AAPG HEDBERG CONFERENCE
"MICROBIAL CARBONATE RESERVOIR CHARACTERIZATION"
JUNE 4-8, 2012 – HOUSTON, TEXAS

Microbial Characterization of Carbonate Surface Sediments from the Bahamas and Turks and Caicos Platforms

Mara R. Diaz, Alan M. Piggot, and James S. Klaus

Division of Marine Geology and Geophysics, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami FL

Microorganisms are abundant and ubiquotous components of carbonate sediments where their metabolisms can modify the geochemical conditions of their surroundings. The consortia of microorganisms along with their vast metabolic diversity allow these communities to efficiently cycle major elements and mediate the degree of carbonate saturation (González-Muñoz 2010; Dittrich and Sibler 2005; Baskar et al 2006) Heterotrophic and autotrophic bacteria are known to play a role in carbonate precipitation (Dupraz et al 2009). These microbes contribute to the formation of carbonates deposits that potentially form reservoir rocks, such as stromatolites (Dupraz and Visccher 2005), dolomites; (Wright and Wacey 2005) and ooid shoals (Kahle 2007). The primary role of bacteria in the precipitation process has been attributed to their metabolic activity (sulfate reduction, denitrification, ammonification, anaerobic sulphide oxidation, and photosynthesis) creating an alkaline environment that induces mineral precipitation (Douglas and Beveridge 1998, Castanier et al. 2000). In addition, the negatively charged nature of their cell wall and extracellular polymeric secretions (EPS), which is influenced by specific functional groups, can further trigger precipitation as they create an ideal microenvironment for crystal nucleation sites of divalent cations, Ca^{+2} and Mg^{+2} (Rivadeneyra et al. 1998). Gerdes et al. (1994) observed that patterns of calcification in biogenic carbonates emphasize both ecological situations and conditions of early diagenesis.

To understand the biological role that these communities exert on different depositional facies and the probable role they play in the formation and precipitation of carbonate deposits it is imperative to delineate variations in microbial composition. This study is the first comparative study that investigates the microbial composition of active, non-active, and mat-stablized carbonate environments of Great Bahama Bank, Cat Cay, Joulters Cay, Shroud Cay, and the Turks and Caicos platform. To date these microbial communities remain poorly described.

Carbonate surface sediments collected from various depositional environments were examined to determine whether different sedimentary facies harbor unique microbial assemblages. Genomic DNA was extracted and the 16S rRNA gene was amplified using polymerase chain reaction (PCR) with universal bacterial primers. Terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA gene was used to assess the variation in bacterial communities between active, non-active, and mat-stabilized environments. Clone libraries were made and the 16S rRNA gene was sequenced to identify dominant populations within sediment samples.

Bacterial 16S rRNA clone libraries were constructed from the three different depositional environments and included a representative sample from stations: Ang (active), GBB10.2ng (non-active) and Bng (mat-stabilized). In total, 267 clones were screened and sequenced, resulting in partial 16S rRNA gene sequence reads between ~400 to 650 bases. All three rRNA clone libraries were phylogenetically diverse and included numerous uncultivated phylotypes associated with marine environments. Comparative 16S rRNA phylogenetic analysis revealed the presence of sequences affiliated to Alphaproteobacteria, Gammaproteobacteria, Acidobacteria, Bacteroidetes, Deltaproteobacteria, Actinobacteria, Plantomycetales, Cyanobacteria, Verrumicrobia, Chloroflexi, Nitrospirae and Firmicutes, whereas Epsilonproteobacteria and Betaproteobacteria were absent (Figure 1). All clone libraries were predominantly characterized by a higher abundance of sequences affiliated to the Alphaproteobacteria group (Figure 1). Even though previous studies have hypothesized that turbulent hydrodynamic conditions contribute to lower bacterial abundance, lower nutrient levels and overall microbial diversity in marine sands (Buhring et al 2005; Llobet-Brossa et al 1995), this study revealed broad bacterial diversity across many of the documented phyla in all three depositional environments. This was supported by our T-RFLP and clone analysis that showed that all three environments harbor a highly diverse microbial assemblage.

