

MICROBIAL CONTROLS ON CALCITE PRECIPITATION IN LACUSTRINE ENVIRONMENTS

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INTRODUCTION

Bacteria can mediate the precipitation of calcium carbonate (CaCO_3), producing both massive limestone deposits and small crystal forms (McGenity and Sellwood 1999). This project examines sulfate reducing bacteria (SRB) in a lacustrine environment to assess their influence on the formation of calcite. The study location is Lough Carra, a shallow, flow-through lake in Western Ireland (Figure 2). Both the lake and its catchment area reside on carboniferous limestone, with the soil surrounding the lake evolving from glacial till (King and Champ 2000). Lough Carra contains sediment that is 95% CaCO_3 and has been deposited since the last glacier left approximately 12,000 years ago. The microbes were sampled from Lough Carra as part of a larger study on paleoclimate reconstruction. Lakes in Western Ireland provide ideal sites for studying stable isotopic records of oxygen and carbon with minimal continental rainout and seasonal effects. Variations in the isotopic record allow for inferences to be drawn about precipitation and temperature ($\delta^{18}\text{O}$) and about lake productivity ($\delta^{13}\text{C}$). It is possible that microbial in situ precipitation of calcite is affecting this record.

The formation of CaCO_3 depends on the concentration of bicarbonate (HCO_3^-) and calcium (Ca^{2+}) ions in water: $\text{Ca}^{2+} + 2\text{HCO}_3^- \leftrightarrow \text{CaCO}_3 + \text{H}_2\text{O} + \text{CO}_2$. This reaction will be driven to precipitate calcite by the addition of bicarbonate, if sufficient Ca^{2+} ions are also present. SRB induce the formation of calcium carbonate (Riding and S.M.Awramik 2000), by producing bicarbonate as they degrade

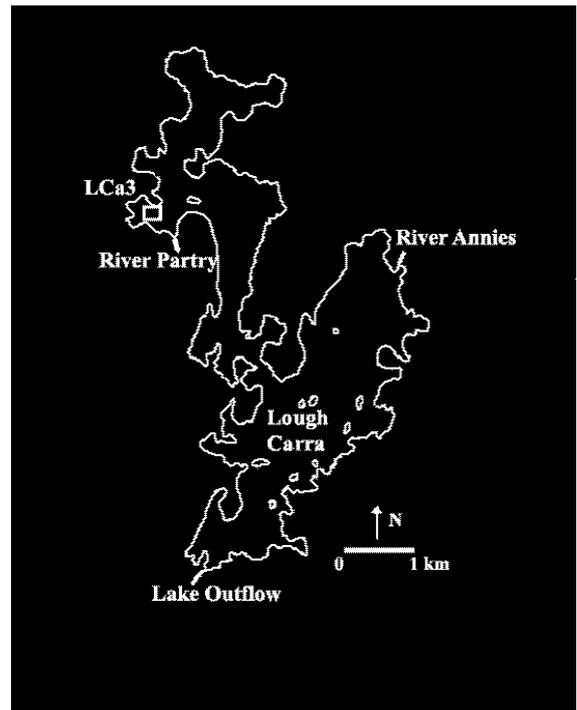


Figure 1: Microorganisms were collected from the top 30 cm of sediment of the LCa3 push core of Lough Carra (modified from King and Champ 2000).

organic matter: $2\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow \text{HS}^- + 2\text{HCO}_3^-$. The addition of bicarbonate (HCO_3^-) to the environment around the bacteria as a by-product of their metabolic processes leads to precipitation of calcium carbonate and the production of hydrogen sulfide gas.

To survive, SRB need an environment that is anoxic, sulfate-rich, and can supply organic material. The lake sediments we sampled are one such environment. Sulfate reducing bacteria belong to a subclass of the Proteobacteria group, and include the well-studied *Desulfovibrio* as well as the genera *Desulfomonas*, *Desulfotomaculum* among

others. Sulfate reducing bacteria preferentially use the lighter of the two main sulfur stable isotopes (^{32}S over ^{34}S). Thus, based on isotopically depleted sulfides found in rocks it is largely believed that SRB have been around for approximately 3.5 billion years (Shen, Buick et al. 2001). In addition, SRB choose ^{12}C over ^{13}C in the organic matter they consume, leaving behind an isotopically enriched HCO_3^- as a by-product.

