Characterization of Stress Response in a Sulfate Reducer/Methanogen Coculture

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ESPP2 Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

VIMSS Virtual Institute for Microbial Stress and Survival

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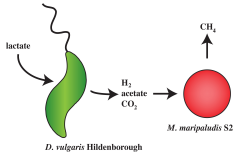
Abstract

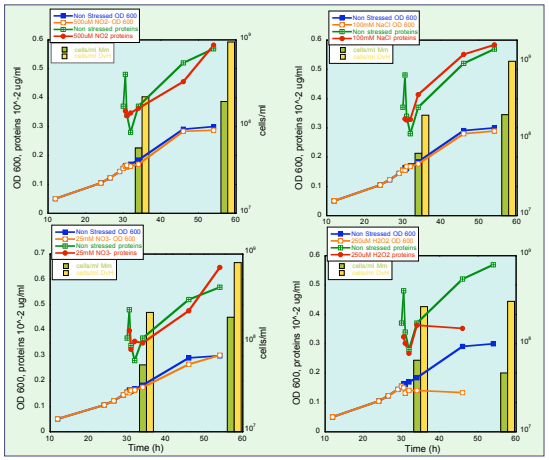
Sulfate-reducing bacteria and methanogens are found to coexist in a variety of anoxic marine sediments. In these systems they either compete for substrates or engage in successful syntrophic relationships. In our experimental setup, *Desulfovibrio vulgaris* Hildenborough ferments lactate, producing acetate and hydrogen. *Methanococcus maripaludis*, a hydrogenotrophic methanogen, then utilizes hydrogen while also incorporating limited amounts of acetate as a carbon source. Mid-log growth phase of this co-culture is achieved in 3 days growing at 37°C at which point, nearly 50% of the initial lactate was depleted. In this study we investigate the stress response of this coculture and compare it to the *D. vulgaris* monoculture. Minimum Inhibitory Concentration (MIC) determinations of two environmentally relevant stressors (NO_3^- and NaCl) on the coculture and monoculture suggest nitrate predominantly affects *M. maripaludis* with a MIC of 25mM while sodium stress affects *D. vulgaris* with a MIC of 100mM. The response of the coculture to stressors like nitrate, nitrite, salt and peroxide was monitored by several methods. The fate of metabolites was tracked in the cultures and rates of gas evolution/utilization were measured with the Micro-Oxymax. Total biomass was measured over time with direct cell counts (including ratios of SRB: methanogen), cell protein and optical density. Metal reducing capability of log phase co-culture under NO3 stress was investigated and compared to that of under NaCl stress. Phenotype Microarray substrate utilization profiles generated by the Omnilog technology for a variety of metabolic substrates showed differential profiles for the coculture and the monoculture. Whole-genome transcriptional analysis of NaCl stressed coculture indicates up-regulation of genes coding for numerous transmembrane electron transfer enzymes.

Coculture Syntrophy

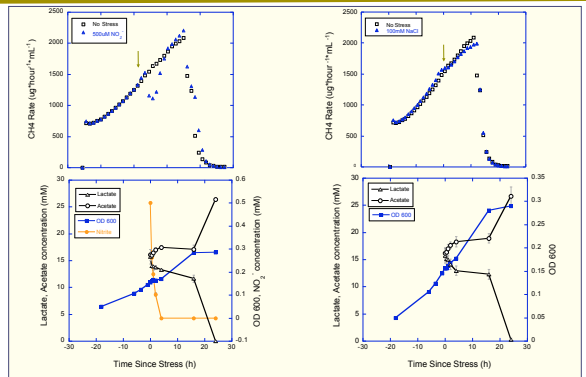


Physiology: Cell Density, Total Proteins, ODs Post Stress

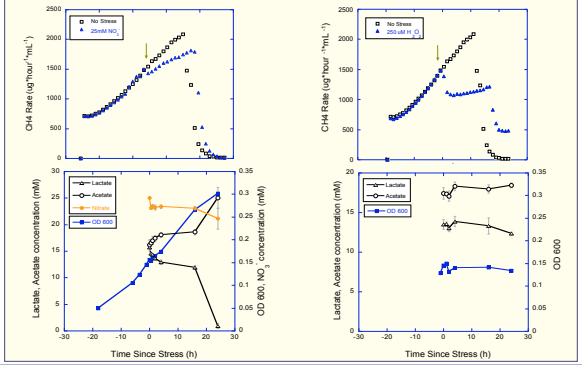
Stress addition to mid-log phase cocultue was followed by sampling for IC analysis, total proteins, cells/ml by AODC and Optical Density at 30m, 60m, 120m, 240m, 3h (IC only), 16h and 24h.



