10 *RNA-cDNA Handling for Microarray Monitoring*

11 50 mL of liquid culture samples were centrifuged-at 14190×g. The centrifuged sample was split
12 into 8 individual RNA extractions with each sample following the RNeasy© Mini Kit (Qiagen)
13 extraction previously outlined (62). The 8 distinct RNA extractions were recombined on the spin
14 filter before the first RW1 buffer wash. The Superscript I © DNase RNA cleanup, amino-allyl
15 cDNA formation, cDNA cleanup, and cDNA labeling with Cy3 or Cy5 followed the method
16 outlined (76). The quality and quantity of the RNA was determined using the RNA 6000 Nano
17 assay on an Agilent 2100 bioanalyzer (Agilent Technologies). The quantity of resulting cDNA
18 was determined by using the Quant-IT™ OliGreen® ssDNA Assay Kit (Invitrogen). A common
19 control RNA pool sampled from the main Donna II reactor after 3 days of starvation was labeled
20 with Cy3.

21 *Microarray Hybridization and Scanning:*

22 For each experiment, Cy5 labeled cDNA from the mixed community mRNA pool was hybridized
23 against an aliquot of common control of Cy3 labeled cDNA from 3-day starved culture. The
24 hybridization, washing, and scanning of the microarray samples was performed by the Cornell
25 University Microarray Core Facility (<http://cores.lifesciences.cornell.edu/brcinfo/>) and followed
26 the methods outlined by the manufacturer (5). The general procedure mixed 25 µl (~400 ng) of

1 the labeled cDNA sample with 25 μ l 2x Gene Expression (GEx) Hybridization Buffer HI-RPM
2 (5), hybridized the sample to the microarray slide at 65° C for 17 hours, washed with GEx Wash
3 Buffer 1 and 2 (5) at room and elevated (37° C) temperatures, and scanned with an Agilent
4 Technologies Scanner G2505C with a 5 μ m resolution.

5 *Statistical Treatment of the Data Set:*

6 Microarray image analysis was conducted using Agilent Feature Extraction 10.5 Image Analysis
7 Software. The Feature Extraction Software was also utilized to perform a within array modified
8 LOESS normalization between the Cy5 and Cy3 signals, to calculate a log ratio between the Cy5
9 and Cy3 channels, and to calculate a modified Student t-test p-value between the Cy5 and Cy3
10 signal distributions (83). A between array normalization was not performed due to the wide
11 variety of experimental conditions analyzed, possibly violating the assumption that less than 20%
12 of transcripts are differentially regulated throughout the experimental set. The more detailed
13 treatment the Agilent Feature Extraction employed can be found in the user manual (4). Replicate
14 spots for the same probe (ranging from 6-20 spots/probe) were geometrically averaged.
15 Hierarchical clustering was performed in R 2.11.1 (<http://www.r-project.org/>). The K-Means
16 cluster, figure of merit (FOM) test, and the Cluster Affinity Search Technique were performed in
17 the Multiple Experiment Viewer (MeV 4.6) freely available from JCVI (66). The raw and
18 normalized data is freely available at the NCBI GEO database.

19 **Results:**

20 *Batch Growth Compared to the Continuous Feed Pseudo Steady State System*

21 The first set of microarrays compared the mixed community culture with similar electron
22 acceptor/donor conditions under batch and pseudo-steady state growth. The batch culture
23 dechlorinated 660 μ eqs/L of PCE to VC or ethene in 6 hours (110 μ eqs/(L-hr)) while the PSS
24 experiments received a continuous input of 53 $\pm$ 13 or 83 $\pm$ 2.8 μ eqs/L-hr of PCE or TCE
25 respectively. The main biological differences are the high initial pulse of electron acceptor and
26 donor seen by the batch cultures to the low concentrations maintained in the PSS-setup (the batch

1 culture experiences the initial pulse of 660 $\mu\text{eqs/L}$ while the PSS cultures maintain a constant
 2 level of about 12 ± 9.5 $\mu\text{eqs/L}$ of chlorinated ethenes based on the last 48 hours of GC data) and
 3 that the rate of continuous feeding in the PSS cultures was maintained for 7 days. The two PCE (1
 4 & 2) and two TCE (1 & 2) experiments are compared via a correlogram (Figure 1). The PCE (1 &
 5 2) cultures do not display a high correlation with each other due to the inadvertent variation in the
 6 feeding of electron acceptor (PCE 1 and 2 were fed 66 and 44 $\mu\text{eq/(L-hr)}$ respectively, (GC data
 7 displayed in Supplemental Image 1). To determine the transcripts differentially regulated
 8 similarly across all four arrays, only transcripts where all replicate probe spots ($n=6-20$) on the
 9 microarray exceed a fold ratio between the PSS and batch cultures of two (for up-regulated
 10 genes) or one-half (for down-regulated genes) and are below a p-value of 0.05 are considered.
 11 Overall, compared to the batch control, PCE 1, PCE 2, TCE 1, and TCE 2 displayed 193, 165,
 12 144, and 275 differentially regulated transcripts, respectively. Of these, 43 (23 up- and 20 down-
 13 regulated) are shared across all samples and displayed in Table 1. Within the 23 up-regulated
 14 transcripts, the RDase DET0180 and its anchoring protein as well as the RDase anchoring protein
 15 DET1544 display a 5.5 ± 4.11 , 5.1 ± 0.92 , and 3.5 ± 0.59 fold increase respectively. The presence of
 16 the anchoring protein DET1544 without the catalytic RDase DET1545 transcript is surprising as
 17 they are predicted to reside on the same polycistronic mRNA. All probes for the RDase DET1545
 18 pass the requirement of displaying a p-value below 0.05, but the transcript is excluded from the
 19 stringent analysis due to two probe spots (out of 20 replicates) in the PCE1 experiment failing to
 20 exceed the ratio cut off of 2. If the stringency of the analysis is relaxed to remove the log ratio
 21 cutoff, the number of transcripts shared across all experiments is increased from 43 to 65
 22 (Supplemental Table 1). DET1545 falls within this category displaying an average ratio across all
 23 experiments of 3.3 ± 0.86 . The 20 significantly down-regulated transcripts under PSS conditions in
 24 the stringent analysis are populated by many key transcripts encoding for enzymes in respiratory
 25 energy conservation. These include transcripts of the *ATPase* operon (DET0558, DET0560,
 26 DET0561, DET0562) and subunits of the hydrogenase *ech* (DET0864). Additional members of

1 the *ech* operon (DET0861 and DET0862) are found in the expanded data set (Supplemental Table
2 1).

3 *Expression profiles comparison across multiple PSS experiments:*

4 In order to explore a wide variety of environmental conditions, mRNA pools from 53
5 experiments (performed on 14 separate days) were analyzed by the microarray technique
6 described above. The parameters and conditions for each experiment are outlined in Table 2. The
7 electron acceptors were varied from none, PCE, TCE, DCE, and DCP while the electron donor
8 for the PCE experiments was varied from none, butyrate (with yeast extract (YE)), lactate (with
9 YE), hydrogen, YE, and fermented yeast extract (FYE). The feed rates of electron acceptor and
10 the ratio of electron acceptor to donor were altered as well. The respiration rates varied between 0
11 $\mu\text{eq}/(\text{L}\cdot\text{hr})$ in cultures not fed electron acceptor to 170 $\mu\text{eq}/(\text{L}\cdot\text{hr})$ in the PCE High
12 Experiments.

13 When the experiments are organized via a hierarchal Ward cluster dendrogram based on a
14 Euclidean distance matrix of the log ratio fold changes (Figure 2), two distinct groupings develop.
15 The two clusters are separated primarily by the respiration rate recorded with higher and lower
16 respiration rates clustering together. However, the High 2 from the High-Low PCE experiment
17 (HLH2_INHIB) plots with the higher respiration cluster when in fact respiration ceased prior to
18 culture sampling due to the formation of non-aqueous phase PCE. HLH1_INHIB and
19 HLH2_INHIB display a unique decay series as both cultures experienced an initial high
20 respiration rate ($\sim 100 \mu\text{eq}/(\text{L}\cdot\text{hr})$), but PCE built up above its solubility limit at day 2 inhibiting
21 PCE respiration. Additionally, the DCE “3 rate” mid-range (DCE B1 and B2) do not fall into the
22 same cluster. The remainder of the experiments and biological replicates plot as expected. The
23 hydrogen abiotic controls (H2A1 and A2) cluster with the 3 day decay experiment (DecayA1) as
24 both are decay experiments on the same time scale. Additionally, the experiments fed: PCE with
25 either no electron donor, YE, or FYE with PCE (P0FY_XX); the chlorophenol experiment (Cp);

1 the PCE low series (PLL); the decay 7-day (DecayB) replicates; the PCE high series (HiP); the
2 half butyrate series (PHB); and the majority of the “3 rate” experiments (P3, T3, and D3) cluster
3 with their respective conditions and biological replicates, supporting the biological
4 reproducibility of the overall transcriptional response.

5 *Gene clusters*

6 After examining the clustering of experiments, the clustering of genes with similar behavior was
7 explored with a K-means clustering technique. This technique was able to align all genes into
8 biologically relevant clusters (63). However, the hindrance to employing a K-means clustering
9 technique was that the number of clusters assigned must be set prior to analysis. Based on a figure
10 of merit test, the number of biological clusters was set at 20. A hierarchal dendrogram displaying
11 the relationship among the twenty clusters is displayed in Figure 3 with the specific gene list of
12 each cluster described in Supplemental Table 2. Other clustering techniques, such as the cluster
13 affinity search technique (CAST) (9), gave similar results (data not shown). The 20 clusters map
14 into four distinct superclusters. Figure 3.b-e displays the cluster’s centroid log ratio expression
15 value versus respiration rate broken into the four primary clusters. Figure 3.b displays a general
16 positive trend in response to respiration rate while Figure 3.c displays a general negative trend
17 (Figure 3.d-e displays clusters with centroids of these clusters remain nearly constant). This
18 bifurcation is important to note as the majority of high respiration rate linked RDases (*tceA*, *pceA*,
19 DET1559) and respiration chain elements (*hup*, *ATPase*) are members of the clusters plotted in
20 Figure 3.b while the low respiration linked RDases (DET0173, DET1535, DET1538, and
21 DET1545) are members of the clusters plotted in Figure 3.c.

22 Across all clusters, three centroid data points occurring around the 35 $\mu\text{eq}/(\text{L}\cdot\text{hr})$ respiration rate
23 appear to be outliers (lower in Figure 3.b and higher in Figure 3.c). These centroids are
24 noteworthy as they represent the DCE_INHIB experiments. If the final 48 hours of respiration
25 prior to sampling are considered, the respiration rate was actually around $2.4 \pm 1.8 \mu\text{eq}/(\text{L}\cdot\text{hr})$

1 which shifts all three centroids to the left in the respiration diagrams of Figure 3.b-e. Following
2 this adjustment, these points do not appear to be outliers but support the overall trend.

3 *K-Means Clusters Mapped onto DET's Genome:*

4 -Describe binning the genes based on supercluster
5 -Show that there are several regions where a significant number of genes appear in the same
6 supercluster

7 -Describe the virus region and explore other genes that are clustered with it.

