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EVALUATING EXTREME WEATHER RESPONSE IN CONNECTICUT RIVER FLOODPLAIN ENVIRONMENT*:*
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Students: *KYRA BRISSON*, Hampshire College, *KYLE METCALFE*, Pomona College, *MICHELLE PARDIS*, Williams College, *CECILIA PESSOA*, Amherst College, *HANNAH PLON*, Wesleyan Univ., *KERRY STREIFF*, Whitman College

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MICROBIAL COMMUNITY ANALYSIS OF DEEP SPRINGS LAKE, CA: EXPLORING THE ROLE OF AEROBIC BIOFILMS IN BIOGENIC DOLOMITE PRECIPITATION

KYRA BRISSON, Hampshire College
Research Advisor: Jason M. Tor

INTRODUCTION

The mechanism responsible for the primary precipitation of the mineral dolomite has long been a problem in the field of sedimentology. This problem has two elements; first, there is little evidence of widespread dolomite precipitation in modern environments in contrast with the abundance of dolomite in rock records, indicating rich primary precipitation in ancient environments (Holland and Zimmermann, 2000; Warren 2000; Land 1998). Second, researchers are unable to abiotically form primary precipitates experimentally under environmentally-relevant conditions thought to be present where the dolomite formed in the natural environment (Ardvidoson and Mackenzie 1999; Land 1998; Roberts et al., 2013; Bontognali et al., 2013). In recent decades research indicates that Bacteria and Archaea are capable of mediating dolomite formation. Evidence of microbially influenced dolomite formation was reported in two marine lagoons in central Brazil (Vasconcelos et al., 1995; Vasconcelos and McKenzie, 1997; Warthmann et al., 2000; van Lithe et al., 2003a; van Lithe et al., 2003b), various distal ephemeral lakes of the Coorong region South Australia (Wright et al 1999; Wright and Wacey, 2005), a saline alpine lake on the Tibetan plane (Dong et al., 2006; Deng et al., 2010), and a hypersaline lake, Deep Springs Lake, near Bishop CA (Meister et al 2011).

There are two primary mechanisms proposed for biogenic dolomite formation. One proposes that through metabolic processes such as sulfate reduction, ammonification and photosynthesis microbes can induce dolomite precipitation by increasing alkalinity and bicarbonate concentration, thus leading to a decrease in thermodynamic barriers to carbonate mineral precipitation (Braissant et al., 2003; Rivadeneyra et al., 2000; Rivadeeyra et al., 2004; Rodriquez-Navarro 2003; Rodriguez-Navarro 2007; Sanchez-Roman et al., 2009). The other model suggests that the production of exopolymeric substances (EPS) from microorganisms is the main process controlling dolomite and other carbonate precipitation due to the buffering capacity and dehydration of magnesium molecules by the EPS (Braissant 2007; Roberts et al., 2013; and Bontognali et al., 2013).

One site of ongoing modern dolomite formation that is gaining recognition is Deep Springs Lake, California (DSL). The site has conditions that are favorable to biogenic dolomite formation including, ephemeral conditions that create a seasonal fluctuation in solute concentration due to evaporation, seasonal rise in salinity to toxic levels for most macrobiota, and elevated pH. Meister et al. (2011) examined the carbon and oxygen isotope composition of sediment and pore water and determined that, based on presence of metabolites, there was a possible microbial influence on the saturation state of dolomite. Furthermore, it was also concluded that the precipitation of authigenic fine-grained dolomite is occurring in the water column (Meister et al., 2011).

The objective of this study was to examine the microbial diversity of spring environments and lake sediment from Deep Springs Lake and to examine the potential for microbial communities to mediated dolomite formation. In order to address these goals numerous methods were employed

including genetic sequencing of 16S rDNA genes, cultivation of aerobic biofilms, and biofilm-mediated precipitation of carbonates in the laboratory. The results from these aforementioned techniques are anticipated to demonstrate the diversity of the microbial communities associated with four springs as well as surrounding sediment; and to gain a better understanding of the role of aerobic biofilms in the formation of dolomite in this and similar environments.