“Studying microorganisms is a difficult task, and in many environments less than 1% of the microbial community can be cultivated” (McGenity and Sellwood 1999). The use of nucleic acids to identify the microbial community allows for a more representative picture of the entire community to be built. Sequence analysis of amplified 16S RNA gene sequences provides a general framework for assessing the microbial diversity and abundance, but does not provide a necessary link to the metabolic capabilities of a bacterium. Thus the functional gene approach will be used to identify bacteria responsible for the sulfate reduction process (Dhillon, Teske et al. 2003). By exploring the phylogenetic and functional diversity of the bacteria from Lough Carra we can choose which species to utilize for culturing. The bacteria will be grown in media and the rates and processes of calcium carbonate precipitation will be monitored as a way of recreating the microbially-induced precipitation of calcium carbonate that is occurring in the sediments of Lough Carra. Additionally it will be possible to measure the effect the biological processes have on the stable isotope values that paleoclimate research relies on.

METHODS

All molecular biology work was performed at the University of Massachusetts at Amherst in the Nusslein Microbiology Laboratory. The goal was to recover from the Lough Carra lake sediments nucleic acids of sufficient quality and quantity so as to analyze through gene sequencing the structure of the microbial community. After retrieval of sediment from the top 30 cm of a push core (see LCa3 Figure 1), the samples were preserved anoxically with

helium and shipped back to the United States on dry ice.

Cell Lysis

To perform genetic analyses the nucleic acids (DNA) must be released from the cells. The bacteria cells were lysed, or broken apart, in the presence of the soil particles. A combination of solubilization of cell wall constituents by a detergent and physical disruption of cell walls by the mechanical disruption procedure of bead-mill homogenization were used for cell lysis. The bead breaking technique is the most rigorous and is likely to give the least biased recovery of nucleic acids (Jackson, Harper et al. 1997). This guaranteed that the DNA of the sulfate-reducers, amongst others, would be recovered.

Separation of Nucleic Acids from Particulates

To separate the DNA from soil particles a chelating agent and a phosphate buffer were used. The chelating agent helped to keep metal ions from attaching to DNA. The anionic phosphate groups of the sugar-phosphate backbone of DNA like to form bridges with the calcium of the marl particulates. The phosphate buffer is added next so that the phosphate in the DNA has fewer chances to attach to iron and aluminum oxides present on the surface of the soil particulates. These processes increase the final DNA yield.

Purification

Finally, the nucleic acids need to be purified so there are no humic substances that could inhibit later procedures such as the Polymerase Chain Reaction (PCR) which amplifies particular segments of DNA. Humic compounds are part of the organic carbon content of soils, sediments, and natural water. Since humic compounds and nucleic acids are both water-soluble, anionic molecules they respond in a similar manner to procedures needed to analyze a bacterial community (Jackson, Harper et al. 1997). Spin columns were constructed by plugging syringes with glass wool and packing the syringes with Sepharose 4B. The wool and Sepharose allow DNA to flow through while blocking the humic contaminants. All columns were loaded

with buffer, centrifuged, and the eluent collected. The process was repeated and the five fractions with the greatest concentration of DNA were selected for further study.

Gene Amplification, Cloning, and Sequencing

The DNA that had been separated from the sediment was amplified to get enough material for genetic identification of bacterial populations. DNA is amplified by using the PCR technique. See (Dhillon, Teske et al. 2003, for details). The genetic material identified will be examined to generate a diversity pattern. The most dominant will be sent to the University of Massachusetts Sequencing Facility and upon determination will be used in further analysis.

Besides determining the most dominant it is important to find the species that are most active, or carry the functional SRB gene. Again, DNA extracted from the sediment was used. The primers *dsr1F* and *dsr4R* were used to amplify the *dsr* genes (specific to SO_4^{2-} -reducing activity) from the environmental samples. This information will

be combined with the sequences from the diversity pattern to choose the most appropriate samples to use for further experiments.

RESULTS

The presence of the metabolite SO_4^{2-} in the lake waters makes the process of sulfate reduction by SRB favorable. The decrease in sulfate concentrations measured in the upper 30 cm of porewater in the marl indicates that sulfate is being consumed (Figure 2).

After extraction, separation, and purification five samples were determined to contain the most DNA with which to perform the next tests with. The dilutions with the brightest bands seen in a denaturing gradient gel electrophoresis (DGGE) were chosen for subsequent analyses (Figure 3.)

Amplified rDNA restriction analysis created over 120 successful sequences that are currently being tallied for their relative abundance. The sequencing gives a rough picture of the original sample. There is some extraction and PCR bias, but by using protocols specifically designed for SRB these

