Growth kinetics of a deep-sea thermophilic, hydrogenotrophic bacterium that reduces S<sub>2</sub>O<sub>3</sub><sup>2</sup>-, S° and NO<sub>3</sub>-. Lucy C. Stewart<sup>1</sup>, James Llewellyn<sup>2</sup>, and James F. Holden<sup>3</sup>, <sup>1</sup>University of Massachusetts, Amherst, lstewart@microbio.umass.edu, <sup>2</sup>University of Massachusetts, Amherst, <sup>3</sup>Dept. of Microbiology, N203 Morrill IV, 639 N. Pleasant St., University of Massachusetts, Amherst, MA 01003, jholden@microbio.umass.edu

**Introduction:** Deep-sea hydrothermal vents are considered an important analogue environment for potentially habitable extraterrestrial sites, such as Europa and Enceladus, as well as for the early Earth and early Mars [1]. Low-temperature venting at deep-sea hydrothermal vent sites also provides a window into the deep subsurface and the microbes that inhabit it [2]. Hydrogenotrophic chemolithoautotrophs are thought to be important primary producers both in near-surface deepsea hydrothermal vent environments and the deep subsurface [3]. Hydrogenotrophs found in diffuse hydrothermal venting include sulfur-reducers, iron-reducers, and methanogens [4]. For all of these organisms, hydrogen is often the limiting substrate, for which they are in competition. In order to understand the distribution of hydrogenotrophs in these extraterrestrial analog environments, it is necessary to characterize their substrate requirements and growth kinetics to determine when and where they are likely to co-exist or outcompete each other. Studies of mesophilic freshwater sulfate-reducing bacteria (SRB) and methanogens suggest that SRB will generally outcompete methanogens for hydrogen[5][6], but there is limited data available on the substrate requirements and growth kinetics of typical sulfur-reducing hydrogenotrophs from deep-sea hydrothermal venting, which are usually thermophilic or hyperthermophilic.

We have characterized a thiosulfate-, elemental sulfur, and nitrate-reducing thermophilic hydrogenotroph, *Desulfurobacterium* sp. HR11, which was isolated from a low-temperature hydrothermal vent which also contains thermophilic, hydrogenotrophic methanogens. We measure the Monod kinetics of this strain's growth for limiting hydrogen and thiosulfate and compare them to previously-measured minimum hydrogen requirements for hyperthermophilic, hydrogenotrophic methanogens from deep-sea hydrothermal vents.

**Results:** *Desulfurobacterium* strain HR11 was isolated from the Endeavour Segment of the Juan de Fuca Ridge, and is closely related to the genus type strain, *Desulfurobacterium thermolithotrophum*. It has an optimum temperature of 75°C (range 40-77°C), an optimum pH of 6.0-7.0 (range 5.0-8.5), and an optimum NaCl concentration of 3.0-4.0% (w/v) (range 1.0-5.0%).

It grows chemolithoautotrophically with hydrogen as its sole electron donor and elemental sulfur, thiosulfate, or nitrate as an electron acceptor. When grown on thiosulfate, it has a  $V_{max}$  of 2.01 (~20 min doubling

time), a  $H_2$   $k_s$  of 29  $\mu$ M, and it has a minimum  $H_2$  requirement of ~3 $\mu$ M. When grown with excess hydrogen (>100  $\mu$ M) its  $k_s$  for thiosulfate is 19  $\mu$ M and it can grow on as little as 5  $\mu$ M.

**Discussion:** We find that where sufficient thiosulfate or sulfur is present, HR11 will outcompete thermophilic methanogens for hydrogen, as it has a much lower hydrogen requirement compared to previously-measured values [7]. Its distribution is likely controlled by the availability of sulfur and/or thiosulfate as electron acceptors, as they are of limited availability at many hydrothermal vent sites. Where these are of low concentration, methanogens will outcompete these organisms for hydrogen. Additionally, if nitrate is the only available electron acceptor, HR11 will not outcompete methanogens, as its growth even on levels of nitrate significantly above those found in seawater or diffuse hydrothermal fluid is comparatively slow.

This is an important indicator of how competition for hydrogen may play out in environments where hydrogenotrophic autotrophs are primary producers, such as much of the deep subsurface.

## **References:**

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