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Energy Conservation Mechanisms for Syntrophic Growth of *Desulfovibrio vulgaris* and *Methanococcus maripaludis*

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ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT

OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

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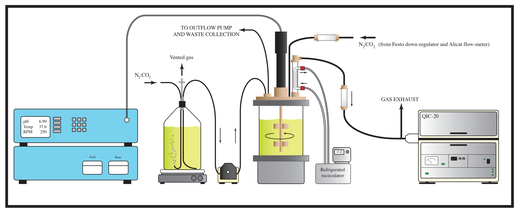
ABSTRACT

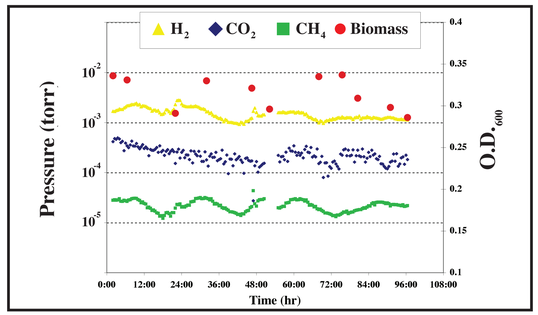
In the absence of electron acceptors, many *Desulfovibrio* species grow on non-fermentable substrates via syntrophic association with hydrogen consuming methanogens. We examined the physiology of *D. vulgaris* Hildenborough growing syntrophically with *Methanococcus maripaludis* LL using a combination of transcriptional and deletion mutant analyses. Syntrophic cocultures were established in chemostats on minimal media amended with lactate but lacking electron acceptor. Replicated whole genome transcriptional analyses identified 169 and 254 genes that were significantly up- or down-regulated, respectively, relative to sulfate-limited monocultures grown at the same generation time. The majority of up-regulated genes were associated with energy production/conservation, signal transduction mechanisms, and amino acid transport/metabolism. A number of the down-regulated genes were associated with signal transduction mechanisms, inorganic ion transport/metabolism and amino acid transport and metabolism. In order to elucidate possible roles of several highly up-regulated genes associated with electron transfer and energy conservation, we constructed mutants of *Desulfovibrio* deleted in a subset of these genes. Cocultures developed with these mutants displayed a range of growth yields, implicating a putative carbon-monoxide induced hydrogenase (Coo, DVU2286-93) and a high-molecular weight cytochrome (Hmc, DVU0531-6) in energy conservation during syntrophic growth. Mutant monocultures grew to the same density on lactate/sulfate as the wildtype. The *cooL* and *hmc* mutants grew significantly slower and to approximately 25% yields of wildtype cocultures. Together, these data suggest a role of these genes in energy conservation of *D. vulgaris* Hildenborough during syntrophic growth.

CHEMOSTAT CONFIGURATION



Chemostats are run using a 24 hr retention time at 37 ºC and a stirring speed of 250 rpm. The headspace of the chemostat is flushed with a mixture of N_2 :CO_2 (90:10) at a rate of 0.20 - 0.50 ml/min. The headspace gas composition is sampled in 15 min. intervals using a Hiden QIC-20 mass spectrometer.

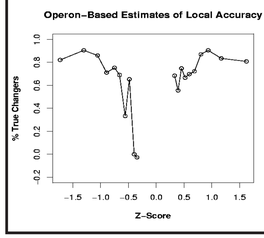
BIOMASS & HEADSPACE GAS MEASUREMENTS

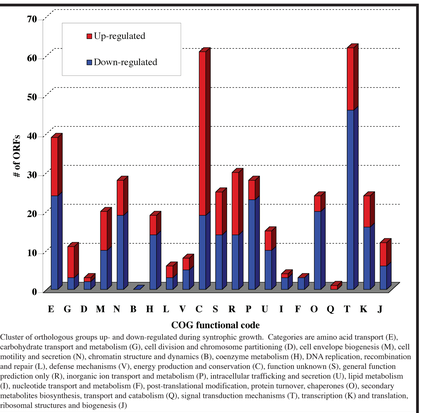


Steady-state was assumed when O.D.$_{600}$ measurements varied by less than 10% of initial value for 3 rentention times. *D. vulgaris*:*M. maripaludis* cell ratio was ~4:1 throughout steady-state as determined by DAPI-stained cell counts.

