# ASSESSING THE ROLE OF MICROBES IN THE FORMATION OF OOIDS AND CARBONATE PRECIPITATION

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## PROJECT OBJECTIVES

This project investigates the role of sediment-associated microbial biofilms on calcium carbonate precipitation with the goal of better understanding the physical, chemical and microbiological conditions involved in the formation of large-scale ooid shoals. Towards this end we seek to:

- Quantify the amount of biofilm coating carbonate grain surfaces and assess the stability/adherence of sediment biofilms through column flow-through and agitation experiments.
- Characterize metabolic processes associated with carbonate precipitation through metabolic gene profiling of active, non active, and mat stabilized sediments.
- In vitro precipitation experiments to assess the roles of microbial metabolism and biofilm/EPS in carbonate precipitation.

## PROJECT RATIONALE

Microbial communities are abundant components of the sedimentary environment where their metabolisms alter the surrounding geochemical environment. The vast metabolic diversity and complex interactions within these communities efficiently cycle nutrients and mediate levels of carbonate saturation (González-Muñoz et al., 2010; Dupraz et al., 2009). Moreover, microbes typically exist in interdependent cooperative communities referred to as a biofilms. Extracellular polymeric substances (EPS) facilitate the aggregation and attachment of microbes within biofilms in order to remain in optimal conditions for growth. Three different types of EPS alteration have been proposed to lead to $CaCO_3$ precipitation: 1) microbially mediated decomposition of EPS, liberating $HCO_3^-$ and $Ca^{2+}$; 2) organomineralization in which the EPS matrix is altered by chemical or biological activity, creating a template for $CaCO_3$ binding and precipitation; and 3) precipitation regulated by the balance of the external cation concentration and binding capacity of EPS (Dupraz and Visscher et al., 2005).

Despite the presumed importance of microbial processes in shallow water carbonates, there is scant information about microbial community composition and associated metabolism. This knowledge gap in carbonates is nowhere more evident than in the formation of oolitic sand bodies. In spite of the importance of ooilitic grainstones as potential carbonate reservoirs, the genesis of ooids remains controversial. Some researchers support abiotic processes (Duguid et al., 2010), while others have attributed ooid genesis to biogenic factors (Gerdes et al., 1994).

The first stage of this project focused on the molecular characterization of surface sediment microbial communities based on 16S rRNA gene sequencing and TRFLP community profiling. This preliminary work showed that sediments from active, non active and mat stabilized depositional facies all harbor complex microbial communities, some of which have been intrinsically linked to mineralization processes. Furthermore, preliminary studies suggest the amount of EPS coating varied in the three depositional

environments. Although all three environments contained EPS coatings, the amount of coating decreased from the mat stabilized to the active environment (Figure 1). Ongoing research over the coming year will give special attention to three areas: 1) characterization of sediment biofilm/eps; 2) metabolic gene profiling of sediment biofilms; and 3) controlled in vitro precipitation studies.

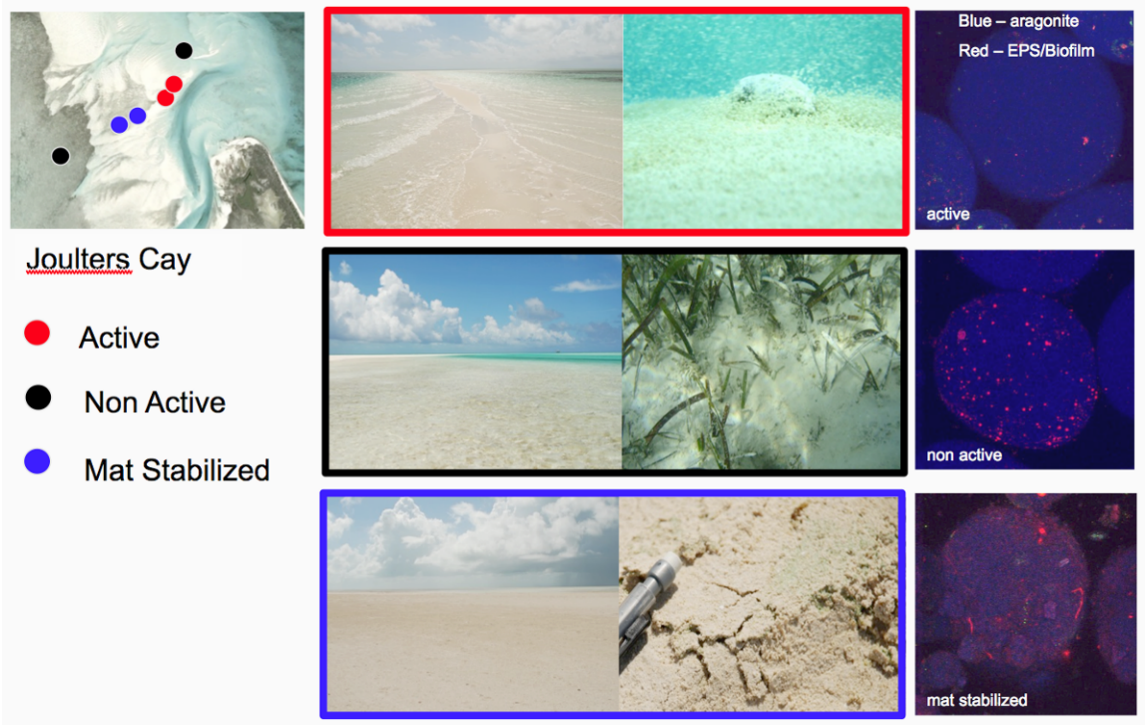


*Figure 1. Transect from the active to the mat stabilized environments of the Joulters Cay ooid shoal and confocal laser scanning microscopy images of the grains and their EPS coating. The confocal images depict a progression of the amount of EPS coating from active to the mat stabilized environment.*