8 *Average Expression Values Across all Experiments*

9 -Describes those genes that are most highly expressed
10 -Compare to Johnson et. al to see if any uniquely turned on

11 *Putative Respiration Chain Element Rate Response*

12 The fact respiration is a key and informative phenotype of DET prompted interest in plotting
13 expression patterns of transcripts versus experimental respiration rate. Focusing on the responses
14 of mRNAs encoding for known or predicted respiration chain elements could determine if there
15 are unique electron transport chain members responding to low or high respiration. Figure 4
16 displays the expression pattern clustering of 18 RDases (17 full length plus DET0162, an RDase
17 with a known point mutation (69)), 5 hydrogenases (*hym*, *hyc*, *ech*, *hup*, *vhu*), other membrane-
18 bound oxidoreductases (*mod* (potential molybdopterin oxidoreductases), *fdh* (annotated formate
19 dehydrogenase), *nuo* (NADH-ubiquinone-oxidoreductase)), and *ATPase*. In this figure a single
20 polypeptide subunit is selected to represent the full operon. However, members of the electron
21 transport chain often are encoded on multi-subunit operons. A version of Figure 4 with all
22 subunits plotted is presented in Supplemental Figure 2. The majority of subunits of the respective
23 operons cluster closely together. However, variability is seen in the *nuo*, *ech*, and *hym* transcripts.
24 In Figure 4, three groups emerge and again vary in their trend relative to respiration rate. The first
25 grouping contains the high-respiration-linked RDases with the majority of the other predicted
26 respiratory elements. As seen by Figure 4.b, these transcripts respond very sharply to increased

1 levels of respiration and then hit a plateau. This plateau was noted in previously published work
 2 by Rahm and Richardson (60,61). The low-respiration-linked RDases, DET0173, DET1535,
 3 DET1538, and DET1545, are tightly clustered in the second group and at rates above 5 $\mu\text{eq}/(\text{L}\cdot\text{hr})$
 4 display an inverse relationship to respiration as first noted in the K-means analysis (Cluster 1
 5 in Figure 3.c). The remaining RDases and the putative respiration chain elements *mod* and *ech*
 6 appear to fall within a cluster that does not respond greatly to varied levels of chlorinated ethene
 7 respiration although clustering between *modA* (DET0103) and DET0180 is observed (Figure 4.a).
 8 *Response of Respiration Elements to Switching the Electron Acceptor Type from Chlorinated*
 9 *Ethenes to 2,3-Dichlorophenol and 1,2,3,4 Tetrachlorobenzene*
 10 The electron acceptor was varied in the experimental set among PCE, TCE, DCE or 2,3-
 11 dichlorophenol (DCP). Figure 5.a displays the natural value differential expression of the
 12 individual RDases across a relatively consistent low-rate of respiration with varying the electron
 13 acceptor type (electron donor was always butyrate). The *tceA* transcript is about equally up-
 14 regulated in the DCP samples as with any of the chlorinated ethenes (PCE, TCE, DCE) (Figure
 15 4.a). Additionally, DET1171 and DET1559 are both highly up-regulated in the chlorophenols fed
 16 cultures (32 ± 16 and 27 ± 4.1 fold respectively). The distribution of the absolute intensity of
 17 RDase spots for the varied electron acceptor types (Figure 5.b) reveals that for the TCE and DCE
 18 fed cultures *tceA* dominates the percentage of the RDase transcriptional pool, for the PCE fed
 19 culture DET0162 dominates, while for the DCP fed culture *pceA* is the dominant transcript
 20 comprising above 40% of the total RDase processed fluorescence. Additionally, *pceA*'s
 21 differential expression displays a slight non-significant up-regulation for the DCP culture as
 22 compared to the chlorinated ethene fed cultures. Furthermore, DET0180 sees a decrease in the
 23 percentage of RDase intensity between DCP and TCE/DCE.
 24 Including the other respiratory chain elements in the analysis (data not shown) reveals that the
 25 majority of the transcripts are down regulated under DCP conditions. However, *nuo* (DET1571),

1 *vhu* (DET0615), and *ech* (DET0860) do not display a significant difference between the DCP fed
2 and chlorinated ethene fed cultures in terms of ratio to standard controls.

3 The analysis for DCP specific response was expanded to include all transcripts in a rank ordered
4 list based off of differential expression ratios for the DCP experimental series (Supplemental
5 Table 3). The first entry is DET1171, as previously indicated in Figure 5.a. Besides the two
6 RDases DET1171 and DET1559 reside in the top twenty of all genes by fold change, response
7 regulators and hypothetical proteins heavily populate the list. Two histidine kinase sensory boxes
8 (DET0301 and DET1560), *pyrR* (DET1198, putative transcriptional binding protein of
9 pyrimidine nucleotide biosynthesis), and *recX* (DET1607, putative regulatory protein for the
10 DNA recombinase RecA) all display an up regulation greater than ten-fold. Among the most
11 down-regulated transcripts, an over abundance of genes related to iron or cobalamin are present.
12 These genes include two ABC transporters (DET0650 (corrinoid specific) and DET1176) and a
13 biotin-Acetyl-CoA-carboxylase ligase (DET0849, also putatively described as a transcriptional
14 repressor).

15 Tetrachlorobenzene experiments (two weeks)

16 Tetrachlorobenzene arrays (one week)

17 Analysis (few days)

18 **Discussion:**

19 In the comparison of the batch fed culture to the PSS setup, the RDases DET1545 and DET0180
20 are displayed to be highly up-regulated under PSS conditions. This suggests that DET1545 and
21 DET0180 are up-regulated in response to lower electron acceptor availability conditions
22 (specifically the batch fed culture processed a 660 $\mu\text{eq}/(\text{L})$ pulse of PCE at a rate of 110 $\mu\text{eq}/(\text{L}-$
23 $\text{hr})$ while the PSS received a continuous low rate of $55 \pm 8.2 \mu\text{eq}/(\text{L}-\text{hr})$), as supported by
24 previous publications (60-62). Although neither transcript is dominant in the absolute intensity
25 sense (data not shown), the minor RDases potentially serve as key enzymes when electron
26 acceptor availability is low or, their regulatory elements are sensitive to external electron acceptor

1 concentrations. When the analysis is expanded to include putative members of the entire
2 respiration chain, it is noted that the *ech* and *ATPase* operons are both down-regulated. The
3 ATPase enzyme encodes for a proton pump utilized primarily for energy generation and the Ech
4 hydrogenase putatively encodes for a proton pump which generates low potential electrons that
5 could be used to reduce ferredoxin or possibly cobalt in RDase corrinoids to the Co I state, or for
6 other biosynthetic metabolites (69). Potentially, the batch cultures are limited by the rate of
7 hydrogen production from butyrate while the continuous-fed cultures are limited for electron
8 acceptor therefore allowing higher hydrogen levels. The higher hydrogen level in the continuous
9 feed cultures may mitigate “*Dehalococcoides*” need for reverse electron transport by Ech.

10 In previous work with the mixed community culture Donna II, qRTPCR was used to monitor
11 expression levels of selected genes (60-62). The trends from that work with respect to the
12 RDases’ relationship to respiration rate are supported here with genome wide profiles. Namely,
13 some highly expressed respiration chain elements and RDases up regulate sharply in response to
14 increased respiration rate or electron acceptor concentration before plateauing at the highest rates
15 while, in contrast, other RDases are up-regulated only at low respiration rates or electron acceptor
16 concentrations before being down-regulated at higher rates. This trend is also supported by batch
17 studies with DET where the low-respiration rate linked cluster of RDases is up-regulated upon
18 entry into stationary phase (37). This bifurcation of behavior in the RDases may indicate a unique
19 response of specializing RDases in the electron transport chain and prove useful for the
20 identification of field site biomarkers in determining the respiration state of the organisms in situ.

21 However, though the expression patterns of these RDases are remarkably repeatable, their
22 substrate range remains unknown and functional studies are needed.

23 Focusing on responses specific to electron acceptor type, previous studies comparing PCE, TCE,
24 and DCE at similar respiration rates showed shifts in relative expression of the RDases being
25 tracked by qRTPCR (e.g. *pceA* was down-regulated slightly when TCE or DCE was fed), but
26 these shifts were less than two fold (61). Shifting to a different class of chloroorganic electron

1 acceptor altogether displayed larger shifts (2,21,23). The current study, in varying the electron
2 acceptor type from chlorinated ethenes to DCP, supports the majority of reported findings (23).
3 DET1559 and *pceA* have been highlighted as two transcripts potentially involved in chlorophenol
4 dechlorination (23). These two findings are supported in the current study by the dominance of
5 *pceA* in the RDase absolute intensity distribution (Figure 5.b), and the high up-regulation of
6 DET1559 (27 ± 4.1 fold for DCP compared to 4.4 ± 1.4 fold for PCE). PceA has been shown to
7 be bifunctional, able to dechlorinate both chlorinated ethenes and phenols (2).
8 Expanding off of these findings, the current study displays that the RDase DET1171 is highly up
9 regulated in the DCP cultures (31 ± 15 fold) while not significantly up-regulated in the
10 chlorinated ethene fed cultures (Figure 5.a). In the Fung et al. 2007 study, the RDase DET1171 is
11 indicated as, but not highlighted for due to low overall expression, displaying significant up
12 regulation. When DET1171's amino-acid sequence was searched by BLAST against the NCBI
13 protein database, ortholog RDases appear in the CBDB1, BAV1, and VS strain (95%, 95%, and
14 97% amino acid identity respectively) as previously indicated (49). Additionally, weaker hits
15 match with the ortho-chlorophenol RDases in the *Desulfitobacterium* genus (39% amino acid
16 identity) with seven out of the top twenty hits described as a chlorophenols-related RDase.
17 However, the closest hits outside of the "*Dehalococcoides*" group are genes from species of
18 *Photobacterium* and *Shewanella*, which may be non-energy conserving RDases involved in the
19 breakdown of a chlororganics. Therefore, the correlation of DET1171 with DCP dechlorination
20 may be the result of shared transcriptional regulation rather than actual functional dechlorination
21 by the final enzyme. To fully explore the function and behavior of DET1171 and the other RDase
22 family members, the dataset which is primarily comprised of chlorinated ethene fed cultures
23 should be expanded to explore more perturbations in chlorophenols, chlorobenzene,
24 chlorodibenzodioxins, and other chlorinated substances.

25 **Acknowledgements:**

1 We would like to thank Laura Hug (University of Toronto) for her valuable input and
2 collaboration in the design of the microarrays, Jim Gossett for his advice in the experimental
3 setup, Heather Fullerton (Cornell University) for her knowledge of DET, and the Cornell
4 University Life Sciences Core Facilities Center for their help in developing and running the
5 microarrays. Additionally, we would like to thank our two funding sources, the Department of
6 Defense Army Research Office and the National Science Foundation Environmental Engineering
7 Program.

8

9

10

11 Figure 1. Comparison of the microarray results for the Batch and PSS fed cultures. The
12 correlogram displays the similarity of experimental replicates TCE 1&2 and PCE 1 & 2 by
13 correlating the gene expression across experiments for all transcripts compared to batch
14 conditions.

15

16 Table 1. The 43 genes that are similarly regulated amongst all four Batch vs. PSS samples with
17 the transcript name, regulation (up or down when comparing PSS to batch level), average natural
18 value ratio recorded, and the standard deviation of that ratio across all 4 arrays (40 spots on 4
19 arrays).

20

21 Table 2. Experimental conditions for the continuous-feed (PSS) investigations. The experimental
22 title provides a brief description of the overall experiment where the culture title designates the
23 electron acceptor (PCE, TCE, DCE, DCP, or none (-)) and electron donor source (butyrate,
24 lactate, hydrogen, yeast extract (YE), fermented yeast extract (FYE), hydrogen, or none (-)) type.
25 Note, the experiments that received butyrate and lactate also were received YE. Additionally, the

1 Batch versus PSS direct arrays are not displayed as those experiments were compared against a
2 different control.

3
4 Figure 2. Full experimental Ward cluster dendogram for all experiments based off of an
5 Euclidean distance matrix for the log ratio fold change of all gene targets. Consult Table 2 for
6 culture descriptions.