TRANSCRIPTIONAL ANALYSIS

Triplicate biological replicates of cocultures and sulfate-limited *D. vulgaris* monocultures were analzyed by the ESPP Functional Genomics Core using custom-designed whole-genome microarrays. Microarray slides were designed with duplicate spots of each open-reading frame (ORF) for both organisms. At least three slides were used for each biological replicate. The ESPP Computational Core calculated RNA/DNA expression ratios for each ORF and the log$_2$ ratio comparing the coculture versus sulfate-limited monoculture growth conditions was determined. Z-scores for each ORF were calculated to determine statistical significance. Operon-based estimates of local accuracy indicate an absolute Z-score of 1.0 accurately predicts expression changes between the two conditions. Using this value, 169 ORFs displayed significant up-regulation. 254 ORFs were statistically down-regulated. Genes were assigned clusters of orthologous group (COG) functional codes based on previous genome annotations.





Cluster of orthologous groups up- and down-regulated during syntrophic growth. Categories are amino acid transport (E), carbohydrate transport and metabolism (G), cell division and chromosome partitioning (D), cell envelope biogenesis (M), cell motility and secretion (N), chromatin structure and dynamics (B), coenzyme metabolism (H), DNA replication, recombination and repair (L), defense mechanisms (V), energy production and conservation (C), function unknown (S), general function prediction only (R), inorganic ion transport and metabolism (P), intracellular trafficking and secretion (U), lipid metabolism (I), nucleotide transport and metabolism (F), post-translational modification, protein turnover, chaperones (O), secondary metabolites biosynthesis, transport and catabolism (Q), signal transduction mechanisms (T), transcription (K) and translation, ribosomal structures and biogenesis (J)

TRANSCRIPTIONAL RESPONSE

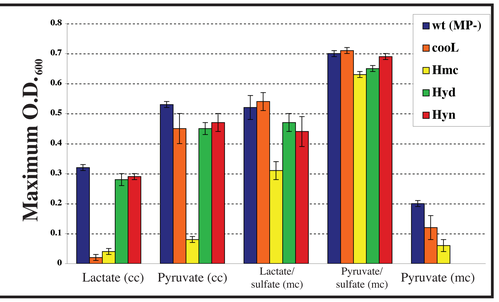
Gene	TIGR annotation	Log R	(Z) score	RNA/gDNA expression ratio in coculture
DVU2285	lactate permease	1.37	1.92	0.44
DVU3025	pyruvate-ferredoxin oxidoreductase (*por*)	1.96	2.51	1.82
DVU1179	aldehyde-ferredoxin oxidoreductase (*aor*)	1.93	1.91	1.64
DVU2287	Coo hydrogenase (*cooL*)	1.67	2.56	1.88
DVU2289	Coo hydrogenase (*cooU*)	1.73	2.77	1.56
DVU2402	heterodisulfide reductase (*hdrA*)	1.42	2.03	0.29
DVU2405	alcohol dehydrogenase	2.94	3.51	3.39
DVU1769	periplasmic [Fe] hydrogenase (*hydA*)	2.33	1.86	0.37
DVU1922	periplasmic [NiFe] hydrogenase (*hynA*)	1.00	1.09	0.23
DVU0536	high-molecular weight cytochrome (*hmcA*)	2.22	2.85	0.54
DVU0531	high-molecular weight cytochrome (*hmcF*)	1.93	2.76	0.26
DVU1295	sulfate adenylyltransferase (*sat*)	0.75	1.17	4.48
DVU0847	adenylyl-sulfate reductase (*aprA*)	1.21	1.20	4.84
DVU0402	dissimilatory sulfite reductase (*dsrA*)	0.94	1.56	3.59
DVU1636	pyrophosphatase (*ppaC*)	1.38	1.82	3.16
DVU0173	thiosulfate reductase (*phsA*)	2.08	3.41	0.48
DVU0053	sulfate permease, putative	-0.84	1.43	0.07
DVU3107	cytochrome c_{553}	-0.82	1.56	0.15
DVU2680	flavodoxin	-5.19	6.67	0.04
DVU0694	molybdopterin oxidoreductase	0.98	1.73	0.56
DVU0599	carbon starvation protein A, putative	2.11	2.77	0.82
DVU2652	hypothetical protein	3.07	4.67	2.12
DVU2655	D-alanyl-D-alanine carboxypeptidase	0.75	1.17	0.95