MATERIALS AND METHODS

Site Description and Sampling. Deep Springs Lake is and ephemeral, shallow, alkaline lake located 31 miles East of Big Pine, California (N 37 17' 545", W118 2' 27.16"). It is situated in a valley at an altitude of 1500 m above sea level. There are a total of 15 different saline and carbonate minerals in the lake's sediment the most abundant of which is dolomite (Land, 1969). Meister et al. (2011) examined the carbon and oxygen isotope composition of sediment and pore water at Deep Springs Lake and determined that based on presence of metabolites that there was a possible microbial influence on the saturation state of dolomite. It was also determined that precipitation of authigenic fine-grained dolomite is occurring in the water column (Meister et al., 2011).

Samples were collected aseptically form DSL between June 16th – 21st, 2013 from 7 locations, including 5 springs (9S, ES, CS, SS, PS) and two sediment sites (MS and FO). Samples included sediment, salt crust, biofilm, suspended particles from spring, and spring water. All sediment samples were collected using sterile forceps, placed in sterile 15 ml and 50 ml Falcon tubes and sealed with Parafilm® for later analysis. Spring water (40-150 ml based on turbidity of spring) was collected with a sterile 60 ml syringe, and filtered through Milipore® Sterivex™ 0.2 µm pore filter to collect microorganisms for genetic analysis. All samples were stored in a cooler after collection and transported back to the laboratory where they were stored at -20° C. Spring water salinity, pH, and temperature were analyzed in the field.

Cultivation of Biofilms and Precipitation of Carbonates. Microbes were first cultivated using Modified Postgate B (PB) growth medium. PB has the following composition: 2 g/L $MgSO_4 * 7H_2O$, 1 g/L NH_4Cl, 0.5 g/L KH_2PO_4, 2.6 g/L NaCl, 0.68 g/L acetate and 0.6 g/L tris-base. The media was brought to pH 8.4 with NaOH. After autoclaving and cooling to room temperature 10 ml/L of Wolfe's trace mineral solution and 10 ml/L of vitamin solution were filter sterilized and added to the media (Atlas, 2010). Media (300 µl) was added to each well of a 96 well base MBEC™ Biofilm Inoculator and then inoculated with 30 mg of sediment/biofilm or 30 µl of spring water. The plate lid, containing 96 pegs that correspond to the wells, was secured to the base using Parafilm® to avoid excessive evaporation which would change the solute concentrations. The plates were placed on a shaker at 200 rpm at room temperature and placed under Philips 50 watt R20 plant grow light for 12 hours daily. Due to evaporation of the media from the wells and a desire to remove the pegs from the parent material, the lids were removed from the original plates and placed in sterile 96 well plates with 300 µl of fresh media in each well.

After a period of 90 days biofilm growth was visually confirmed on the pegs using DAPI staining and microscopy. The pegs were then were removed from the lid and placed in a solution containing HCO_3 and varying Mg:Ca ratios based on previous precipitation experiment and environmental concentrations: 1.4 (Roberts et al., 2013), 6.0 (Bontognali et al., 2013), 1.5 based on data from MS site, and 2.3 based on 9S site. After a period of 30 days the pegs were removed from the solutions, allowed to dry at 37° C for one hour, and analyzed using x-ray diffraction.

Microbial Community Analysis. DNA was extracted from cells caught on the filter using the PowerWater® Sterivex™ DNA Isolation Kit according to manufacturer's instructions and quantified using a Nanodrop spectrophotometer. Extracted DNA was used as a template for the amplification of 16S rDNA genes via PCR. The reactions were carried out to a final volume of 50 µl, containing 25 µl REDTaq Ready Mix PCR reaction Mix (Sigma-Aldrich) 0.2 mM primers (see below), 50 ng of template DNA, and the remainder of the 50 µl reaction mixture contained sterile ddH2O. The bacteria-specific primer sequences were 8F: 5'-AGAGTTTGATCCTGGCTCAG-3' and

