

**PREVALENCE OF THE ANCIENT WOOD-LJUNGDAHL PATHWAY IN A SUBSEAFLOOR OLIVINE COMMUNITY.** A. R. Smith<sup>1,2</sup>, R. Mueller<sup>2</sup>, M. R. Fisk<sup>2</sup>, O. U. Mason<sup>3</sup>, R. Popa<sup>4</sup>, B. Kieft<sup>2</sup>, and F. S. Colwell<sup>2</sup>, <sup>1</sup>Woods Hole Oceanographic Institution (266 Woods Hole Road, MS #51, [asmith@whoi.edu](mailto:asmith@whoi.edu)), <sup>2</sup>Oregon State University ([ryan.mueller@oregonstate.edu](mailto:ryan.mueller@oregonstate.edu), [m.fisk@coas.oregonstate.edu](mailto:m.fisk@coas.oregonstate.edu), [kieft1bp@gmail.com](mailto:kieft1bp@gmail.com), [r.colwell@coas.oregonstate.edu](mailto:r.colwell@coas.oregonstate.edu)), <sup>3</sup>Florida State University ([o.mason@fsu.edu](mailto:o.mason@fsu.edu)), <sup>4</sup>University of Southern California ([rpopa@usc.edu](mailto:rpopa@usc.edu)).

**Introduction and Rationale:** The igneous oceanic crust spans the majority of Earth's surface and contains an active subsurface chemosynthetic ecosystem with the potential to influence global carbon cycles. This suboceanic ecosystem may be largely supported by iron-bearing minerals in the crust and their reactions with seawater. Olivine, a common igneous mineral, is capable of producing molecular hydrogen during water-rock reactions, even at low temperatures indicative of thermal basaltic aquifers (55 – 100 °C) [1]. Seawater bicarbonate and the hydrogen generated by these low-temperature reactions can be used for chemosynthesis and energy generation via the ancient Wood-Ljungdahl pathway. Since this pathway may have first appeared in oceanic crust [2] and relies on hydrogen, organisms that use this pathway may be excellent analogs for life on Enceladus or other ocean worlds containing thermal or hydrothermal aquifers in igneous crust. However, acetogenic bacteria that use this pathway in the suboceanic aquifer have largely remained elusive [3] and are known to be presented with unique thermodynamic challenges [4]. In order to test whether olivine can support chemosynthetic communities genetically capable of using hydrogen in the Wood-Ljungdahl pathway, we produced eleven high-quality metagenome-assembled genomes (MAGs) from olivine grains incubated in a ~ 65 °C subseafloor borehole off the Juan de Fuca Ridge (JdFR) for 4 years.

**The Wood-Ljungdahl Pathway:** The Wood-Ljungdahl pathway is a bifunctional carbon fixation and energy generating pathway that may have been one of the first biosynthetic pathways to arise on Earth. This pathway contains two branches, the methyl and the carbonyl (also known as the acetyl-CoA pathway) [5]. It is used by sulfate reducers (acetyl-CoA pathway only), methanogens, and acetogens. The methyl branch of the pathway contains different enzymes in methanogens and acetogens, and the acetogenic pathway requires less enzymatic steps [2], which makes it an excellent candidate for the first biosynthetic pathway, especially considering the product acetyl-CoA, which is one of the most important biosynthetic building blocks for life on Earth.

**Prevalence of the Wood-Ljungdahl Pathway on Olivine Incubated in Oceanic Crust of the JdFR:**

We found that ten out of eleven of the olivine MAGs produced in this study contained the Wood-Ljungdahl pathway. Three archaeal MAGs contained the acetyl-CoA pathway, or carbonyl branch, of the Wood-Ljungdahl pathway, and seven bacterial MAGs contained the pathway for acetogenesis, most of which were Clostridia. The results of this study suggest the Wood-Ljungdahl pathway has a previously unrecognized role in oceanic crust and that this pathway is relevant to understanding life in ocean worlds.

**References:** [1] Mayhew L. E. et al. (2013) *Nat. Geo.*, 6 doi:10.1038/NCEO1825. [2] Nitschke W. and Russell M. J. (2013) *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 368. [3] Jungbluth S. P. et al. (2017) *PeerJ.*, 1 - 22. [4] Lever M. A. (2012) *Front. Microbiol.*, 2 doi:10.3389/fmicb.2011.00284. [5] Ragsdale S. W. (2008) *Ann. N. Y. Acad. Sci.* 1125: 129 - 136.