7
8 Figure 3. Breakdown of the of individual genes' expression patterns into clusters. (a) A Euclidean
9 distance and Ward cluster dendogram comparing the similarities of the clusters. (b-e) Scatterplots
10 plotting the centroid value (with 95% confidence intervals about the mean) against the
11 experimental respiration rates for all chlorinated ethenes.

12
13 Figure 4. Respiratory chain elements' response to respiration rate. (a) Ward hierarcial clustering
14 with an Euclidean distance matrix of the differential expression in log ratio. Transcripts with an
15 (*) do not exceed the intensity cutoff limit of 100. The bootstrap analysis of the cluster results in
16 two scores, the approximately unbiased (AU) p-value in red and the bootstrap probability (BP) in
17 green. (b-d) Scatterplots of the differential expression log ratio for individual genes in each large
18 cluster plotted against the respiration rate ($\mu\text{eq/L/hr}$). The black point represents the average of
19 the log ratio values in each gene cluster with the error bars representing the 95% confidence
20 interval about the mean.

21
22 Figure 5. RDase distribution across different electron acceptors for the PCE, TCE, DCE, DCP,
23 and No Acceptor fed conditions (a) natural value ratio compared to a 3-day starved culture for the
24 17 RDase and the *rpoB* transcript is plotted. Error bars represent standard error across biological
25 replicates. (b) The absolute processed intensity value distribution of the RDases.

1 Table 3. Correlation scores of RDases that meet the background cutoff to neighboring annotated
2 genes including putative response regulators. The solid black line separates the RDases based on
3 their clustering and response to respiration rate (RDases responding positively to respiration rate
4 above the black line, negatively below) displayed in Figure 4. A negative correlation score
5 indicates an inverse correlation. Values >0.25 or <-0.25 are considered significant by the one-
6 tailed t-test with $N=49$.

7

8 Table 4. Correlation scores of *tceA* to RDases and response regulators adjacent to RDases (left
9 column) and correlation scores of *tceA* to all transcripts on DET's genome represented on the
10 microarray (right column).

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

- 1 1. **Adrian, L., U. Szewzyk, J. Wecke, and H. Gorisch.** 2000. Bacterial dehalorespiration
2 with chlorinated benzenes. *Nature* **408**: 580–583.
- 3 2. **Adrian, L., S.K. Hansen, J.M. Fung, H. Görisch, and S.H. Zinder.** 2007. Growth of
4 *Dehalococcoides* Strains with Chlorophenols as Electron Acceptors. *Environmental*
5 *Science & Technology*. **41**:2318-2323.
- 6 3. **Adrian, L., J. Rahnenfuhrer, J. Gobom, and T. Holscher.** 2007. Identification of a
7 chlorobenzene reductive dehalogenase in *Dehalococcoides* sp. strain CBDB1. *Appl*
8 *Environ Microbiol* **73**: 7717–7724.
- 9 4. **Agilent Technologies.** 2009. Agilent Feature Extraction Software (v10.7) Reference
10 Guide. Santa Clara, CA.
- 11 5. **Agilent Technologies.** 2010. Agilent Genomic Workbench 6.0 User Guide. Santa Clara,
12 CA.
- 13 6. **Agilent Technologies.** 2010. Microarray-Based Gene Low Input Quick Amp Labeling.
14 Gene Expression Analysis Protocol. Santa Clara, CA.
- 15 7. **Ballerstedt, H., J. Hantke, M. Bunge, B. Werner, J. Gerritse, J.R. Andreessen, and U.**
16 **Lechner.** 2004. Properties of a trichlorodibenzo-p-dioxin-dechlorinating mixed culture
17 with a *Dehalococcoides* as putative dechlorinating species. *FEMS Microbiol Ecol* **47**:
18 223–234.
- 19 8. **Bedard, D.L., K.M. Ritalahti, and F.E. Löffler.** 2007. The *Dehalococcoides* population
20 in sediment-free mixed cultures metabolically dechlorinates the commercial
21 polychlorinated biphenyl mixture Aroclor 1260. *Appl Environ Microbiol* **73**: 2513–2521.
- 22 9. **Ben-Dor, A, R. Shamir R, and Z. Yakhini.** 1999. Clustering gene expression patterns.
23 *Journal of computational biology : a journal of computational molecular cell biology*.
24 **6**:281-97.
- 25 10. **Bowman K. S., W. M. Moe, B. A. Rash, H. Bae, and F. A. Rainey.** 2006. Bacterial
26 diversity of an acidic Louisiana groundwater contaminated by dense nonaqueous-phase

- liquid containing chloroethanes and other solvents. FEMS Microbiology Ecology. 58:120-33.
11. **Bunge, M., L. Adrian, A. Kraus, M. Opel, W.G. Lorenz, J.R. Andreessen, H. Görisch, and U. Lechner.** 2003. Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. Nature **421**:357-360.
12. **Bunge, M., M. A. Kähkönen, W. Rämisch, M. Opel, S. Vogler, F. Walkow, M. Salkinoja-Salonen, and U. Lechner.** 2007. Biological activity in a heavily organohalogen-contaminated river sediment. Environ. Sci. Pollut. R. **14**:3-10.
13. **Bunge, M., A. Wagner, M. Fischer, J. R. Andreessen, and U. Lechner.** 2008. Enrichment of a dioxin-dehalogenating *Dehalococcoides* species in two-liquid phase cultures. Environ. Microbiol. **10**:2670-2683.
14. **Bürgmann, H., J. Kleikemper, L. Duc, M. Bunge, M. Schroth, and J. Zeyer.** 2008. Detection and quantification of *Dehalococcoides*-related bacteria in a chlorinated ethene-contaminated aquifer undergoing natural attenuation. Bioremediation J. **12**: 193–209.
15. **Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T.L. Madden.** 2008. BLAST+: architecture and applications. BMC Bioinformatics **10**:421.
16. **Cheng, D., and J. He.** 2009. Isolation and characterization of ‘*Dehalococcoides*’ sp. strain MB that dechlorinates tetrachloroethene to trans-1, 2-dichloroethene. Appl Environ Microbiol. **75**:5910–5918.
17. **Duhamel M., K. Mo, and E. A. Edwards.** 2004. Characterization of a Highly Enriched *Dehalococcoides*-Containing Culture that Grows on Vinyl Chloride and Trichloroethene. Appl Environ Microbiol. **70**:5538-5545.
18. **Duhamel, M., S.D. Wehr, L. Yu, H. Rizvi, D. Seepersad, S. Dworatzek, E. Cox, and E.A. Edwards.** 2002. Comparison of anaerobic dechlorinating enrichment cultures

- 1 maintained on tetrachloroethene, trichloroethene, cis-dichloroethene and vinyl chloride.
2 Water Res. **36**: 4193–4202.
- 3 19. **Ellis, D.E., E.J. Lutz, J.M. Odom, R.J. Buchanan, C.L. Bartlett, M.D. Lee, M.R.**
4 **Harkness, and K.A. DeWeerd.** 2000. Bioaugmentation for accelerated in situ anaerobic
5 bioremediation. Environ Sci Technol. **34**: 2254–2260.
- 6 20. **Fennell, D. E., J. M. Gossett, and S. H. Zinder.** 1997. Comparison of butyric acid,
7 ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive
8 dechlorination of tetrachloroethene. Environ. Sci. Technol. **31**:918-926.
- 9 21. **Fennell, D. E., I. Nijenhuis, S. F. Wilson, S. H. Zinder, and M. M. Häggblom.** 2004.
10 *Dehalococcoides ethenogenes* strain 195 reductively dechlorinates diverse chlorinated
11 aromatic pollutants. Environ. Sci. Technol. **38**:2075-2081.
- 12 22. **Field, J.A., and R. Sierra-Alvarez.** 2008. Microbial degradation of chlorinated dioxins.
13 Chemosphere **71**: 1005–1018.
- 14 23. **Fung, J.M., R.M. Morris, L. Adrian, and S.H. Zinder.** 2007. Expression of reductive
15 dehalogenase genes in *Dehalococcoides ethenogenes* strain 195 growing on
16 tetrachloroethene, trichloroethene, or 2,3-dichlorophenol. Applied and Environmental
17 Microbiology. **73**:4439-45.
- 18 24. **Futamata H., N. Yoshida, T. Kurogi, S. Kaiya, and A. Hiraishi.** 2007. Reductive
19 dechlorination of chloroethenes by *Dehalococcoides*-containing cultures enriched from a
20 polychlorinated-dioxin-contaminated microcosm. The ISME Journal. **1**:471-9.
- 21 25. **Groster, A., and E.A. Edwards.** 2006. Growth of *Dehalobacter* and *Dehalococcoides*
22 spp. during degradation of chlorinated ethanes. Appl Environ Microbiol. **72**: 428–436.
- 23 26. **Harkness, M.R., A.A. Bracco, M.J. Brennan, K.A. DeWeerd, and J.L. Spivack.** 1999.
24 Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene
25 in Dover soil columns. Environ Sci Technol. **33**: 1100–1109.

- 1 27. **He, J., K. M. Ritalahti, K. L. Yang, S. S. Koenigsberg, and F. E. Löffler.** 2003.
2 Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium.
3 Nature **424**:62-65.
- 4 28. **He, J., Y. Sung, R. Krajmalnik-Brown, K. M. Ritalahti, and F. E. Löffler.** 2005.
5 Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene
6 (TCE)- and 1,2-dichloroethene-respiring anaerobe. Environ. Microbiol. **7**:1442-1450.
- 7 29. **He, J., V. Holmes, P. K. H. Lee, and L. Alvarez-Cohen.** 2007. Influence of vitamin
8 B12 and cocultures on the growth of *Dehalococcoides* isolates in defined medium. Appl.
9 Environ. Microbiol. **73**:2847–2853.
- 10 30. **Hendrickson, E. R., J. A. Payne, R. M. Young, M. G. Starr, M. P. Perry, S.**
11 **Fahnestock, D. E. Ellis, and R. C. Ebersole.** 2002. Molecular analysis of
12 *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout
13 North America and Europe. Appl. Environ. Microbiol. **68**:485-495.
- 14 31. **Himmelheber D. W., K. D. Pennell, and J. B. Hughes.** 2007. Natural attenuation
15 processes during in situ capping. Environmental Science & Technology **41**:5306-13.
- 16 32. **Holliger, C., G. Wohlfahrt, and G. Diekert.** 1999. Reductive dechlorination in the
17 energy metabolism of anaerobic bacteria. FEMS Microbiol. Rev. **22**:383-398.
- 18 33. **Holmes, V.F., J. He, P.K. Lee, and L. Alvarez-Cohen.** 2006. Discrimination of
19 multiple *Dehalococcoides* strains in a trichloroethene enrichment by quantification of
20 their reductive dehalogenase genes. Appl Environ Microbiol. **72**: 5877–5883.
- 21 34. **Hölscher, T., R. Krajmalnik-Brown, K. M. Ritalahti, F. von Wintzingerode, H.**
22 **Görisch, F. E. Löffler, and L. Adrian.** 2004. Multiple nonidentical reductive-
23 dehalogenase-homologous genes are common in *Dehalococcoides*. Appl. Environ.
24 Microbiol. **70**:5290-5297.
- 25 35. **Imfeld, G., I. Nijenhuis, M. Nikolausz, S. Zeiger, H. Paschke, J. Drangmeister, J.**
26 **Grossmann, H.H, Richnow, and S. Weber.** 2008. Assessment of in situ degradation of