## CHARACTERIZATION OF SEDIMENT BIOFILM/EPS

We will conduct quantitative analysis of EPS coatings on sediment samples from various hydrodynamic settings. EPS analysis will follow the sulfuric acid assay. In addition, some of the samples will be preserved in 70% formaldehyde to microscopically analyze the microspatial distribution of attached microbes within biofilms. Confocal laser scanning microscopy (CLSM) provides the ability to acquire in-focus images from selected depths, a process known as optical sectioning or tomography. Images are acquired point-by-point allowing for three-dimensional surface reconstructions of topologically-complex objects like sand grains. Sediments observed with CLSM are stained with a cyanine die-conjugated lectin, wheat germ agglutinin (WGA). The WGA lectin binds to extracellular polysaccharide secretions (EPS) associated with the biofilms (Neu et al., 2001). Environmental scanning electron microscopy (ESEM) will also be used to obtain high-resolution images of natural sediment biofilms. Together, these analyses will allow quantification and visualization of the spatial distribution of EPS and microbes on sediment grains and the relationship of these two components in sediment biofilms.

In addition, we will also test the adherence/stability of EPS within various hydrodynamic conditions. Toward this end, we will perform an agitation assay that

emulates various hydrologic conditions found within oolitic shoals. Treatment 1 and treatment 2 will simulate low and high groundwater flow within the pore space and will be undertaken in flow-through columns. Published reports for groundwater flow range from 0.025 – 4.3 L $m^{-2}$ $min^{-1}$ (Shellenbarger et al., 2006). Core sediments exposed to treatment 3 will be emptied into a large glass baking dish along with 2 liters of filtered seawater and placed on a laboratory rocker/shaker table set at 10 rpms for a duration equal to the pumping times of treatments 1 and 2. Treatment 4 sediments will be placed into a sterile 4 L polyethylene bottle and vigorously shaken on a reciprocating shaker for the same duration. In treatment 5 we will attempt to remove all associated microbes from the sediment grains by subjecting them to pulsed ultrasonic treatment (150 s incubation time, 30% of this time pulsed, Bandelin M72 probe, 3mm diameter, 20 kHz, 70W, vials on ice) following an optimized protocol for sandy sediments (Ferguson et al., 2005, Rusch et al., 2006). After the sand grains have settled and the supernatant removed, the remaining sediment will be washed 6 times with filtered seawater and all supernatants combined. Ultrasonic treatments followed by 6 washings each will be carried out until no additional microbes can be removed from the sediment. For each treatment the removed EPS will be quantified using the sulfuric acid assay.

## BIOFILM GENE PROFILING

Profiling of microbial functional genes will provide insight on the intrinsic enzymatic capacity of key genes associated to carbonate precipitation. To achieve this goal, we will use GeoChip, a robust high-throughput functional gene array that comprises over 24,243 oligonucleotide probes with the potential capability to target 150 functional gene groups involved in nitrogen, carbon and sulfur cycling (He et al., 2007). Special attention will be given to microbial genes that are key players in metabolic processes (e.g. denitrification, sulfate reduction, photosynthesis) known to create an alkaline environment with conditions that promote calcium carbonate precipitation. Some genes of interest will include: a) carbon fixation (RbCL); b) sulfate reduction (dSr); c) nitrogen reduction (narG, nasA, niRs, niRk, norB, nosZ); d) ammonia monooxygenase (amoA); e) methane oxidation (mmO, pmO; and f) urea hydrolysis (ureC). Gene profiles will be compared between sub-environments of the Joulters Cay ooid shoal.

## PRECIPITATION EXPERIMENTS

To test the effect of microbes and EPS on calcification, a series of precipitation experiments will be performed in small flow-through sediment columns. The column experiments will be performed on three sediment treatments. Treatment 1 will represent the control treatment in which all microbes and EPS will be removed from the sediment surface. In treatment 2, all active microbes will be killed through UV and antibiotic treatment, but the sediment-associated biofilm will remain in place. In treatment 3 sediments with active biofilms will be loaded into the precipitation columns and supplemented with seawater growth media. Precipitation in the three treatments will be compared using the buoyant weight method and will be visually inspected under SEM.

## KEY DELIVERABLES OR EXPECTED RESULTS

- Quantitative data on sediment Biofilm/EPS concentrations across different depositional facies based on the sulfuric acid assay and CLSM.
- A quantitative assessment of the stability/adherence of biofilm/EPS to sediment surfaces under varying hydrodynamic regimes.

- Preliminary assessment of the variation in sediment microbial metabolisms operating in a range of depositional settings based on functional gene profiles.
- Assessment of the role of active microbes and associated EPS coatings in controlled ooids precipitation studies.

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