

**Microbiological and genomic analysis of a terrestrial subsurface Fe(II)-silicate based lithotrophic microbial community.** S. A. Napieralski<sup>1,3</sup>, H. L. Buss<sup>2</sup>, and E. E. Roden<sup>1,3</sup>. <sup>1</sup>Department of Geoscience, University of Wisconsin, Madison, Wisconsin, 53706, USA (snapieralski@wisc.edu), <sup>2</sup>School of Earth Sciences, University of Bristol, Bristol, BS8 1RJ, UK. <sup>3</sup>NASA Astrobiology Institute, University of Wisconsin, Madison, WI 53706, USA,

The Earth's crust is dominantly composed of silicate rocks, many of which contain ferrous iron [Fe(II)] that constitutes a vast energy reservoir for microorganisms both in near-surface and deep subsurface environments. It is postulated that the subsurface microbial biosphere constitutes a considerable portion of Earth's biomass, both in terms of total cells and carbon content [1]. Active subsurface communities have been shown to exist deep beneath the seafloor as well as in the deepest reaches of the continental crust. In such environments, lithotrophic pathways, including the oxidation of Fe(II), are thought to be of crucial importance, potentially serving as the source of primary production in diverse subsurface communities and exerting influence on the flux of nutrients from the weathering of primary silicate minerals.

This study thus investigated the role of lithotrophic iron oxidizing bacteria (FeOB) in a terrestrial subsurface silicate weathering system within the Rio Icacos Watershed of the Luquillo Mountains in Puerto Rico. Rio Icacos is underlain by the rapidly weathering 200 ka Rio Blanco Quartz Diorite containing appreciable amounts of Fe(II)-bearing minerals including biotite and hornblende (9.5 and 6.3 wt %, respectively) [2]. Prior geochemical evidence including Fe isotope measurements [3] and mass transfer calculations suggest that the flux of Fe(II) from the weathering bedrock is capable of supporting a robust lithotrophic microbial community at depth near the bedrock-saprolite interface [4]. Current work confirms the existence of an active microbial community at depth determined via ATP bioluminescence, with a marked increase in ATP occurring near the bedrock-saprolite interface. 16S rRNA gene based analysis of regolith recovered from the bedrock-saprolite interface (785 cm) reveals a diverse assemblage of Bacteria, including taxa related to known FeOB from the order *Acidimicrobiales* as well as several *Betaproteobacteria* genera.

Enrichment cultures established using powdered solid phase Fe(II)-bearing minerals as a sole energy source, including whole rock Rio Blanco Quartz Diorite, were inoculated with material recovered from the bedrock-saprolite interface. Monitoring of Fe(II)/Fe(total) ratio, ATP content and aqueous chemistry suggests growth of microorganisms coupled to the oxidation of mineral bound Fe(II). 16S rRNA sequencing of DNA extracted from the enrichment cultures lends further support to the ability of indigenous microorganisms to grow via solid phase Fe(II)-silicate

lithotrophy with significant enrichment for organisms with taxonomic affinity to known FeOB including organisms from the genera *Cupriavidus*, *Burkholderia* and *Uliginosibacterium*.

Forthcoming metagenomic analysis of the *in-situ* microbial community as well as selected cultures displaying evidence of lithotrophic iron oxidation will provide further insight into the metabolic capacity of these organisms, with genes involved in extracellular electron transport and carbon fixation being of particular interest. Combining genomic approaches with geochemical and mineralogical investigations will thus provide novel insight into the role of microorganisms into the weathering of the Rio Blanco Quartz Diorite which has important implications for life, not only on Earth, but other rocky bodies.

#### References:

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