Hydrogen Syntrophy Among Hyperthermophilic Archaea: A Model of Early Cell-Cell Interactions.

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Introduction: Microbes are the most likely forms of life beyond Earth, and hyperthermophilic archaea are among the best extant representatives of early life on Earth [1-4]. Most laboratory-based studies on the evolution and adaptation of organisms to extreme environments, like those thought to have existed early in Earth's history (e.g., hydrothermal environments), have focused on pure cultures. Our understanding of interspecies cell-cell interactions among extremophiles is very limited. It is likely that hyperthermophilic archaea diversified their cellular functions and became influenced by, and perhaps even dependent upon, each other under certain environmental conditions. This study quantitatively models interspecies cell-cell interactions among two hyperthermophilic archaea to better understand how life adapts to different environments.

We focus on hydrogen syntrophy among two hyperthermophilic archaea. Hydrogen gas is predicted to be too low to support the growth of hyperthermophilic methanogens in many basalt-hosted deep-sea hydrothermal vent systems. Yet, these organisms can be found at low concentrations at these sites. Hydrothermal fluids are not the only source of H_2 in this environment. Hyperthermophilic, anaerobic heterotrophs produce H_2 that can be used as the sole source of their electrons for growth by methanogens. We examined H_2 syntrophy between a H_2-producing hyperthermophilic heterotroph, *Thermococcus paralvinellae*, and a H_2-consuming hyperthermophilic methanogen, *Methanocaldococcus bathoardescens*.

H_2 syntrophy at Axial Seamount: In 2014, our laboratory tested for H_2 syntrophy at 55°C and 80°C among natural assemblages of microbes using three 20-35°C hydrothermal fluids collected from Axial Seamount in the northeastern Pacific Ocean. Hydrothermal fluids were incubated anaerobically in duplicate with either 1.6 atm H_2 (1.2 mM), 0.5% (wt vol^{-1}) tryptone (protein) + 1.6 atm N_2, or 0.03 atm H_2 (20 µM) + 1.57 atm N_2 added as the energy source for growth (each bottle also contained 0.4 atm CO_2). Eight of the 12 incubations with 1.2 mM H_2 produced on average up to 9 mmol l^{-1} of CH_4, and 8 of the 12 incubations with tryptone produced up to the same amount of CH_4. Two of the 12 incubations with 20 µM H_2 produced up to 0.25 mmol l^{-1} of CH_4. There was no growth or CH_4 production when neither H_2 nor organics were supplied. The results indicate that methanogenesis at thermophilic temperatures is limited by H_2 availability, and that heterotrophs can provide the substrates (presumably H_2) necessary for growth and methanogenesis in the absence of added H_2.

H_2 syntrophy among model organisms: We established a co-culture of *T. paralvinellae* and *M. bathoardescens* that grow together in serum bottles at 82°C. *T. paralvinellae* produced up to 9.5 mmol of H_2 l^{-1} when grown alone on 0.5% tryptone + 0.5% (wt vol^{-1}) maltose (a carbohydrate). It produced up to 1.1 mmol of H_2 l^{-1} when grown in co-culture with *M. bathoardescens* on the same medium at 82°C. *M. bathoardescens* produced up to 9.4 mmol of CH_4 l^{-1} when grown in co-culture. This experiment indicates that *M. bathoardescens* used the H_2 produced by *T. paralvinellae* for growth and CH_4 production in co-culture.

Conclusion: To better predict when, how, and to what extent their cellular functions are influenced by H_2 syntrophy, we are developing constraint-based metabolic network models for *T. paralvinellae* and *M. bathoardescens*. Complete genomes of both organisms were sequenced, and we are in the process of curating constraint-based metabolic models for both organisms individually and in co-culture. These models are used to estimate growth, catabolism, biosynthesis, H_2 production and consumption, CH_4 production, and energy requirements of the organisms when grown in pure culture and co-culture under optimal and inhibitory conditions. Growth and H_2 and CH_4 production rates for both organisms grown alone and in co-culture are determined in a chemostat to provide kinetic constraints for the model. Gene expression profiling using RNA-Seq are used to constrain the metabolic reactions and pathways used by the cells under varying conditions.

References: [1] Martin W. et al. (2008) *Nature Rev Microbiol*, 6, 805-814. [2] Kelley D.S. et al. (2002) *Ann Rev Earth Planet Sci*, 30, 385-491. [3] Stetter K.O. (2006) *Proc Natl Acad Sci USA*, 361, 1837-1843. [4] Nisbet E.G. and Sleep N.H. (2001) *Nature*, 409, 1083-1091.