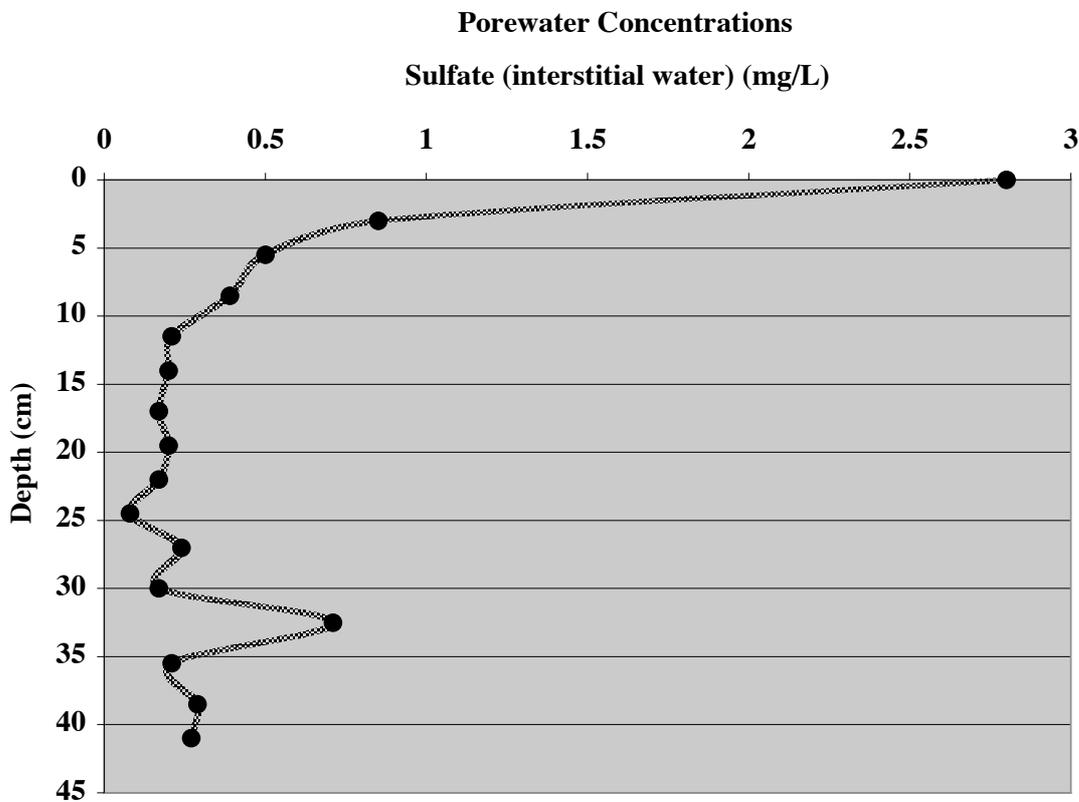


Figure 2. Porewater concentration.

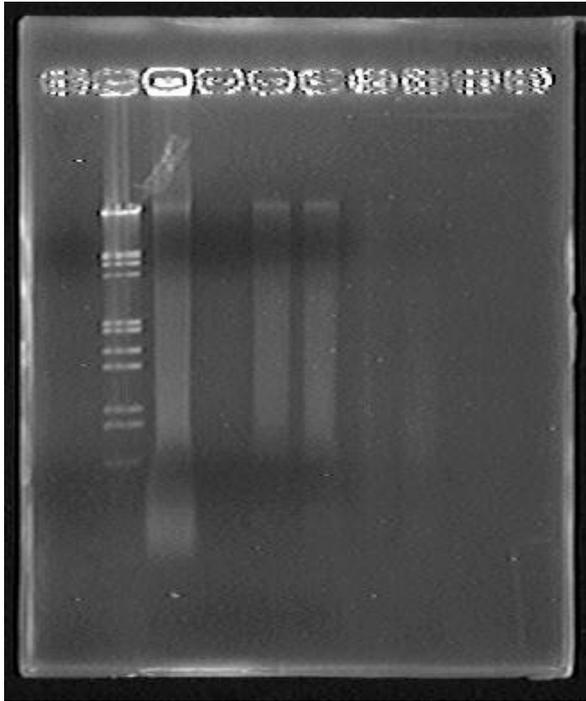


Figure 3.

were minimized.

Isolation of the functional sulfate reducing gene is ongoing as two bands of DNA are present around the location where the sulfate reducing gene would be expected. Currently the bands are being extracted and concentrated separately so they can be sent to the Sequencing Facility.

DISCUSSION

While this study is still in its preliminary stages, the presence of SRB in the marl has been confirmed. The dominant SRB species will be identified, isolated and finally cultured for precipitation experiments. The ^{13}C isotope values of the dissolved inorganic carbon (DIC), the calcium carbonate, and the dissolved organic carbon (DOC) will be measured before and after growth to see how the microbes affect the carbon isotopes. Calcite with high ^{13}C values will indicate that SRB are indeed forcing precipitation of calcite. In addition, the ^{18}O isotopic values of the CaCO_3 and the experimental water will be analyzed to determine isotopic equilibrium. Determining the influence that microbes may have on the oxygen isotope record in calcium carbonate marl is a major goal of this study.

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