A Geobiological Approach to Understanding Dolomite Formation at Deep Springs Lake, CA


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Project Details
Number of students: 6
Approximate dates: June 15 – July 13, 2013
Locations: Deep Springs Lake, CA, Amherst College, Hampshire College, Mt. Holyoke College, and Smith College

Introduction
We are proposing a 6-student Keck project to investigate modern biologically mediated precipitation of dolomite and other carbonate minerals in Deep Springs Lake, CA. Elucidating the mechanisms of dolomite precipitation is a fundamental and longstanding problem in sedimentology and Earth history. The project will be an interdisciplinary collaboration between faculty members from four institutions, including one non-Keck college. Students and faculty will develop an integrated suite of field and laboratory data utilizing techniques in microbiology, aqueous geochemistry, sedimentology, mineralogy, and isotope geochemistry. During the project, participants will have the opportunity to perform cutting-edge geobiological research, including one week of fieldwork in a modern alkaline playa lake and three weeks of lab research at the sponsoring institutions.

Geologic Background and Project Impetus
In order to decipher the history of Earth’s early surface environments, it is critical to develop an understanding of potential modern analogs. Many modern carbonate-forming environments and sediments may be analogs for Precambrian depositional environments. The strata host many of the records used to reconstruct the early history of our planet, potentially providing insight into the geological, chemical, and biological processes occurring at the time of their formation. Understanding interactions among these processes, particularly microbial contributions, is essential for determining the signatures of early life and environments on Earth, and may provide new ways to detect biological activity on Mars and other planets.

In both field observations and laboratory experiments, microorganisms play a role in the formation of carbonates, possibly by reducing the thermodynamic barriers to mineral precipitation. The discovery of such microbe-mineral interactions provides fresh insights into the origin of many carbonate minerals. For example, the metabolic activity of sulfate-reducing bacteria involved in the anaerobic respiration of organic matter was shown to result in an elevated pH and a concentration of ions (e.g., Ca$^{2+}$, Mg$^{2+}$, HCO$_3^-$), leading to the nucleation of carbonate crystals. This process was also shown to stimulate dolomite precipitation, and may be a major contributor to the global carbonate sedimentary budget (Warthmann et al., 2000; van Lith et al., 2002). Dolomite occurs extensively in the geological record, despite its scarcity
in contemporary settings (Land, 1998). This disparity, along with an inability to experimentally form dolomite in a laboratory setting at temperatures and pressures closest to those found in environments where formation has occurred, is often referred to as the “dolomite problem.” In particular, microorganisms with a broad range of metabolisms (including sulfur-oxidizers, methanogens, methanotrophs, and phototrophs) were implicated in dolomite formation.

One particularly interesting site where microorganisms may be contributing to contemporary formation of dolomite is Deep Springs Lake, CA (Torrens and Tor, 2009; Meister et al., 2011). Deep Springs Lake (Figure 1 and 2) is an ephemeral saline lake whose sediments include approximately fifteen different carbonate minerals (Jones, 1965). Of all these, dolomite is the most abundant. A biogenic origin of dolomite formation was recently proposed for low temperature environments involving sulfate-reducing bacteria (Vasconcelos et al., 1995; Vasconcelos et al., 1997; Warthmann et al., 2000; Wright, 2000). This hypothesis proposes that sulfate-reducing bacteria may help overcome three major kinetic constraints that otherwise inhibit dolomite formation: 1) they lower sulfate concentrations, which inhibits dolomite formation at high concentration; 2) they increase the availability of magnesium, since sulfate is often associated in seawater with magnesium; and 3) they increase the pH and carbonate alkalinity through respiration (van Lith et al., 2003b; Wright and Wacey, 2005).

Figure 1. Map of California showing the approximate location of Deep Springs Lake.
Figure 2. Deep Springs Lake in March (A) and in October (B) of 2008. The lake is ephemeral, filling in the spring with snowmelt and drying into a playa over the summer months. The two photos were taken from the same vantage point.

While a significant amount of evidence both in vitro and in situ exists to link sulfate-reducing bacteria with dolomite precipitation, this model is based on observations from only three very similar locations, Lagoa Vermelha and Brejo do Espinho in Brazil, and the Coorong region of Southern Australia (van Lith et al., 2003a). These sites share a great deal in common by being large, alkaline, hypersaline lagoons that are located at sea level and are very close to oceans. In contrast, Deep Springs Lake is an ephemeral lake 1,500 m above sea level in an arid region fed in part by springs of meteoric water, thus subject to vastly different biogeochemical conditions and variation.

