Metabolic and membrane adaptations of the hydraulically fractured shale isolate Halanaerobium in response to temperature and growth rate fluctuations under continuous culture

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Project Goals: The injection of fluids and proppants to fracture the deep shale introduces microbial cells and substrates to low-permeability rocks. Microorganisms in hydraulically fractured wells govern biogeochemical reactions and often produce acids and sulfides, leading to corrosion and gas souring, and form biofilms, resulting in clogging and fouling events. The overarching goal of this research is to advance our comprehension of the microbial diversity and function in non-sterile hydraulically fractured wells. Our current understanding of microbial growth within fractured hydrocarbon-bearing rock is based primarily on genomic information, we identified three specific objectives that will shed light on in situ physiologies and kinetic rates, governing biogeochemical reactions: (1) characterize variables influencing growth parameters and membrane features of shale taxa, (2) characterize interactions between shale matrices and microorganisms, and (3) elucidate engineered and environmental processes driving biogeochemical signatures at field scale.

Abstract: Here we characterized the physiology of *Halanaerobium congolense* WG10, a dominant taxon isolated from a 2.5-km deep hydraulically fractured natural gas well in Ohio, for the first time under continuous culture (chemostat) conditions. The anaerobe *H. congolense* WG10 was cultivated at 20% salinity under three growth rates (hydraulic retention times (HRTs) of 48, 24, and 19.2 hrs) and two temperatures (25ºC and 40ºC) under complete control of system pH and redox conditions using a 1-L Sartorius Biostat® Q-plus system. We applied an integrative 'omics approach to characterize metabolomic, proteomic, and lipidomic features (MPLEx analysis) and quantify metabolite production (using ^{1}H-NMR and GC-FID) under steady state growth rates of 0.021 to 0.052 1/hr. Our experiments highlighted 1.5 fold increased biomass at higher dilution rates (HRT 19.2 hrs) and warmer temperatures (40ºC) as compared with slower dilution rates (HRT 48 hr) and cooler temperatures (25ºC).

Proteomics analysis showed a total of 2,227 out of 2,800 predicted protein-coding genes (79.5%) were identified in our data set. Among those proteins identified, 356 were found to be significantly higher in abundance in one or more treatments (Student's t test, $p < 0.05$). Of these, 91 proteins were identified during cooler temperature growth (25ºC) while 109 were identified in growth at warmer temperature (40ºC). An additional 71 were in greater abundance when *H. congolense* WG10 was grown at 40°C in the highest dilution rate (HRT 19.2 hrs). When grown at 25ºC, cells exhibited cold shock proteins (CspA family, WG10-13112 -12818), a typA, bipA GTP binding protein involved in stress response (WG10-10337), and a nucleotide-binding universal stress protein (UspA family, WG10-10469). Both proteomic and metabolic data supported significant activity for the utilization of 1,3- propanediol, especially in warmer temperatures (40ºC) and longer HRTs (24 and 48 hrs). Proteins associated with the methylglyoxal bypass pathway (e.g.

glyoxalase) and two subunits of the propanediol dehydratase (PduD, PduE), which catalyzes the formation of propionaldehyde from 1,3-propanediol, were important during lower temperature growth (25ºC), suggesting this pathway is activated under stress. The propanediol dehydratase is a cobamide-dependent enzyme that has been shown to also dehydrate ethylene glycol to acetaldehyde. The formation of both propionaldehyde and acetaldehyde was confirmed with both ^{1}H-NMR and GC-FID analysis. In addition to aldehydes, we identified ketones (acetone), volatile fatty acids (acetate, lactate, formate), alcohols (ethanol, propanol), and amino acids (alanine, valine) metabolites via ^{1}H-NMR and GC-FID. Lipidomics analysis and fatty acid methyl ethyl analysis is currently underway to characterize key membrane lipids for these treatments. In parallel with experimental efforts, produced fluid samples were collected from hydraulically fractured shale wells from MSEEL II, a DOE NET-funded field research site in West Virginia for analysis of intact polar lipids and phospholipids. Our continuous culture MPLEx approach sheds new light on the metabolism and membrane features of *Halanaerobium* under biogeochemical drivers relevant to engineered shale.

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