- 1 chlorinated ethenes and bacterial community structure in a complex contaminated
2 groundwater system. *Water Res.* **42**: 871–882
- 3 36. **Johnson, D. R., P. K. Lee, V. F. Holmes, A. C. Fortin, and L. Alvarez-Cohen.** 2005.
4 Transcriptional expression of the *tceA* gene in a *Dehalococcoides*-containing microbial
5 enrichment. *Appl. Environ. Microbiol.* **71**:7145-7151.
- 6 37. **Johnson, D.R., E.L. Brodie, A.E. Hubbard, G.L. Andersen, S.H. Zinder, and L.**
7 **Alvarez-Cohen.** 2008. Temporal transcriptomic microarray analysis of
8 ‘*Dehalococcoides ethenogenes*’ strain 195 during the transition into stationary phase.
9 *Appl Environ Microbiol.* **74**: 2864–2872.
- 10 38. **Johnson, D.R., A. Nemir, G.L. Andersen, S.H. Zinder and L. Alvarez-Cohen.** 2009.
11 Transcriptomic microarray analysis of corrinoid responsive genes in *Dehalococcoides*
12 *ethenogenes* strain 195. *FEMS Lett.* **294**:198-206.
- 13 39. Korhonen, I.O.O. 1983. Glass-Capillary Gas Chromatography of Chlorobenzenes on SE-
14 30 and Carbowax 20M Columns. Simultaneous Determination of Chlorobenzenes and
15 Chlorophenols. *Chromatographia* **17**:195-199.
- 16 40. **Krajmalnik-Brown, R., T. Hölscher, I.N. Thomson, F.M. Saunders, K.M. Ritalahti,**
17 **and F.E. Löffler.** 2004. Genetic identification of a putative vinyl chloride reductase in
18 *Dehalococcoides* sp. strain BAV1. *Appl Environ Microbiol.* **70**: 6347–6351.
- 19 41. **Krajmalnik-Brown, R., Y., Sung, K.M. Ritalahti, F.M. Saunders, and F.E. Löffler.**
20 2007. Environmental distribution of the trichloroethene reductive dehalogenase gene
21 (*tceA*) suggests lateral gene transfer among *Dehalococcoides*. *FEMS microbiology*
22 *ecology.* **59**:206-14.
- 23 42. **Kube, M., A. Beck, S.H. Zinder, H. Kuhl, R. Reinhardt, and L. Adrian.** 2005.
24 Genome sequence of the chlorinated compound-respiring bacterium *Dehalococcoides*
25 species strain CBDB1. *Nat Biotechnol.* **23**: 1269-1273.

- 1 43. **Lee, P. K., D. R. Johnson, V. F. Holmes, J. He, and L. Alvarez-Cohen.** 2006.
2 Reductive dehalogenase gene expression as a biomarker for physiological activity of
3 *Dehalococcoides* spp. *Appl. Environ. Microbiol.* **72**:6161-6168.
- 4 44. **Lee, P. K., T. W. Macbeth, K. S. Sorenson, Jr., R. A. Deeb, and L. Alvarez-Cohen.**
5 2008. Quantifying genes and transcripts to assess the in situ physiology of
6 *Dehalococcoides* spp. in a trichloroethene-contaminated groundwater site. *Appl. Environ.*
7 *Microbiol.* **74**:2728-2739.
- 8 45. **Lendvay, J.M., F.E. Löffler, M. Dollhopf, M.R. Aiello, G. Daniels, B.Z. Fathepure,**
9 **B.Z., M. Gebhard, R. Heine, R. Helton, J. Shi, R. Krajmalnik-Brown, C. L. Major,**
10 **Jr., M. J. Barcelona, E. Petrovskis, R. Hickey, J. M. Tiedje, and P. Adriaens.** 2003.
11 Bioreactive barriers: a comparison of bioaugmentation and biostimulation for chlorinated
12 solvent remediation. *Environ Sci Technol.* **37**: 1422–1431.
- 13 46. **Liu, F. and D.E. Fennell.** 2008. Dechlorination and detoxification of 1,2,3,4,7,8-
14 hexachlorodibenzofuran by a mixed culture containing *Dehalococcoides* ethenogenes
15 strain 195. *Environ Sci Technol.* **42**: 602–607.
- 16 47. **Löffler, F. E. and E. A. Edwards.** 2006. Harnessing microbial activities for
17 environmental cleanup. *Current Opinion in Biotechnology*, **17**:274–284.
- 18 48. **McMurdie, P.J., S.F. Behrens, S. Holmes, and A.M. Spormann.** 2007. Unusual codon
19 bias in vinyl chloride reductase genes of *Dehalococcoides* species. *Appl Environ*
20 *Microbiol.* **73**: 2744–2747.
- 21 49. **McMurdie, P.J., S.F. Behrens, J.A. Müller, J. Göke, K.M. Ritalahti, R. Wagner, E.**
22 **Goltsman, A. Lapidus, S. Holmes, F.E. Löffler, A.M. Spormann.** 2009. Localized
23 plasticity in the streamlined genomes of vinyl chloride respiring *Dehalococcoides*. *PLoS*
24 *Genetics.* **5**:e1000714.

- 1 50. **Magnuson, J. K., R. V. Stern, J. M. Gossett, S. H. Zinder, and D. R. Burris.** 1998.
2 Reductive dechlorination of tetrachloroethene to ethene by two-component enzyme
3 pathway. *Appl. Environ. Microbiol.* **64**:1270-1275.
- 4 51. **Magnuson, J. K., M. F. Romine, D. R. Burris, and M. T. Kingsley.** 2000.
5 Trichloroethene reductive dehalogenase from *Dehalococcoides ethenogenes*: Sequence of
6 tceA and substrate range characterization. *Appl. Environ. Microbiol.* **66**:5141-5147.
- 7 52. **Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R.**
8 **Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici.** 2002. Field
9 demonstration of successful bioaugmentation to achieve dechlorination of
10 tetrachloroethene to ethene. *Environ Sci Technol.* **36**: 5106–5116.
- 11 53. **Maymó-Gatell, X., Y. Chien, J. M. Gossett, and S. H. Zinder.** 1997. Isolation of a
12 bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* **276**:1568-
13 1571.
- 14 54. **Maymó-Gatell, X., T. Anguish, and S.H. Zinder.** 1999. Reductive dechlorination of
15 chlorinated ethenes and 1, 2-dichloroethane by "Dehalococcoides ethenogenes" 195.
16 *Appl. Environ. Microbiol.* **65**:3108-13.
- 17 55. **McCarty, P. L.** 2010. *In Situ Remediation of Chlorinated Solvent Plumes.* Clean Air.
18 Springer. New York, NY.
- 19 56. **Morris, R. M., S. Sowell, D. Barofsky, S. Zinder, and R. Richardson.** 2006.
20 Transcription and mass-spectroscopic proteomic studies of electron transport
21 oxidoreductases in *Dehalococcoides ethenogenes*. *Environ. Microbiol.* **8**:1499-1509.
- 22 57. **Morris, R. M., J. M. Fung, B. G. Rahm, S. Zhang, D. L. Freedman, S. H. Zinder,**
23 **and R. E. Richardson.** 2007. Comparative proteomics of *Dehalococcoides* spp. reveals
24 strain-specific peptides associated with activity. *Appl. Environ. Microbiol.* **73**:320-326.
- 25 58. **Müller, J. A., B. M. Rosner, G. Von Abendroth, G. Meshulam-Simon, P. L.**
26 **McCarty, and A. M. Spormann.** 2004. Molecular identification of the catabolic vinyl

- 1 chloride reductase from *Dehalococcoides* sp. strain VS and its environmental
2 distribution. Appl. Environ. Microbiol. **70**:4880-4888.
- 3 59. **Nijenhuis, I., and S.H. Zinder.** 2005. Characterization of Hydrogenase and Reductive
4 Dehalogenase Activities of *Dehalococcoides ethenogenes* Strain 195. Appl. Environ.
5 Microbiol. **71**:1664-1667.
- 6 60. **Rahm, B.G., and R.E. Richardson.** 2008a. Correlation of respiratory gene expression
7 levels and pseudo-steady state PCE respiration rates in *Dehalococcoides*. Environ Sci
8 Technol. **42**: 416–421.
- 9 61. **Rahm, B.G., and R.E. Richardson.** 2008b. *Dehalococcoides*' gene transcripts as
10 quantitative bioindicators of PCE, TCE and cDCE dehalorespiration rates: trends and
11 limitations. Environ Sci Technol. **42**: 5099–5105.
- 12 62. **Rahm, B.G., R.M. Morris, and R.E. Richardson.** 2006. Temporal expression of
13 respiratory genes in an enrichment culture containing *Dehalococcoides ethenogenes*.
14 Appl. Environ Microbiol. **72**: 5486–5491.
- 15 63. **Regeard, C., J. Maillard, C. Dufraigne, P. Deschavanne, and C. Holliger.** 2005.
16 Indications for acquisition of reductive dehalogenase genes through horizontal gene
17 transfer by *Dehalococcoides ethenogenes* strain 195. Appl. Environ. Microbiol. **71**:2955-
18 2961.
- 19 64. **Richardson, R.E., V.K. Bhupathiraju, D.L. Song, T.A. Goulet, and L. Alvarez-**
20 **Cohen.** 2002. Phylogenetic characterization of microbial communities that reductively
21 dechlorinate TCE based upon a combination of molecular techniques. Environ Sci
22 Technol. **36**: 2652–2662.
- 23 65. **Rowe, A.R., B.J. Lazaar, R.M. Morris, and R.E. Richardson.** 2008. Characterization
24 of the community structure of a dechlorinating mixed culture and comparisons of gene
25 expression in planktonic and biofloc-associated *Dehalococcoides* and *Methanospirillum*
26 species. Appl. Environ. Microbiol. **74**:6709–6719.

- 1 66. **Saeed, A.I., N.K. Bhagabati, J.C. Braisted, W. Liang, V. Sharov, E.A. Howe, J. Li,**
2 **M. Thiagarajan, J.A. White, and J. Quackenbush.** 2006. TM4 microarray software
3 suite. *Methods in Enzymology*. **411**:134-93
- 4 67. **Scheutz, C., N. D. Durant, P. Dennis, M. H. Hansen, T. Jorgensen, R. Jakobsen, E.**
5 **E. Cox, and P. L. Bjerg.** 2008. Concurrent ethene generation and growth of
6 *Dehalococcoides* containing vinyl chloride reductive dehalogenase genes during an
7 enhanced reductive dechlorination field demonstration. *Environ. Sci. Technol.* **42**:9302-
8 9309.
- 9 68. **Schumacher, W., C. Holliger, A. J. Zehnder, and W. R. Hagen.** 1997. Redox
10 chemistry of cobalamin and iron-sulfur cofactors in the tetrachloroethene reductase of
11 *Dehalobacter restrictus*. *FEMS Lett.* **409**:421-425.
- 12 69. **Seshadri, R., L. Adrian, D.E. Fouts, J.A. Eisen, A.M. Phillippy, B.A. Methe, N.L.**
13 **Ward, W.C. Nelson, R.T. Deboy, H.M. Khouri, J.F. Kolonay, R.J. Dodson, S.C.**
14 **Daugherty, L.M. Brinkac, S.A. Sullivan, R. Madupu, K.E. Nelson, K.H. Kang, M.**
15 **Impraim, K. Tran, J.M. Robinson, H.A. Forberger, C.M. Fraser, S.H. Zinder, and**
16 **J.F. Heidelberg.** 2005. Genome sequence of the PCE-dechlorinating bacterium
17 *Dehalococcoides ethenogenes*. *Science (New York, N.Y.)*. **307**:105-8.
- 18 70. **Smidt, H., and W. M. de Vos.** 2004. Anaerobic microbial dehalogenation. *Annu. Rev.*
19 *Microbiol.* **58**:43-73.
- 20 71. **Sung, Y., K.M. Ritalahti, R.P. Apkarian, and F.E. Löffler.** 2006. Quantitative PCR
21 confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring
22 *Dehalococcoides* isolate. *Appl Environ Microbiol.* **72**: 1980–1987.
- 23 72. **Tang, Y.J., S. Yi, W. Zhuang, S.H. Zinder, J.D. Keasling, and L. Alvarez-Cohen.**
24 **2009. Investigation of carbon** metabolism in "*Dehalococcoides ethenogenes*" strain 195
25 by use of isotopomer and transcriptomic analyses. *Journal of bacteriology*. **191**:5224-31.