**Previous Work at Deep Springs Lake**

In a preliminary study, sediment and water samples were aseptically collected from Deep Springs Lake in October 2008 (Torrens and Tor, 2009). A novel growth medium was synthesized based upon the chemistry of the lake water to enrich sulfate-reducing bacteria. An isolate (BSML1) was obtained that utilized a mixture of organic acids (sodium succinate, malic acid and lactic acid) and was found to be phylogenetically related to *Desulfovibrio brasilensis*, a sulfate reducing bacterium that was linked to dolomite formation (Warthmann et al., 2005). A broth culture of BSML1 in low sulfate medium showed a milky white precipitate that was not observable when it was grown on a high-sulfate medium. While verification that the milky white precipitate is dolomite is pending, the close phylogenetic relationship of
BSML1 to *D. brasiiliensis* and the fact that the precipitate did not form on a high-sulfate medium are positive indicators that it may be involved in biogenic dolomite formation.

Molecular community analysis of lake water was conducted in order to estimate the microbial diversity. Preliminary results suggest that lake water is quite diverse, and over 50% of the sequenced clones did not show a high similarity (<85%) to any previously characterized microorganisms. With such a large portion of the clones grouping with yet uncharacterized bacterial species, predicting their function in the ecosystem or geochemical influence is difficult until they are studied further and more are cultivated. Characterizing the community of microorganisms in Deep Springs Lake will aid in elucidation of the role that microorganisms play in biogenic origin of dolomite, which in turn will provide crucial insight into environmental conditions necessary for dolomite formation under modern and ancient conditions.

**Hypothesis and Significance**

We hypothesize that microbial metabolic activity creates conditions favorable for dolomite formation at Deep Springs Lake, and that these microbe-mineral interactions exert primary controls on elemental cycling in this environment. In this scenario the metabolism of sulfate is expected to drive a localized increase in the carbonate alkalinity and availability of magnesium, which would favor the precipitation of dolomite. The significance of testing this hypothesis at Deep Springs Lake is that it informs our understanding of the range of biogeochemical and environmental conditions under which dolomite can precipitate and elucidates a role for microbes in the formation of some examples of geologically ancient dolomite. In addition, characterization of the pathways for biomineralization of carbonates on Earth has the potential to provide new insight into the preservation of possible biosignatures in Martian carbonates.

**Research Questions and Approach**

The research goals for this study are to: 1) cultivate and identify the bacteria potentially responsible for the formation of dolomite; 2) assess the geochemical conditions and biochemical mechanisms that contribute to microbially-mediated dolomite formation; 3) characterize early mineral precipitates, both microbially-mediated and inorganic; and 4) elucidate the differences in the microbial communities of Deep Springs Lake, based on location within the water column and sediment. Potential research questions the students’ work may address include:

- What types of microbial metabolic activities contribute to dolomite formation?
- What geochemical and environmental conditions favor the formation of dolomite?
- What is the effect of lake drying and evaporation on the community of microorganism in the water column and sediment, and on the formation of dolomite?

**Potential Student Research Projects**

Students will test the overarching hypothesis by focusing on a number of interrelated geochemistry, sedimentology, and geomicrobiology projects. All projects will be grounded in sample collection during our initial fieldwork at Deep Springs Lake.

*Sample collection.* All samples will be collected aseptically to prevent contamination and returned to the laboratory. Sediment cores will be collected to a depth of 1.5 m with polycarbonate tubes, pore waters will be extracted using squeeze cores, and bulk water samples collected in polycarbonate containers. At 2 sites near sediment cores *in situ* “peeper” devices (dialysis chambers) will be employed for pore water
extraction and comparison to the squeezed water samples. Bulk and pore water samples intended for microbial community analysis will be immediately filtered through Millipore Sterivex filters for later analysis in the laboratory. In situ pH and temperature will be recorded. All samples will be stored in a cooler after collection and transported back to the laboratory where they will be stored at 4°C. Sediment cores and pore water samples for microbiological analysis will be processed using aseptic and anaerobic processes (e.g., gassing manifolds, glove bags).

**Characterization of sediment (1 student)** Mineral analysis of sediment cores will be conducted using X-ray diffraction (XRD) and scanning electron microscopy (SEM). Mineralogy will also be characterized with Raman spectroscopy in Dyar’s lab. Analysis down core and between sites will produce a range of sedimentological data on dolomite abundance and formation and will particularly address early diagenetic processes.

