In situ electrochemical enrichment and isolation of a magnetite-reducing bacterium from a high pH serpentinizing spring. Annette R. Rowe[1], Miho Yoshimura[2], Doug E. LaRowe[1], Lina J. Bird[1], Jan P. Amend[1,3], Kazuhito Hashimoto[2], Kenneth H. Nealson[1], Akihiro Okamoto[2], [1]Department of Earth Sciences, University of Southern California, [2]Department of Applied and Chemical Engineering, University of Tokyo, [3]Department of Biological Sciences, University of Southern California, [4] Center for Green Research on Energy and Environmental Materials, National Institute for Material Sciences

Introduction: Serpentinization is a geologic process that produces highly reduced, hydrogen-rich fluids that support microbial communities under high pH conditions [1]. These serpentinizing environments host characteristic Bacteria and Archaea [2]. However, it is not always clear what electron acceptors support life in these environments, especially in the oxygen limited subsurface [1,2]. We investigated the activity of microbes capable of extracellular electron transfer (transfer to solid minerals exterior to the cell) in a terrestrial serpentinizing system known as "The Cedars". By measuring *in vivo* current generation with an on-site two-electrode system (**Fig. 1**), we observed daily oscillations with current maxima and minima occurring during the day, along with the enrichment of a distinct microbial community. The electrochemical activity was reproduced in lab-scale electrochemical reactors with carbohydrate-amended media, but the oscillations observed in the in situ current generation were not maintained, which coincided with decline in the phototrophic members. Gammaproteobacteria and Firmicutes were consistently enriched on γ-MnO2 and amorphous Fe(OH)3 at pH 11. However, isolation of an electrogenic strain proved difficult as transfer cultures failed to grow after multiple rounds of media transfer. Lowering the bulk pH in the media allowed us to isolate a *Firmicutes* strain (*Paenibacillus* sp.) that was enriched in our electrochemical cultivation experiments. This strain was capable of electrode and mineral reduction (including magnetite) in Cedars-specific minimal media adjusted to pH 9. This report provides evidence of the in situ activity of microbes using extracellular substrates as sinks for electrons at The Cedars, but also highlights the potential importance of community dynamics for supporting microbial life in this high pH system.

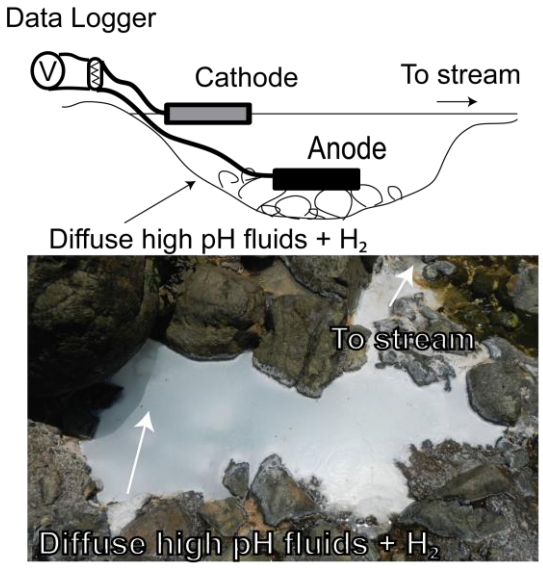

Fig. 1. Schematic of two-electrode on-site electrochemical enrichment incubated it Campsite Spring, at The Cedars (A). Electrodes were constructed from carbon felt (black/anode) or carbon felt coated with platinum catalyst (grey/cathode). Photograph of Campsite Spring where incubations were performed highlight flow direction of serpentinizing fluids (B).

References:

[1] Morrill, P.L., et al. (2013) *Geochim. Cosmochim. Acta* **109**: 222–240.

[2] Suzuki, S., et al. (2013) *Proc. Natl. Acad. Sci.* **110**: 15336–15341