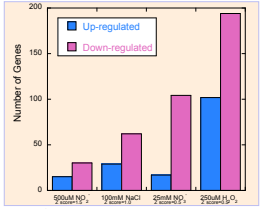
Micro-Oxymax & IC: Gas Evolution & Metabolite Profile



Stressors were added to mid log phase cultures followed by continuous sampling for metabolites and anions measured by IC.
Growing cultures were continuously monitored for headspace methane until mid-log phase at which point stressors were added followed by continued headspace methane measurements. Methane was measured with the Micro-Oxymax Respirometer made by Columbus Instruments. The headspace in the experimental chamber is periodically pumped through a closed system to the methane sensor, which uses a single beam infrared device to detect changes in gas percentage.



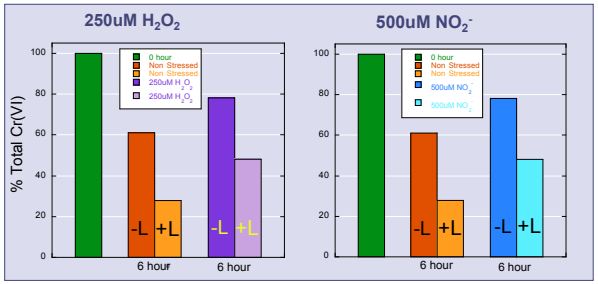
Transcriptomics



Top 10 Up-regulated Genes in Stressed Co-culture Compared to Non-stressed Control

Stressor was added to mid-log phase coculture and harvested 2h post stress for transcriptomic analysis.

Chromium Reduction with H_2O_2 & NO_2^- Stress



Coculture suspension was treated with 20mM lactate or not treated with lactate as an abiotic control and spiked with 200uM Cr(VI). Samples were analyzed by a colorimetric DPC assay.

Conclusions

Minimum inhibitory concentrations were determined for the stressors. At these concentrations the generation time of the coculture was doubled. General physiological response shows that the culture was able to recover to some degree with the exception of oxidative stress. SRB: Methanogen ratios remained fairly consistent over time with the added stressors indicating that neither organism was significantly more hindered by the added stressor than its partner or that cell ratios must remain consistent for the syntrophic interaction to occur.

Rates of methane generation and metabolite profiles detail perturbation of growth and in most cases partial recovery of respiration after stressor addition. Lactate consumption is complete in all cases of recovery whereas methane generation recovers to varying degrees, suggesting differing capacities for recovery by each organism in the coculture. For NO_2^-, complete recovery is seen upon the depletion of NO_2^- from the system which may be an abiotic effect. H_2O_2 physiology shows that the culture does not recover after stressor addition, however slight carbon consumption and steady methane evolution continue until the end of the non stressed control culture life cycle.

Non stressed coculture reduced up to 72% Cr(VI) in a 6 hour period as compared to the abiotic reduction of 38%. H_2O_2 and NO_3^- stressed coculture showed decreased reduction resulting in 56% & 52% of initial added Cr(VI) for H_2O_2 and NO_3^- respectively as compared to the non stressed coculture.

Up and down- regulated genes with a Z score above 1.5 were found only with the NO_2^- stress condition.

Relevant Publications

Walker Et al. 2008. Cooperation at thermodynamic edge : Metabolic coupling between Archaea and Bacteria uses a novel electron transfer system. Submitted.

Stolyar et al. 2007. Metabolic modeling of a mutualistic microbial community. Molecular Systems Biology 3:92.

Mukhopadhyay et al. 2006. Salt Stress in *Desulfovibrio vulgaris* Hildenborough: an Integrated Genomics Approach. Journal of Bacteriology 188:11 p. 4068-4078.

Acknowledgement

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