- 1 73. **Vignais PM, B. Billoud, and J. Meyer.** 2001. Classification and phylogeny of
2 hydrogenases. *FEMS Microbiol Rev.* **25**:455-501.
- 3 74. **Wagner, A., L. Adrian, S. Kleinsteuber, J.R. Andreessen, and U. Lechner.** 2009.
4 Transcription analysis of genes encoding homologues of reductive dehalogenases in
5 *Dehalococcoides* sp. strain CBDB1 using terminal restriction fragment length
6 polymorphism and quantitative PCR. *Appl Environ Microbiol* **75**: 1876–1884.
- 7 75. **Waller, A. S., R. Krajmalnik-Brown, F. E. Löffler, and E. A. Edwards.** 2005.
8 Multiple reductive-dehalogenase-homologous genes are simultaneously transcribed
9 during dechlorination by *Dehalococcoides*-containing cultures. *Appl. Environ. Microbiol.*
10 **71**:8257-8264.
- 11 76. **Waller A. S.** 2009. Molecular Investigation of Chloroethene Reductive Dehalogenation
12 by the Mixed Microbial Community KB1. Ph.D. Thesis. University of Toronto.
- 13 77. **Werner J. J., A. C. Ptak, B. G. Rahm, S. Zhang, and R. E. Richardson.** 2009.
14 Absolute quantification of *Dehalococcoides* proteins: enzyme bioindicators of
15 chlorinated ethene dehalorespiration. *Environmental microbiology* **11**:2687-97.
- 16 78. **West, K.A., D.R. Johnson, P. Hu, T.Z. DeSantis, E.L. Brodie, P.K.H. Lee, H. Feil,**
17 **G.L. Andersen, S.H. Zinder, and L. Alvarez-Cohen.** 2008. Comparative genomics of
18 ‘*Dehalococcoides ethenogenes*’ 195 and an enrichment culture containing unsequenced
19 ‘*Dehalococcoides*’ strains. *Appl Environ Microbiol* **74**: 3533–3540.
- 20 79. **Wohlfarth, G., and G. Diekert.** 1997. Anaerobic dehalogenases. *Curr Opin Biotechnol*
21 **8**: 290–295.
- 22 80. **Yan, T., T.M. LaPara, and P.J. Novak.** 2006. The reductive dechlorination of 2,3,4,5-
23 chlorobiphenyl in three different sediment cultures. Evidence for the involvement of
24 phylogenetically similar *Dehalococcoides*-like bacterial populations. *FEMS Microbiol*
25 *Ecol* **55**: 248–261.

- 1 81. **Yoshida N., N. Takahashi, and A. Hiraishi.** 2005. Phylogenetic Characterization of a
2 Polychlorinated-Dioxin- Dechlorinating Microbial Community by Use of Microcosm
3 Studies. *Appl Environ Microbiol.* **71**:4325-4334.
- 4 82. **Yoshida, N., K. Asahi, Y. Sakakibara, K. Miyake, and A. Katayama.** 2007. Isolation
5 and quantitative detection of tetrachloroethene (PCE)-dechlorinating bacteria in
6 unsaturated subsurface soils contaminated with chloroethenes. *J. Biosci. Bioeng.* **104**:91-
7 97.
- 8 83. **Zahurak M., G. Parmigiani, W. Yu, R. B. Scharpf, D. Berman, E. Schaeffer, S.**
9 **Shabbeer, and L. Cope.** 2007. Pre-processing Agilent microarray data. *BMC*
10 *bioinformatics* **8**:142.

	PCE1	PCE2	TCE1	TCE2
PCE1	1	0.72	0.78	0.78
PCE2	0.72	1	0.66	0.55
TCE1	0.78	0.66	1	0.84
TCE2	0.78	0.55	0.84	1

- 1 Figure 1. Comparison of the Batch and PSS fed cultures. The correlogram displays the similarity
- 2 of experimental replicates TCE 1&2 and PCE 1 & 2 by correlating the gene expression across
- 3 experiments for all transcripts.

Transcript ID and Description	Regulation	Ratio	StDev	Transcript ID and Description	Regulation	Ratio	StDev
DET0036 hypothetical protein	DOWN	0.44	0.04	DET1038 ileS isoleucyl tRNA synthetase	UP	2.5	0.39
DET0137 mgsA methylglyoxal synthase	DOWN	0.31	0.11	DET1051 hypothetical protein	UP	3.8	0.61
DET0180 reductive dehalogenase	UP	5.5	4.11	DET1063 DNA binding response regulator, LuxR	UP	2.7	0.81
DET0181 reductive dehalogenase anchoring protein	UP	5.1	0.92	DET1092 DNA methylase	DOWN	0.41	0.07
DET0297 hypothetical protein	UP	4.9	3.03	DET1106 hypothetical protein	DOWN	0.33	0.06
DET0368 proS prolyl tRNA synthetase	UP	2.7	0.25	DET1107 hypothetical protein	DOWN	0.29	0.05
DET0423 hypothetical protein	UP	4.2	5.14	DET1146 hypothetical protein	DOWN	0.36	0.07
DET0558 atpB ATP synthase F0, A subunit	DOWN	0.21	0.04	DET1186 hypothetical protein	UP	2.9	1.12
DET0560 atpF ATP synthase F0, B subunit	DOWN	0.33	0.08	DET1206 metM homoserine dehydrogenase	DOWN	0.34	0.09
DET0561 atpH ATP synthase F1, delta subunit	DOWN	0.33	0.05	DET1224 cobA 3 cob(II)alamin adenosyltransferase	DOWN	0.43	0.04
DET0562 atpA ATP synthase subunit A	DOWN	0.41	0.04	DET1278 nusB N utilization substance protein B	DOWN	0.25	0.07
DET0673 hypothetical protein	UP	3.6	1.21	DET1284 hypothetical protein	DOWN	0.38	0.06
DET0721 hypothetical protein	DOWN	0.36	0.04	DET1285 serine protease, DegP/HtrA family	UP	2.9	0.58
DET0736 oxidoreductase	UP	2.7	0.42	DET1286 serine protease, DegP/HtrA family	UP	3.9	1.28
DET0754 hypothetical protein	UP	2.7	1.12	DET1377 hypothetical protein	UP	3.0	0.70
DET0755 hypothetical protein	UP	3.8	0.58	DET1379 auxin responsive GH3 protein homolog	UP	3.7	0.29
DET0864 hydrogenase subunit HymB, putative	DOWN	0.37	0.09	DET1419 transcriptional regulator, AbrB family	DOWN	0.29	0.15
DET0908 arsenical pump membrane protein, putative	UP	7.8	2.82	DET1511 hypothetical protein	DOWN	0.14	0.12
DET0909 hypothetical protein	UP	2.8	0.35	DET1544 reductive dehalogenase anchoring protein	UP	3.5	0.59
DET0945 ABC transporter	DOWN	0.43	0.05	DET1569 hypothetical protein	UP	3.0	0.65
DET1021 hypothetical protein	DOWN	0.20	0.08	DET1624 hypothetical protein	UP	2.6	0.39
DET1035 tryptophan synthase subunit beta	UP	3.2	0.40				

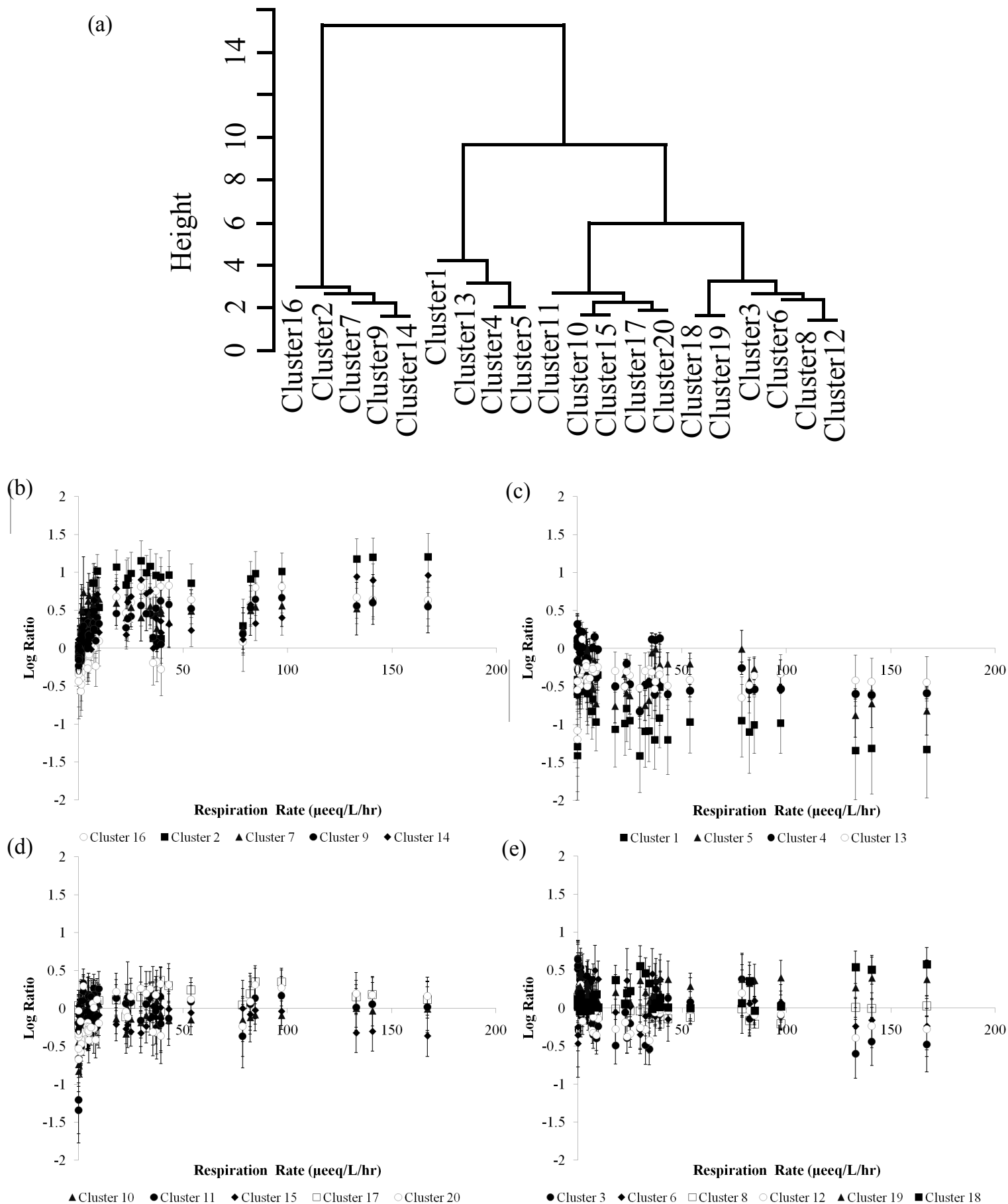
- 1 Table 1. The 43 elements that are similarly regulated amongst all four Batch vs. PSS samples
- 2 with the transcript name, regulation (up or down when comparing PSS to batch level), average
- 3 natural value ratio recorded, and the standard deviation.

