

Iron Reduction by Hyperthermophilic Archaea from Hydrothermal Vents: Environmental Constraints to Physiological Potential

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Introduction: Microbial iron reduction is an important biogeochemical process in the anoxic subsurface [1]. It may have been an important process on the early Earth, and could be a means for life beyond Earth [2]. High-temperature anaerobes are common in deep-sea hydrothermal vents, but microbial iron reduction in vents has been largely overlooked due to the reduced nature of most minerals present and the insolubility of Fe³⁺ in circumneutral fluids. However, iron oxide minerals are common in mildly reducing hydrothermal systems due to seawater oxidation of iron sulfide minerals and could serve as an oxidizing agent for microbial respiration [3]. Indeed, hyperthermophilic iron reducers are ubiquitous in hydrothermal sulfide deposits collected from the Endeavour Segment hydrothermal vents in the northeastern Pacific Ocean [4].

Here, we examine the microbial community compositions of three hydrothermal chimneys from the Endeavour Segment and correlate these with mineralogy to determine likely habitats for anaerobes; describe the growth characteristics of a novel hyperthermophilic, autotrophic iron reducing archaeon; and identify its key physiological traits based on whole genome sequencing.

Anaerobes in Hydrothermal Chimneys: A 282°C venting chimney from the Bastille edifice at Endeavour was composed primarily of sulfide-bearing minerals, and its microbial community was most closely related to anaerobes of the *Deltaproteobacteria*, especially sulfate reducers, and anaerobic hyperthermophilic archaea, including iron reducers. In contrast, a 300°C venting chimney from Dante and a 321°C venting chimney from Hot Harold contained primarily the sulfate-bearing mineral anhydrite, and their microbial communities were most closely related to aerobic *Beta*-, *Gamma*-, and *Epsilonproteobacteria*. The predominance of anaerobes in the Bastille chimney indicates that environmental conditions within sulfide edifices may promote anaerobic metabolisms.

Characteristics of *Pyrodictium* sp. Su06. A hyperthermophilic iron reducer, *Pyrodictium* sp. Su06, was isolated from an active hydrothermal chimney from Endeavour [4]. It is a mildly acidophilic (pH_{opt} 5), hydrogenotrophic autotroph with optimal growth temperatures of 90-92°C [5]. Mössbauer spectroscopy of the iron oxides before and after growth demonstrated that it

forms nanophase (<12 nm) magnetite [Fe₃O₄] from laboratory-synthesized ferrihydrite [Fe₁₀O₁₄(OH)₂] with no detectable mineral intermediates. It remains unclear if the conversion is 100% or if the magnetite merely coats the ferrihydrite grains. *Pyrodictium* sp. Su06 produced up to 40 mM Fe²⁺ in a growth-dependent manner, while all abiotic and biotic controls produced < 3 mM Fe²⁺ [5]. On-going experiments are testing the types and grain sizes of iron oxide minerals that can be used by this organism to model hyperthermophile mineral transformations in their native habitats. We are using many different types of characterization to probe the mineralogy: x-ray diffraction, Mössbauer spectroscopy, and x-ray absorption spectroscopy at the just-commissioned NLSL II at Brookhaven National Laboratory.

In order to better understand the physiological potential of strain Su06, its complete genome was sequenced and shown to be 2.0 Mbp and contain 2,172 protein coding genes. These include genes encoding for a membrane-bound hydrogenase and V-type ATP synthases, and 8 proteins with at least three CXXCH cytochrome *c* binding motifs (which are generally used by mesophilic iron reducers). Remarkably, 1,120 (52%) of its protein coding open reading frames (ORFs) encode for hypothetical proteins demonstrating how little is known about the physiology of this organism. Phylogenetic analysis based on 16S rRNA gene sequences showed the strain is more than 97% identical to all other *Pyrodictium* and *Hyperthermus* species, but phenotypically it is most like *Pyrodictium*. Overall Genome Relatedness Index (OGRI) analyses however clearly indicate that the strain is a new species.

Our group is working to effectively combine initial mineral characterizations of hyperthermophilic iron reducers with sophisticated methods for understanding the chemistry of iron reduction, combined with an understanding of physiology.

References: [1] Lovley D. R. et al. (1987) *Nature*, 330, 252-254. [2] Vargas et al. (1998) *Nature*, 395, 65-67. [3] Kristall et al. (2006) *Geochem Geophys Geosyst* 7, Q07001. [4] Ver Eecke et al. (2009) *Appl Environ Microbiol*, 75, 242-245. [5] Lin et al. (2014) *Geobiology*, 12, 200-211.