Non-Deterministic Factors Affect Competition Between Thermophilic Autotrophs from Deep-Sea Hydrothermal Vents

Briana C. Kubik[‡], James F. Holden[‡]

‡ University of Massachusetts, Amherst, United States of America

Corresponding author: James F. Holden (jholden@umass.edu)

Abstract

Hydrothermal vents provide windows into the rocky subseafloor on Earth and serve as terrestrial analog sites for extraterrestrial environments. By studying patterns of community assembly in hydrothermal vents and using geochemical models, we can better understand how the deep-sea biosphere contributes to local and global biogeochemical cycling and gather valuable information about how similar communities may arise on Earth and beyond Earth. One prevailing thought is that vent microbial community assembly is driven by deterministic factors such as the thermodynamic favorability of redox reactions. We hypothesized that subsurface microbial communities may also be significantly influenced by other factors, such as differential cell yields, varying optimal growth temperatures, and stochasticity.

At Axial Seamount in the Pacific Ocean, H_2-consuming methanogens of the genera *Methanocaldococcus* (T_{opt} 82°C) and *Methanothermococcus* (T_{opt} 65°C) and H_2-consuming sulfur reducers of the genus *Desulfurobacterium* (T_{opt} 72°C) are the most abundant autotrophs that grow optimally at or above 65°C (Fortunato et al. 2017). At one low-temperature hydrothermal vent site, Marker 113, methanogens are the predominant thermophilic autotrophs while at another site, Marker 33, thermophilic autotrophic sulfur reducers predominate. There is no apparent geochemical or thermodynamic explanation for the differences in community composition. In this study, we performed a series of co-culture competition experiments using *Methanocaldococcus jannaschii*, *Methanothermococcus thermolithotrophicus*, and *Desulfurobacterium thermolithotrophum* HR11 as representative methanogens and sulfur reducers common to hydrothermal vents to explain the variations in community composition between thermophilic autotrophs.

M. jannaschii increases its cell yield (cells produced per mole of CH_4 produced) when grown on very low H_2 concentrations as part of a growth rate-growth yield tradeoff (Topçuoğlu et al. 2019). This increase in cell yield could provide methanogens with a

competitive growth advantage over H_2-consuming sulfur reducers, who otherwise catalyze a more thermodynamically favorable growth reaction. Competition co-culture experiments were conducted between *M. jannaschii* and *D. thermolithotrophum* at 72°C and between *M. thermolithotrophicus* and *D. thermolithotrophum* at 65°C, both at 1:1 ratios and initial aqueous H_2 concentrations of 1.2 mM (high H_2) and 85 µM (low H_2) to determine the effects of temperature and H_2 availability on autotroph competition. For both methanogens, the growth rate, maximum cell concentration, and total CH_4 produced decreased when they were grown in co-culture, at low H_2, or both relative to monocultures grown with high H_2. The methanogen cell yields generally increased in co-culture and at low H_2. At both experimental temperatures, the growth rate of *D. thermolithotrophum* remained unchanged in co-culture and at low H_2 relative to monocultures but the maximum cell concentration decreased in co-culture relative to monocultures at both H_2 concentrations. However, at low H_2, both in mono- and co-culture, there was no detectable H_2S produced by the sulfur reducer suggesting a significant shift in growth yield. At both temperatures and H_2 concentrations, the sulfur reducer reached higher cell concentrations than the methanogens.

Stochasticity or vent fluid chemistry could lead to early colonization of a vent by methanogens followed by niche exclusion of autotrophic sulfur reducers due to a numerical advantage of the methanogens. Therefore, competitive co-culture experiments were run as before at high H_2 with varying initial methanogen:sulfur reducer ratios. At 72°C, *D. thermolithotrophum* reached the same maximum cell concentration and produced the same amount of H_2S in monoculture and co-culture even when the methanogens initially outnumbered the sulfur reducer up to 10,000-fold. *M. jannaschii* reached a lower maximum cell concentration and produced less CH_4 in all co-cultures relative to growth in monoculture. At 65°C, *D. thermolithotrophum* reached the same maximum cell concentrations and produced the same amount of H_2S in monoculture and co-culture when the methanogens initially outnumbered the sulfur reducers up to 100-fold. However, when the methanogens initially outnumbered the sulfur reducers 1,000-fold, *M. thermolithotrophicus* grew as well as in monoculture and the maximum cell concentration and amount of H_2S produced by *D. thermolithotrophum* was significantly lower than in monoculture and the other co-culture conditions.

In conclusion, both methanogens and sulfur reducers shift their redox reactions away from CH_4 and H_2S production, respectively, and towards biomass production when H_2 is limiting. This should be accounted for in thermodynamic predictive models. Furthermore, a combination of growth temperatures lower than the optimum of sulfur reducers and high initial methanogen cell concentrations relative to sulfur reducers can lead to a long-term predominance of methanogens over autotrophic sulfur reducers in vent environments through niche exclusion.

Presenting author

James F. Holden

Presented at

Oral

Conflicts of interest

The authors have declared that no competing interests exist.

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