	PCE1	PCE2	TCE1	TCE2
PCE1	1	0.72	0.78	0.78
PCE2	0.72	1	0.66	0.55
TCE1	0.78	0.66	1	0.84
TCE2	0.78	0.55	0.84	1

Figure 1. Comparison of the Batch Control and PSS fed cultures log ratio correlations. The correlogram displays the similarity of the experimental replicates TCE 1&2 and PCE 1&2 by correlating the log ratio for every gene transcript across experiments.

Experiment Title	Culture Title	Electron Acceptor	EA Feed Rate (μeq/L/hr)	Electron Donor Source	ED:EA Ratio (eeq ratio)	Time (Days)	Respiration Rate (μeq/L/hr)
PCE High	HiP1	PCE	258	Butyrate	3.4	1	140
	HiP2	PCE	234	Butyrate	3.8	1	130
	HiP3	PCE	282	Butyrate	3.1	1	170
PCE High Low	HLH1_INHIB	PCE	456	Butyrate	1.9	7	95
	HLH2_INHIB	PCE	504	Butyrate	1.7	7	110
	HLL1	PCE	7.2	Butyrate	4.1	7	6.3
	HLL2	PCE	7.2	Butyrate	4.1	7	6.2
	HLL3	PCE	7.2	Butyrate	3.3	7	6.7
PCE Half Butyrate	PHB1	PCE	102	Butyrate	0.73	7	85
	PHB2	PCE	45.6	Butyrate	1.7	7	39
	PHB3	PCE	108	Butyrate	0.72	7	97
PCE Lactate or Butyrate	PLL1	PCE	45	Lactate	2.1	7	43
	PLL2	PCE	38.4	Lactate	1.3	7	37
	PLB1	PCE	30	Butyrate	1.5	7	30
	PLB2	PCE	58.2	Butyrate	1.8	7	54
PCE Hydrogen	H2A1	-	-	H ₂	-	1.5	-
	H2A2	-	-	H ₂	-	1.5	-
	H2PB1	PCE	1.7	H ₂	0.74	1.5	9.7
	H2PB2	PCE	1.5	H ₂	0.85	1.5	7.3
PCE 0, YE, FYE	P0FY01	PCE	3	-	-	3	1.3
	P0FY02	PCE	3	-	-	3	1.3
	P0FYF1	PCE	4.8	FYE	3.2	7	4.1
	P0FYF2	PCE	5.4	FYE	2.5	7	4.9
	P0FYY1	PCE	4.8	YE	2.8	7	4.5
	P0FYY2	PCE	4.8	YE	2.8	7	4.3
PCE 3 Rates	P3A1	PCE	25.2	Butyrate	2.5	4	25
	P3A2	PCE	22.8	Butyrate	2.8	4	23
	P3B1	PCE	4.2	Butyrate	4.8	4	4.5
	P3B2	PCE	4.8	Butyrate	4.2	4	4.9
	P3C1	PCE	0.6	Butyrate	17	4	1
	P3C2	PCE	0.6	Butyrate	16	4	0.9
TCE 3 Rates	T3A1	TCE	34.4	Butyrate	3.8	4	34
	T3A2	TCE	23.6	Butyrate	3.2	2	23
	T3B1	TCE	6.8	Butyrate	5.1	4	6.9
	T3B2	TCE	7.2	Butyrate	4.8	4	7.3
	T3C1	TCE	1.6	Butyrate	11	4	1.5
	T3C2	TCE	1.4	Butyrate	12	4	1.4
DCE 3 Rates	D3A1	DCE	9	Butyrate	2.6	4	18
	D3A2	DCE	16	Butyrate	3.0	2	32
	D3B1	DCE	4.4	Butyrate	3.0	4	8.9
	D3B2	DCE	4.1	Butyrate	3.2	4	8.2
	D3C1	DCE	1.1	Butyrate	5.5	4	2.3
	D3C2	DCE	1.2	Butyrate	5.4	4	2.3
DCE 10	D1_INHIB	DCE	120	Butyrate	2.0	7	37
	D2_INHIB	DCE	114	Butyrate	1.8	7	35
	D3_INHIB	DCE	120	Butyrate	2.7	7	39
Chlorophenols	CpA1	DCP	0.31	Butyrate	9.2	3	N/D
	CpA2	DCP	0.31	Butyrate	9.9	3	0.36
	CpB1	DCP	0.56	Butyrate	6.8	3	0.42
	CpB2	DCP	0.56	Butyrate	6.8	3	0.42
Decay	DecayA1	-	-	-	-	7	-
	DecayB1	-	-	-	-	3	-
	DecayB2	-	-	-	-	3	-

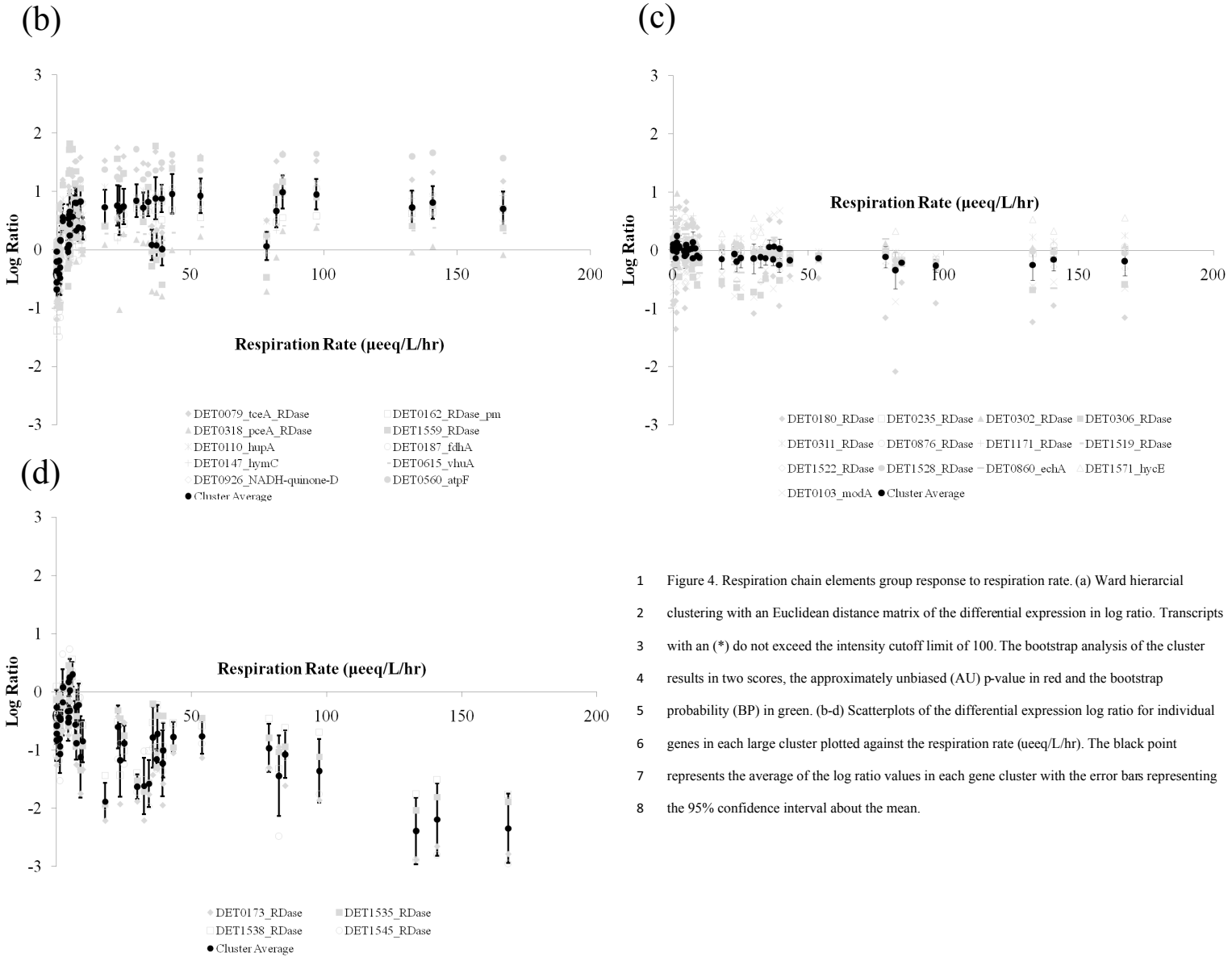
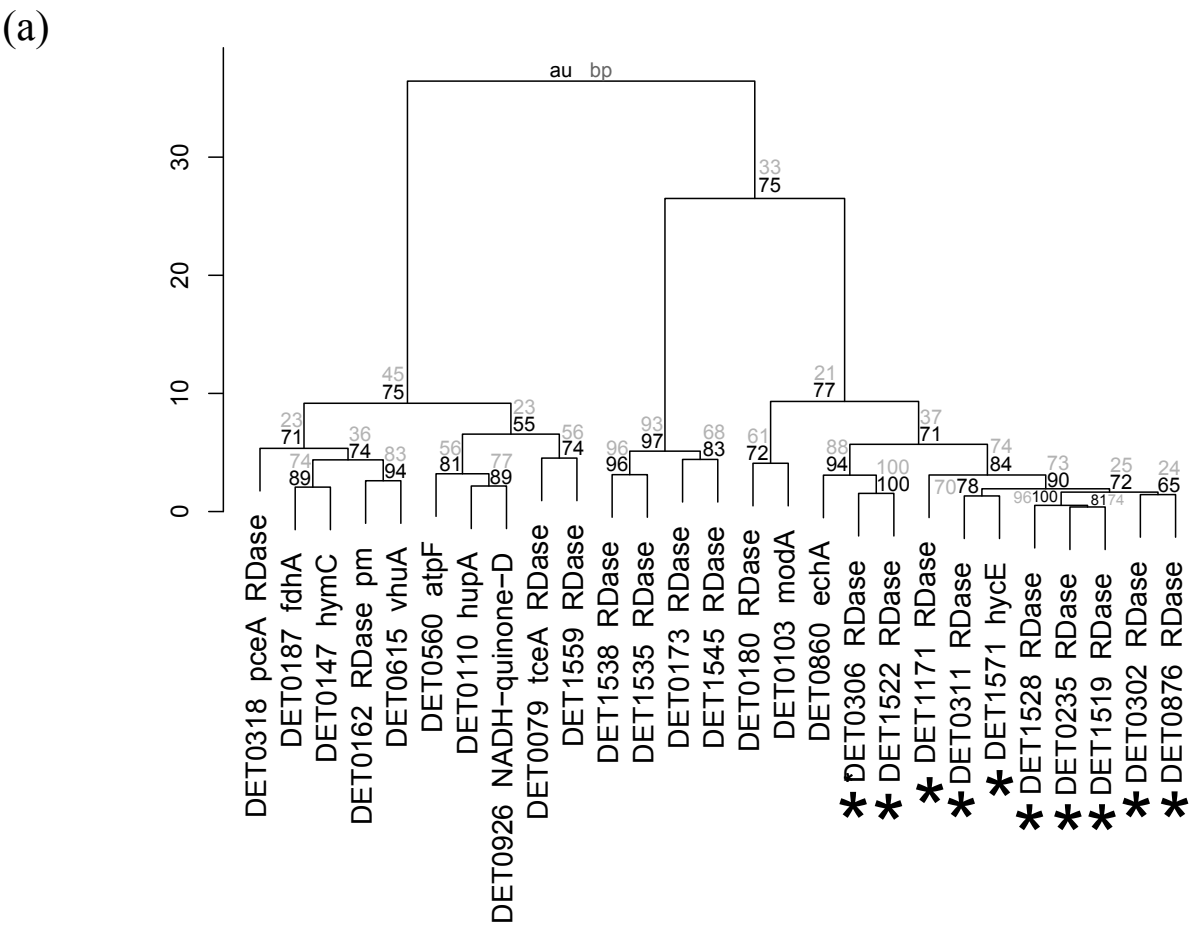
1 Table 2. Experimental conditions for all the 12 investigations comparing the PSS versus a 3 day
 2 starved control. The experimental title provides a brief description of the overall experiment
 3 where the culture title designates the electron acceptor (PCE, TCE, DCE, DCP, or none (-)) and
 4 electron donor source (butyrate, lactate, hydrogen, yeast extract (YE), fermented yeast extract
 5 (FYE), hydrogen, or none (-)) type. Note, the experiments that received butyrate and lactate also
 6 were treated with YE.