**Characterization of water (1 student).** Total carbon (TC), methane, and nitrogen will be determined via gas chromatography, total inorganic carbon (TIC) via coulometry, with total organic carbon (TOC) determined by difference (TOC = TIC – TC). Major, minor and trace cations will be determined on a simultaneous ICP-OES, while anions will be run on a Dionix IC. Sulfide, ammonium and phosphate trace determination will be preformed via colorimetric spectrometry.

**Enrichment and isolation of bacteria (1 student).** In addition to common growth media that have already proven successful (e.g., Postgate), novel growth media based upon the water chemistry data will be designed for the enrichment and isolation of bacteria. An array of aerobic and anaerobic conditions will be created to select for heterotrophic and autotrophic growth, with a range of potential electron donors and acceptors to screen for dolomite mineral-forming organisms. A combination of serial dilution and petri plates will be utilized to obtain isolates. Mineral precipitates will be analyzed by XRD and SEM, growth limitations of isolates will be determined, and the species identified.

**Cultivation-independent analysis of bacteria (1 student).** Microbial community analysis will be conducted by isolating DNA directly from the lake sediment, pore water, and surficial waters with extraction procedures and techniques previously demonstrated (Torrens and Tor, 2009). Gene libraries of 16S rDNA will be prepared by polymerase chain reaction of purified DNA using primers specific for Bacteria and Archaea. Clone libraries will be prepared using a TOPO-TA cloning kit, clones analyzed by restriction fragment length polymorphism (RFLP), with representative clones from each distinct RFLP group sequenced at the University of Massachusetts Amherst DNA Sequencing Facility. Non-chimeric sequences will be matched by a standard BLAST search within the NCBI GenBank database to determine closest matches. Sequences will be submitted to GenBank.

**In situ dolomite formation (1 student).** Laboratory isolates and enrichments will be incubated in Deep Springs Lake to determine their ability to form dolomite in situ. Sterilized dialysis bags will be deployed in lake for one week and for 6 months (Tor will return in October with his own funding to retrieve them), with the following conditions: 1) autoclaved, 2.7-μm filtered lagoon water; 2) autoclaved, 2.7-μm filtered lagoon water that had been inoculated with a laboratory isolate; or 3) unfiltered lake water. Dialysis bags will be collected and transported back to the laboratory for community and mineral formation analysis as described above.
Isotopic analysis of sulfur cycling during dolomite precipitation (1 student). Sulfate reducing bacteria impart an isotopic fractionation to sulfur during their metabolism. This leads to distinct sulfur isotope signatures in pore water sulfate and sulfide. In order to characterize the architecture of the sulfur metabolizing microbial community, we will analyze the sulfur isotope ratios in pore water sulfate and sulfide, in sulfate bound in the crystal lattice of the carbonate minerals, and in lake water sulfate. This study will provide insights into the modern microbe-mineral interactions and provide a framework for interpreting sulfur isotopes in ancient dolomite rocks.

Magnesium isotope fractionation during microbially-mediated dolomite precipitation (1 student). The incorporation of magnesium into dolomite has been shown to be accompanied by a kinetic isotope fractionation, suggesting a possible microbial role in the ordering of the dolomite crystal (Carder et al., 2005). We will measure the Mg isotope composition of dolomite and pore waters in Deep Springs Lake in order to develop models of isotope fractionation under these environmental and biogeochemical conditions.

Project Teaching and Learning Goals
Microbe-mineral interactions are beyond the scope of a single discipline, and this project will provide students with an opportunity to conduct interdisciplinary research into the role of contemporary microorganisms in formation of carbonates at Deep Springs Lake. Our interdisciplinary research approach will provide students with a clear vision for this project, through effective communication and team-building skills. This will serve as a catalyst for accelerating scientific discovery and will prepare students to work more effectively in advancing their studies and solving problems.

Students will gain experience in planning and conducting field work, documenting field observations, critiquing primary research literature, developing research questions, sampling in the field, and performing geomicrobiological laboratory techniques and data analysis, while enhancing their written and oral communication. The project will devote one quarter of the time to fieldwork and the remainder to laboratory research.

This study is likely to result in at least four publications: one on the characterization of mineral assemblages from Deep Springs Lake and laboratory cultures, one on the rates and constraints of dolomite formation by sulfate-reducing bacteria, one on the seasonal effects of lake drying and evaporation on the community of microorganisms, and one on the sulfur and magnesium isotope records of dolomite formation. The student researchers participating in this project will have co-authorship on the papers and participate in the writing and peer-review process.