Gene	TIGR annotation	Log R	(Z) score	RNA/gDNA expression ratio in coculture
DVU2297	glycine/betaine/L-proline ABC transporter	-2.48	3.69	0.45
DVU2298	glycine/betaine/L-proline ABC transporter	-1.99	2.84	0.27
DVU2299	glycine/betaine/L-proline ABC transporter	-2.22	2.90	0.20
DVU2300	hypothetical protein	-1.05	1.20	1.73
DVU2571	ferrous iron transport protein (*feoB*)	-4.71	6.36	0.15
DVU2572	ferrous iron transport protein (*feoA*)	-4.60	7.29	.016
DVU2383	tonB dependent receptor domain protein	-4.37	6.14	0.03
DVU2384	ABC transporter	-2.44	2.5	0.14
DVU2386	ABC transporter	-1.05	1.46	0.05
DVU2390	tonB domain protein	-2.34	3.23	0.09
DVU3329	hypothetical protein	-3.00	5.08	0.03
DVU3330	conserved hypothetical proteins	-4.66	6.70	0.07
DVU3331	hypothetical protein	-3.89	5.51	0.05
DVU3332	heavy metal translocating P-type ATPase	-2.15	3.38	0.05
DVU3333	hypothetical protein	-3.11	5.06	0.04
DVU1592	methyl-accepting chemotaxis protein	-1.47	2.51	0.09
DVU1593	chemotaxis protein (*cheY-4*)	-1.61	2.62	0.12
DVU1594	chemotaxis protein (*cheA-3*)	-1.63	2.63	0.10
DVU0806	chemotaxis protein CheYL putative	-2.59	3.01	0.74

PHENOTYPIC DIFFERENCES BETWEEN *D. VULGARIS* MUTANTS

Based on transcriptional data, four *D. vulgaris* Hildenborough mutants were compared with the the megaplasmid minus wildtype strain (all mutants lack MP). Cocultures (cc) and monocultures (mc) were examined for a variety of growth conditions.

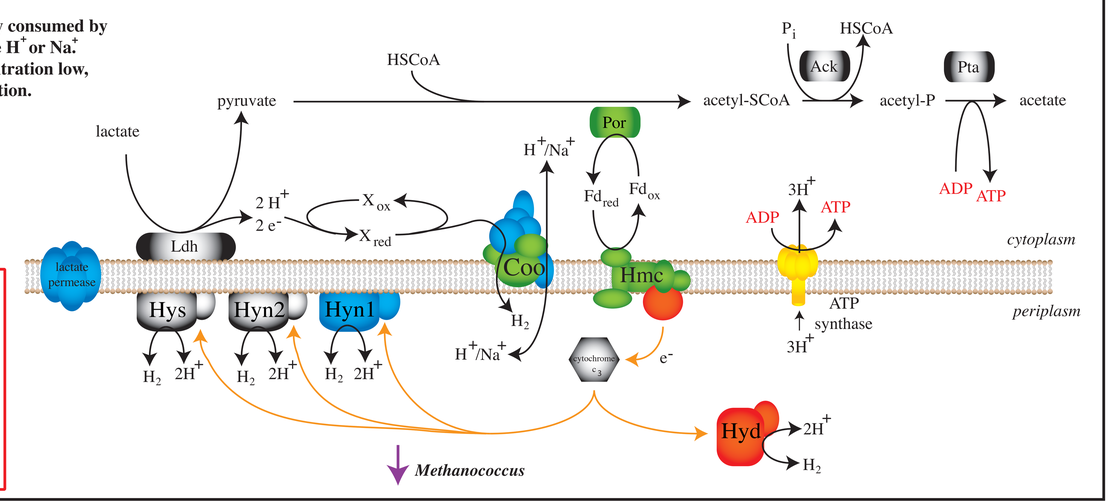
Mutant	Description
cooL	transposon-interrupted mutant of [NiFe] hydrogenase small subunit contained within the CO-induced hydrogenase operon
Hmc	high-molecular weight cytochrome deletion mutant (*hmcBCDE*)
Hyd	[Fe] hydrogenase deletion mutant (*hydAB*)
Hyn	[NiFe] hydrogenase deletion mutant (*hynAB*)



ENERGETIC OVERVIEW

Lactate ⟶ pyruvate + H_2

- **Energetically unfavorable unless pyruvate and/or H_2 kept at low concentrations.**
- **Coo acts to produce hydrogen (likely consumed by the methanogen) and translocate H^+ or Na^+.**
- **Hmc serves to keep pyruvate concentration low, allowing continued lactate oxidation.**



FUTURE WORK

- Develop syntrophic growth pathway on other compounds, such as ethanol.
- Refine metabolite interaction model between *D. vulgaris* and *M. maripaludis* LL using transcriptional, proteome and phenotypic analyses.
- Explore salt, nitrate, pH, etc., stress response of coculture.
- Compare responses with other organisms, especially other *Desulfovibrio* strains.

ACKNOWLEDGMENT

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