

## Growth kinetics and gene expression in a hyperthermophilic methanogen during H<sub>2</sub>-limited and syntrophic growth

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Thermophilic methanogens are primarily H<sub>2</sub> limited in hot subsurface environments [1,2] but persist in low H<sub>2</sub> hydrothermal vents, methane seeps, and petroleum reservoirs. This may be due to physiological adaptations to low H<sub>2</sub> conditions and a reliance on H<sub>2</sub> syntrophy for survival. The hyperthermophilic methanogen *Methanocaldococcus jannaschii* was grown in a chemostat at high (80 μM) and low (15-30 μM) aqueous H<sub>2</sub> concentrations and syntrophically in bottles with the hyperthermophilic, heterotrophic H<sub>2</sub> producer *Thermococcus paralvinellae*. The purpose was to measure changes in growth parameters and gene expression in *M. jannaschii* with changes in H<sub>2</sub> flux. Growth rates and cell-specific CH<sub>4</sub> production rates of *M. jannaschii* decreased with decreasing H<sub>2</sub> availability, and decreased further during syntrophy. Growth yield (biomass produced per mole of CH<sub>4</sub> produced) however increased significantly when *M. jannaschii* was grown on low H<sub>2</sub>, but were at their lowest levels during syntrophy. Differential gene expression analyses using RNA-Seq showed that the enzyme responsible for the reduction of methenyl group to a methylene group during carbon fixation switches from a H<sub>2</sub>-dependent enzyme to a coenzyme F<sub>420</sub>-dependent enzyme with decreasing H<sub>2</sub> availability and into syntrophy. During syntrophy, the genes for energy generation on the membrane decreased in their expression levels. The results suggest that *M. jannaschii* cycles H<sub>2</sub> internally for CH<sub>4</sub> and energy production in high H<sub>2</sub> environments but increases its carbon use efficiency under low H<sub>2</sub> conditions, and produces CH<sub>4</sub> at low rates when dependent on H<sub>2</sub> syntrophy.

*T. paralvinellae* produced formate when inhibited by excess H<sub>2</sub> [3]. *T. paralvinellae* growth rates were the same during monoculture and syntrophic growth, but formate was only produced in monoculture suggesting amelioration of H<sub>2</sub> inhibition during syntrophy.

[1] Ver Eecke, H.C., *et al.* (2012) *PNAS*, 109:13674-13679.

[2] Topçuoğlu, B.D., *et al.* (2016) *Front. Microbiol.* 7:1240.

[3] Topçuoğlu, B.D., *et al.* (2018) *Environ. Microbiol.* 20:949-957.