1 Figure 3. Biological breakdown of the clusters. (a) A Euclidean distance and Ward cluster
2 dendrogram comparing the similarities of the clusters. (b-e) Scatterplots plotting the centroid value
3 with 95% confidence intervals about the mean against the experimental respiration rates for all
4 chlorinated ethenes.

RDase	Anchor	Predicted Histidine Kinase Sensory Box	Predicted DNA Binding Response Reg.	Predicted MarR Regulator	Annotated Lipo- protein	Other Annotations (Including Hypothetical)		
DET0079 - <i>tceA</i>	DET0078							
	0.97							
DET0318 - <i>pceA</i>	DET0319	DET0315	DET0316		DET0321	DET0317	DET0320	
	0.94	0.18	0.30		-0.10	-0.12	0.69	
DET1559	DET1558	DET1560	DET1561		DET1557	DET1556		
	0.69	0.60	0.70		0.61	0.78		
DET0173	DET0175	DET0171	DET0170		DET0174	DET0172	DET0176	
	0.95	-0.25	-0.19		0.94	0.39	0.58	
DET0180	DET0181	DET0178	DET0177			DET0179		
	0.68	0.54	0.48			0.34		
DET1535	DET1534			DET1536		DET1533	DET1532	
	0.94			-0.63		0.41	0.49	
DET1538	DET1537	DET1539	DET1540					
	0.68	0.48	-0.10					
DET1545	DET1544					DET1541	DET1542	DET1543
	0.77					0.33	0.10	-0.31

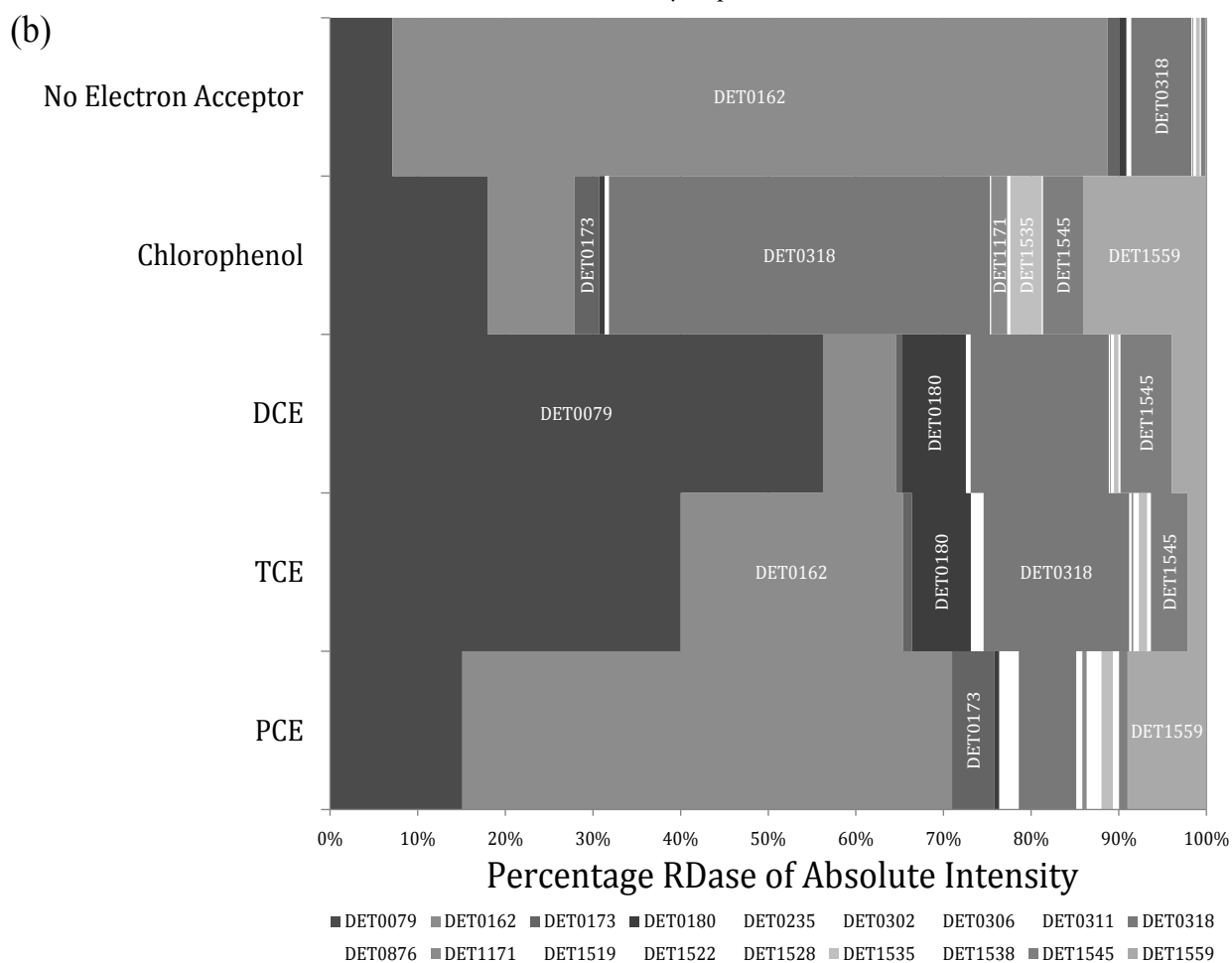
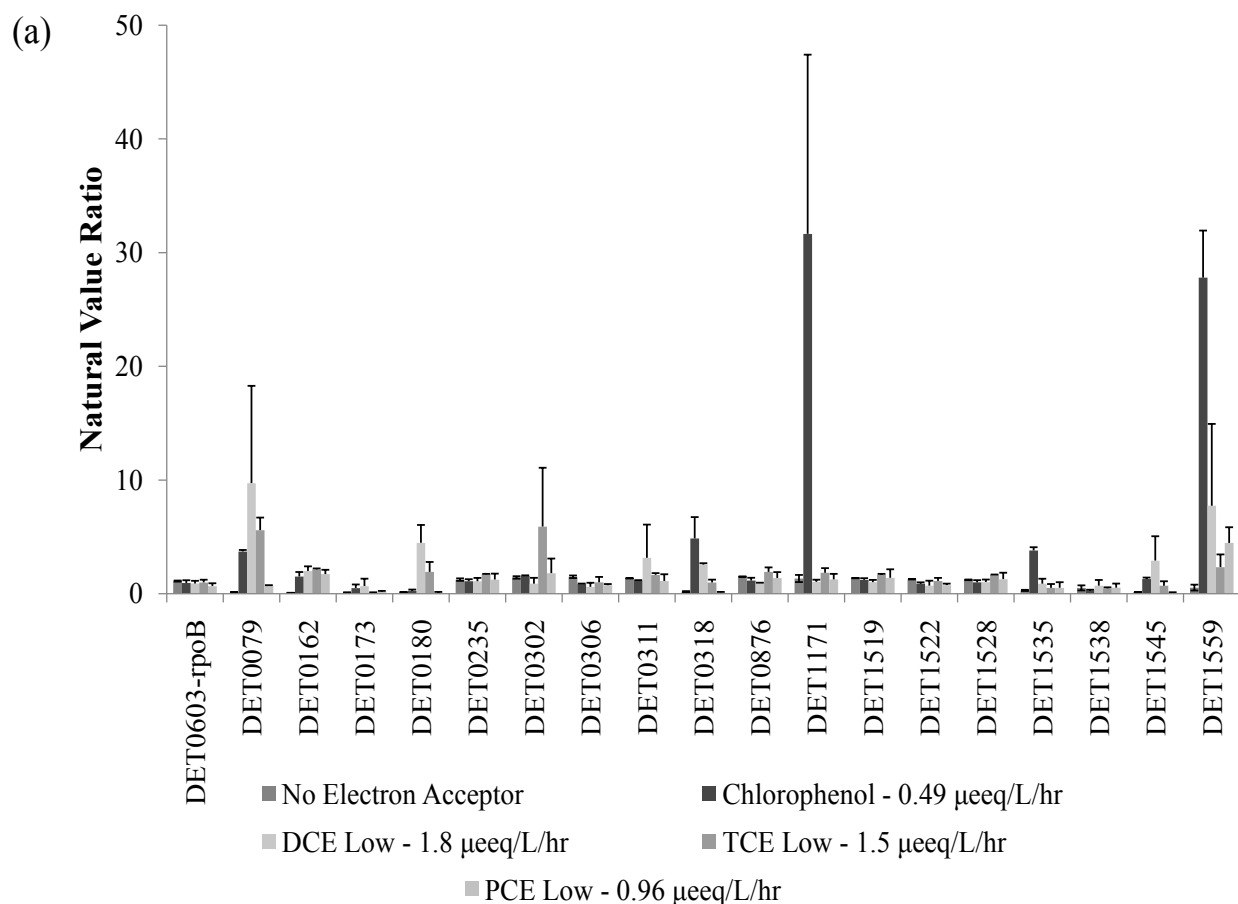
1 Table 3. Correlation scores of RDases that meet the background cutoff to neighboring annotated
 2 genes including putative response regulators. The solid black line separates the RDases based on
 3 their clustering and response to respiration rate (RDases responding positively to respiration rate
 4 above the black line, negatively below) displayed in Figure 4. A negative correlation score
 5 indicates an inverse correlation. Values >0.25 or <-.25 are considered significant by the one-
 6 tailed t-test with N=49.