Preferred Student Background
This is an interdisciplinary research project, so students with an interest in a range fields are desirable, including but not limited to mineralogy, geochemistry, sedimentology, microbiology, and molecular biology. Students should be prepared for the challenge of working in difficult field conditions.
**Schedule**
The project will begin and end in Amherst, MA with the group members meeting on June 15 for introductions and to organize our camping and field gear. We will depart for the field on the next day. The group will fly to Las Vegas, NV and drive to Bishop, CA, with stops in Death Valley National Park and the eastern Sierra Mountains to gain an understanding of the geology and microbiology in the region. Over the following days students and faculty will work in groups to conduct the fieldwork and develop research projects.

Upon finalizing the research projects and finishing fieldwork, we will return to the Pioneer Valley (Amherst/Northampton/South Hadley) to begin processing the samples, pool data and notes, and conduct laboratory-based research. We will introduce all students to the operations of all the laboratories before the students begin focused work on their project. Students will work in the labs at Hampshire, Amherst, Smith, and Mount Holyoke and will meet regularly as a large group to discuss their projects and share data.

**Logistics**

*Housing and Meals.* Lodging during fieldwork will be at an inexpensive motel in Bishop, CA (approximately 36 miles from Deep Springs Lake). The ability to refrigerate biological and geochemical samples on the day of collection is an important factor in selecting our accommodations. We will be preparing most of our own meals.

Lodging for students during lab work will be in dormitories on the campuses of Amherst, Hampshire, and Mount Holyoke colleges.

*Vehicles and Field Equipment.* Two four-wheel drive vehicles will be rented. Roads in the region are well maintained but high clearance vehicles are preferred for safe and easy passage over the 1.5-mile long dirt road leading to Deep Springs Lake.

*Laboratories.* Hampshire College has a fully equipped research and education laboratory dedicated to geomicrobiology and analytical chemistry, complete with technician support in addition to standard laboratory equipment (water baths, incubators, autoclave, ultra-pure water, centrifuges, etc) and standard equipment used in geomicrobiology (e.g. thermocyclers, Gel-Doc imaging system, DGGE unit, a Coy anaerobic chamber, gassing manifolds, ultracentrifuge, ovens for high temperature incubations, and drip-flow biofilm reactors). Phase-contrast and fluorescent microscopes with digital video capability are also available. The analytical chemistry labs at Hampshire are fully equipped with HPLC, GC, IC, FTIR, FPLC, NMR, and ICP-MS.

Amherst College has a fully equipped laboratory for aqueous geochemistry research and education, directed by Anna Martini. Specialized equipment relevant to this proposal includes an ICP-OES, an ICP-MS, and an ion chromatograph. Jones’ lab is equipped to prepare pore water and sediment samples for sulfur isotopic analysis. Smith College has an SEM and petrographic microscopes for detailed sedimentological analyses as well as a research-quality x-ray diffractometer. Mount Holyoke College has a Raman spectrometer for mineral identification.
**Sampling Permits.** Deep Springs Lake is on Bureau of Land Management land where collecting is permitted for scientific purposes.

**Safety.** Deep Springs Lake is a relatively safe research environment with few natural hazards aside from the possibility of encountering snakes and scorpions. The lake is typically quite shallow (< 40 cm depth) at the sample sites. Students will be instructed on the hazards of working in an arid environment, in particular the importance of remaining hydrated (they will be reminded regularly to drink water), wearing sunblock, and dressing appropriately. Only faculty will drive the vehicles.

**Faculty Experience**
Professor Tor has 20 years of field research experience in a variety of volcanic environments (Italy, Philippines), hydrothermal systems (Yellowstone National Park), and arid deserts (Death Valley, CA); over the past 7 years he has led student research trips and class field trips to the eastern Sierra Mtns, including Deep Springs Lake. He is trained as a geomicrobiologist and specializes in microbe-mineral interactions and adaptations to extreme environments.

Professor Jones is a junior faculty member with eight years of field experience studying ancient carbonate rocks in the field and laboratory. Since 2006 he has led 11 undergraduates on research trips totaling 14 weeks in the field. Ongoing projects focus on Ordovician-Silurian strata of the Great Basin and Atlantic Canada. He is trained in stable isotope geochemistry and maintains a lab for the preparation of geological samples for carbon and sulfur isotope analysis.
**References Cited**


