

CULTIVATION OF EXTREME ALKALIPHILES FROM THE CEDARS – AN ULTRABASIC TERRESTRIAL SERPENTINIZATION SITE. Anja. Bauermeister¹, Lina Bird¹, Yuri Gorby², Kenneth Nealson¹, ¹Department of Earth Sciences, Zumberge Hall of Science, 3651 Trousdale Pkwy, University of Southern California, Los Angeles, CA 90089, ²Dept. of Civil and Environmental Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180.

Introduction: In serpentinization systems, water reacts with ultramafic minerals (olivine and pyroxene), resulting in the formation of minerals like serpentine and magnetite, the production of reduced gases such as H₂, and CH₄, and highly alkaline fluids. Terrestrial serpentinization sites such as the Cedars in Northern California could serve as analogs for habitable environments on other planets. For example, the methane detected in the atmosphere of Mars could be an indication for active serpentinization processes in the subsurface [1]. The spring waters emerging from the Cedars peridotite body are characterized by high pH (~11.5), low redox potential (~-550 mV), and low ionic concentrations (i.e. no obvious electron acceptors are present) [2]. A previous study investigating the microbial community structure of the Cedars springs demonstrated distinct differences between springs fed by deep or shallow serpentinizing groundwater, respectively [3]. The deep groundwater community was found to be unique among the terrestrial serpentinization sites previously described. To date, no cultured specimen of this community exist. Although genomics can predict the metabolic potential of microorganisms to some extent, physiological studies are still required to gain a deeper understanding of how these microorganisms cope with their challenging environment. Therefore, our research aims to culture microorganisms from the deep communities of the Cedars groundwater.

Methods: Samples were collected from spring NS1 (fed by a mixture of shallow and deep groundwater) by flow through a column packed with magnetite sand. Enrichment cultures were prepared in chemically defined medium (replicating *in situ* conditions), with the addition of vitamins, trace minerals, and 0.02% yeast extract to stimulate growth. Medium was amended with different electron donors and acceptors, including minerals like magnetite and olivine, and incubated anaerobically at ambient temperature. Growth was confirmed by nucleic acid staining and fluorescence microscopy. If growth occurred consistently over several transfers, DNA was extracted for 16S rDNA sequencing to identify the most interesting bacterial strains for further characterization. As bacteria were seen to preferentially attach to mineral particles, quantification of growth will be carried out by protein or nucleic acid extraction and quantification. The attachment to solid surfaces might indicate the ability of the

organisms to utilize solid electron acceptors or donors. This will be further studied in electromicrobiology experiments, in which bacteria are incubated with an electrode poised at a specific potential.

Expected Results: The results will provide important insights into survival strategies employed by life in extremely low energy subsurface environments at ultrabasic pH.

References: [1] Mumma M. J. et al. (2009) *Science*, 323, 1041-1045. [2] Morrill P. L. et al. (2013) *Geochim et Cosmochim Acta*, 109, 222-240. [3] Suzuki S. et al. (2013) PNAS, 110, 15336-15341.