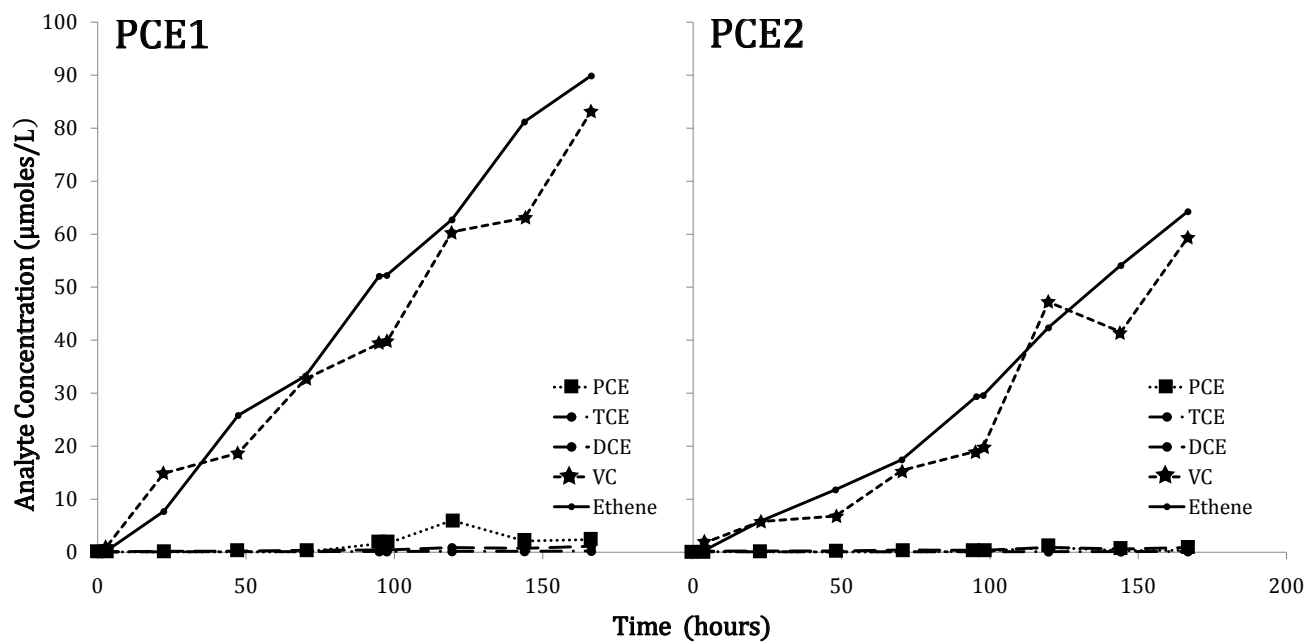
1 Figure 4. Respiration chain elements group response to respiration rate. (a) Ward hierarcial
2 clustering with an Euclidean distance matrix of the differential expression in log ratio. Transcripts
3 with an (*) do not exceed the intensity cutoff limit of 100. The bootstrap analysis of the cluster
4 results in two scores, the approximately unbiased (AU) p-value in red and the bootstrap
5 probability (BP) in green. (b-d) Scatterplots of the differential expression log ratio for individual
6 genes in each large cluster plotted against the respiration rate (μeq/L/hr). The black point
7 represents the average of the log ratio values in each gene cluster with the error bars representing
8 the 95% confidence interval about the mean.

mRNA ID	Correlation Score	mRNA ID	Correlation Score
DET0320 hypothetical protein	0.90	DET1407 BNR/Asp box repeat domain protein	0.98
DET1559 RDase	0.78	DET0078 tceA anchoring protein	0.97
DET0170 DNA binding response regulator	0.76	DET0511 hypothetical protein	0.93
DET1543 hypothetical protein	0.69	DET0112 [Ni/Fe] hydrogenase	0.93
DET0171 sensory box sensor histidine kinase	0.68	DET1600 twin arginine translocation TatB	0.93
DET0177 DNA binding response regulator	0.67	DET0117 transcriptional regulator	0.92
DET1556 hypothetical protein	0.67	DET0352 hypothetical protein	0.91
DET0319 RDase anchoring protein	0.67	DET0191 iron sulfur cluster binding protein	0.91
DET1557 lipoprotein, putative	0.63	DET0320 hypothetical protein	0.90
DET0318 RDase	0.62	DET0145 [Fe] hydrogenase, HymA	0.89
DET1560 sensory box sensor histidine kinase	0.61	DET0111 [Ni/Fe] hydrogenase	0.89
DET0178 sensor histidine kinase	0.60	DET0187 formate dehydrogenase	0.89
DET1561 DNA binding regulator, LuxR family	0.55	DET0186 formate dehydrogenase	0.88
DET0316 DNA binding response regulator	0.51	DET0343 ftsZ-1 cell division protein	0.87
DET0305 sensory box sensor histidine kinase	0.48	DET0996 rpmG ribosomal protein L33	0.87
DET1540 DNA binding response regulator	0.40	DET1225 hypothetical protein	0.87
DET0315 sensory box sensor histidine kinase	0.33	DET0997 tuf translation elongation factor Tu	0.87
DET0322 hypothetical protein	0.33	DET0994 nusG transcription antitermination	0.87
DET1558 RDase anchoring protein	0.29	DET1417 purH cyclohydrolase	0.86
DET1164 iron sulfur cluster binding protein	0.25	DET0001 dnaA replication initiator	0.86

- 1 Table 4. Correlation scores of *tceA* to RDases and response regulators adjacent to RDases and
- 2 correlation scores of *tceA* to all transcripts on DET s genome represented on the microarray.
- 3

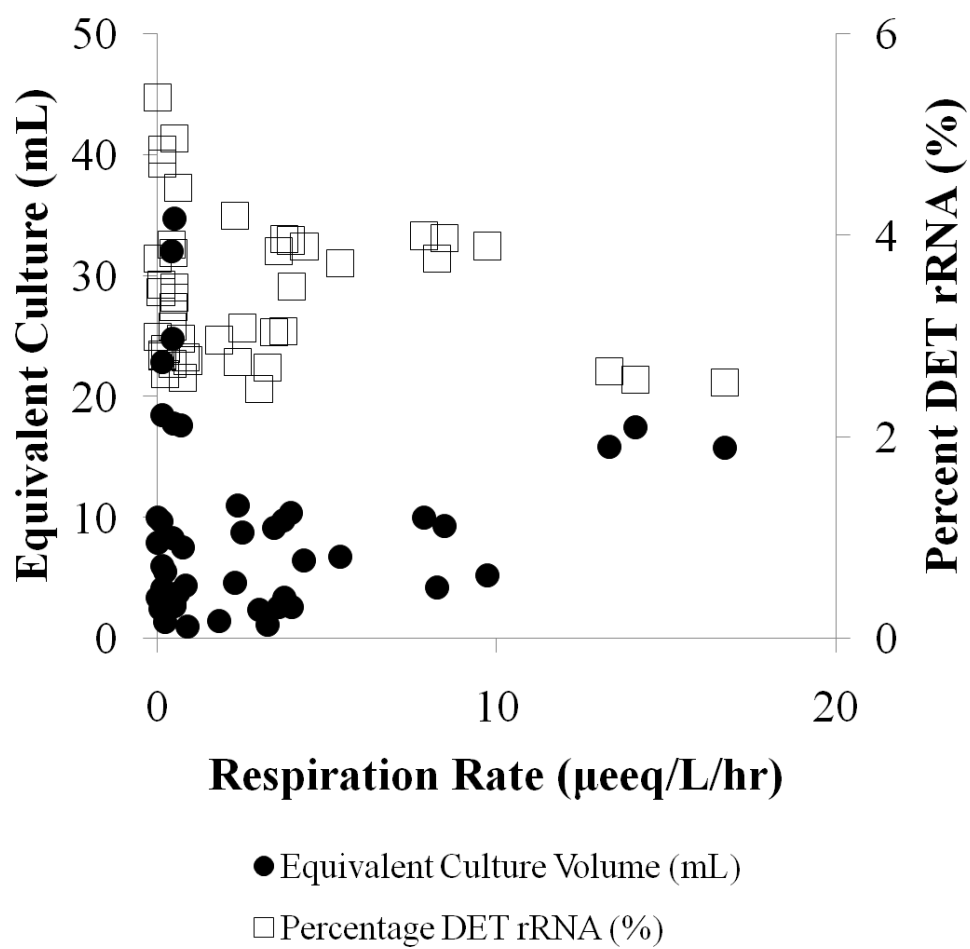


1 Figure 5. RDase distribution across different electron acceptors with the (a) natural value ratio
 2 compared to a 3-day starved culture for the 17 RDase and the *rpoB* transcript is plotted for the
 3 PCE, TCE, DCE, DCP, and No Acceptor fed conditions. Error bars represent standard error
 4 across biological replicates. (b) The absolute processed intensity value distribution of the RDases
 5 for the No Acceptor, DCP, DCE, TCE, and PCE cultures respectively moving inwards from the
 6 outer ring of the pie chart.



Transcript ID and Description	Regulation	Ratio	Transcript ID and Description	Regulation	Ratio	Transcript ID and Description	Regulation	Ratio
DET0014 hypothetical protein	UP	2.7	DET0754 hypothetical protein	UP	2.7	DET1146 hypothetical protein	DOWN	0.36
DET0035 gmk guanylate kinase	DOWN	0.42	DET0755 hypothetical protein	UP	3.8	DET1173 fwdE family protein	UP	2.6
DET0036 hypothetical protein	DOWN	0.44	DET0861 hydrogenase, EchB subunit	DOWN	0.37	DET1186 hypothetical protein	UP	2.9
DET0108 endonuclease/exonuclease/phosphatase	UP	2.2	DET0862 hydrogenase, group 4, EchC subunit	DOWN	0.35	DET1206 metM homoserine dehydrogenase	DOWN	0.34
DET0137 mgsA methylglyoxal synthase	DOWN	0.31	DET0864 hydrogenase subunit HymB	DOWN	0.37	DET1224 cobA 3 cob(I)alamin adenosyltransferase	DOWN	0.43
DET0180 reductive dehalogenase	UP	5.5	DET0908 arsenical pump membrane protein	UP	7.8	DET1278 nusB N utilization substance protein B	DOWN	0.25
DET0181 reductive dehalogenase anchoring protein	UP	5.1	DET0909 hypothetical protein	UP	2.8	DET1284 hypothetical protein	DOWN	0.38
DET0297 hypothetical protein	UP	4.9	DET0945 ABC transporter, permease protein	DOWN	0.43	DET1285 serine protease, DegP/HtrA family	UP	2.9
DET0298 hypothetical protein	UP	4.5	DET0964 recJ single stranded DNA exonuclease RecJ	DOWN	0.38	DET1286 serine protease, DegP/HtrA family	UP	3.9
DET0322 hypothetical protein	DOWN	0.35	DET1021 hypothetical protein	DOWN	0.20	DET1300 universal stress protein family	DOWN	0.27
DET0368 proS prolyl tRNA synthetase	UP	2.7	DET1031 hypothetical protein	DOWN	0.40	DET1377 hypothetical protein	UP	3.0
DET0420 hypothetical protein	DOWN	0.25	DET1035 tryptophan synthase subunit beta	UP	3.2	DET1379 auxin responsive GH3 protein homolog	UP	3.7
DET0423 hypothetical protein	UP	4.2	DET1038 ileS isoleucyl tRNA synthetase	UP	2.5	DET1419 transcriptional regulator, AbrB family	DOWN	0.29
DET0485 rplX ribosomal protein L24	DOWN	0.43	DET1051 hypothetical protein	UP	3.8	DET1448 hypothetical protein	UP	2.8
DET0558 atpB ATP synthase F0, A subunit	DOWN	0.21	DET1057 hypothetical protein	UP	2.3	DET1511 hypothetical protein	DOWN	0.14
DET0560 atpF ATP synthase F0, B subunit	DOWN	0.33	DET1063 DNA binding regulator, LuxR family	UP	2.7	DET1544 reductive dehalogenase anchoring protein	UP	3.5
DET0561 atpH ATP synthase F1, delta subunit	DOWN	0.33	DET1068 recombinase, phage integrase family	DOWN	0.30	DET1545 reductive dehalogenase	UP	3.3
DET0562 atpA ATP synthase subunit A	DOWN	0.41	DET1092 DNA methylase	DOWN	0.41	DET1569 hypothetical protein	UP	3.0
DET0673 hypothetical protein	UP	3.6	DET1105 hypothetical protein	DOWN	0.27	DET1624 hypothetical protein	UP	2.6
DET0721 hypothetical protein	DOWN	0.36	DET1106 hypothetical protein	DOWN	0.33			
DET0736 oxidoreductase, dehydrogenase/reductase	UP	2.7	DET1107 hypothetical protein	DOWN	0.29			

(a)



(b)