1492R: 5'-CGGTTACCTTGTTAC GACTT-3'. The products were amplified using an Applied Biosystems GeneAmp PCR System 2700 the amplicons were produced by the following touchdown conditions: initial denaturation for 5 min at 94°C was followed by a total of 25 cycles of amplification consisting of (1) denaturation at 94°C for 45s, (2) touchdown annealing cycles at 60°, 5 cycles at 58°C, 5 cycles at 56°, 10 cycles at 55°C for 45 s, and (3) extension at 72°C for 90s. The program ended with and extension step at 72°C for 5 min. PCR amplicons were visualized on a 1% agarose gel containing ethidium bromide (0.001 µg/ml) in 1X TAE buffer at a constant voltage of 120V for 2 hours to determine the amplification of 1,500 bp product.

The cloning reaction was carried out using a TOPO TA Cloning® Kit for sequencing (with a PCR®4 vector) and chemically competent Top 10 cells. Amplified PCR products from sterivex DNA extraction were cloned into the vector and the vector transformed into One Shot TOP-10 Competent Cells. Clones were plated on Luria-Bertani (LB) with Kanomycin and incubated overnight at 37°C. Clones were chosen and confirmed to have the insert by performing a whole colony PCR reaction with primers M13F -20 and M13R. PCR thermocycling conditions were set as follows: denaturing at 95°C for 5 minutes, 30 cycles of denaturing at 94°C for 30s, annealing at 55°C for 30s, extension at 72°C for 30s, with a final extension at 72°C 7 min. PCR amplicons were visualized on a 1% agarose gel containing ethidium bromide (0.001µg/ml) in 1X TAE buffer at a constant voltage of 120V for 2 hours to determine the amplification of 1,700 bp product.

RESULTS AND FUTURE WORK

Cultivation of Biofilms and Precipitation of Carbonates. Multiple growth media were designed based on the ionic concentrations measured in the pore and spring waters. In some experiments Ca^{2+} was omitted and only a minimum amount of Mg^{2+} (12.2 µM in mineral solution) was supplied as required for microbial metabolism so that biofilm producing microorganisms could grow but Ca-Mg carbonate precipitation would not occur. Samples taken from sediment and spring water were incubated in a Trough Base MBEC™ Biofilm Inoculator with 35 ml of media corresponding to the spring from which the sample was taken. The media was successful in cultivating various types of biofilms from environmental samples taken from the springs and sediment. Planktonic biofilms formed but did not adhere to the MBEC™ Biofilm Inoculator pegs of the lid. Microscopy and DAPI staining showed that the biofilms were similar in structure to previous culturing attempts.

Preliminary analysis of carbonate formation on the biofilms proved to be inconclusive due to overly noisy data. It was determined that the peg on which the biofilm and possible carbonate sat was a less than ideal sample for the XRD machine. This could be a result of the intact biofilm-mineral matrix not providing a homogeneous crystal structure, as is provided when environmental samples are dried and crushed into a powder. In order to create a uniform biofilm suitable for the precipitation experiments and community analysis, small colonies of planktonic biofilms were subsampled from the trough plate and placed into Petri dishes in 30 ml of media. These dishes will sit until they become turbid, and biofilm growth will be confirmed again using DAPI staining. Approximately 0.2 g of biofilm will be aseptically removed from the plate and placed in a solution of HCO_3 and the same Mg:Ca ratios used in previous precipitation attempts with a pH of 8.5 based on ideal carbonate precipitation conditions (Briassant et al., 2007), and allowed to sit for 20 days at 37°C. Precipitated solids will be filtered onto filter paper, dried for 2 days, and analyzed by XRD and SEM (Deng et al., 2010).

Microbial Community Analysis. Extraction of DNA from the filtered spring water was successful with concentrations ranging from 13.9 - 74.1 ng/µl, thus indicating that there are sizable microbial communities in the springs. Furthermore, amplification of the 16S rDNA sequence and cloning were successful. After a restriction enzyme digest to identify unique clones, the samples will then be sent to the UMASS Amherst Genomics and Bioinformatics Facility. A similar protocol will be followed to extract and sequence DNA from the biofilms associated with the precipitation experiment.

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