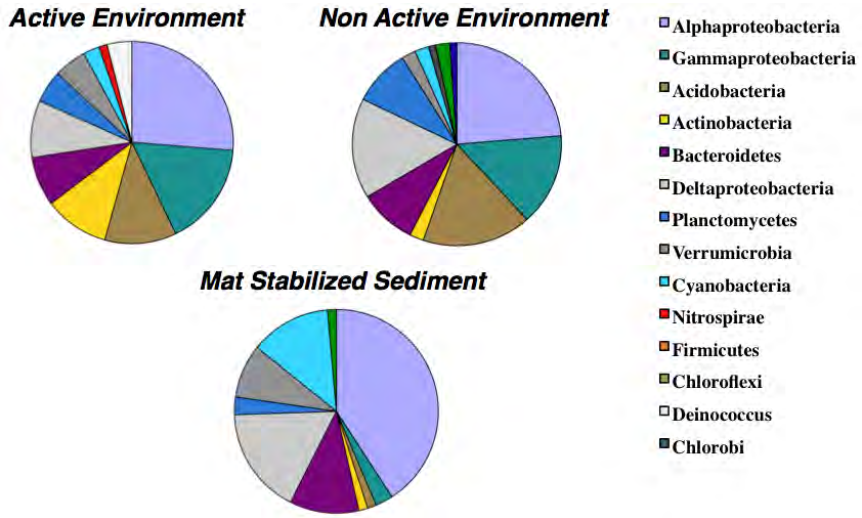

Figure 1. Pie diagrams illustrating the bacterial diversity within carbonate surface sediments of the active, non-active, and mat-stablized environments from the Bahamas Platform.

To visually complement the molecular microbial characterizations, sediment samples were additionally 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 (Figure 2). Environmental scanning electron microscopy (ESEM) was also used to obtain high-resolution images of natural sediment biofilms. Together, these studies allow visualization of the spatial

distribution of EPS and microbes on sediment grains and the relationship of these two components in sediment biofilms.

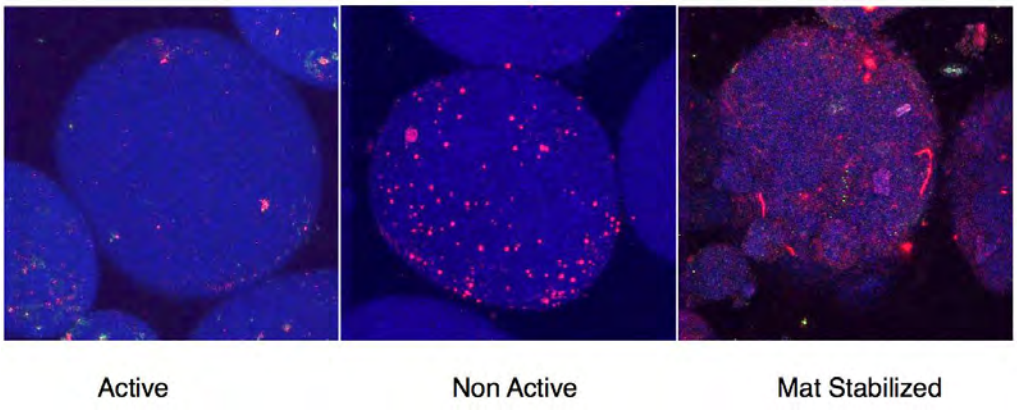

Figure 2. Confocal Laser Scanning Microscopy (CLSM) micrographs of ooid grains sampled from active, non-active, and mat-stabilized environments of the Bahamas platform. Blue color represents aragonite auto-fluorescence, pink color represents WGA lectin stain bound to extracellular polysaccharide secretions (EPS).

References

Baskar, S, R. Baskar, L. Mauclaire, JA. McKenzie 2006. Microbially induced precipitation in culture experiments:Possible origin for stalactites in Sahastradhara caves, Dehradun, India. Current Science 90:58-64.

Buhring, S. I., M. Elvert, and U. Witte. 2005. The microbial community structure of different permeable sandy sediments characterized by the investigation of bacterial fatty acids and fluorescence in situ hybridization. Environ. Microbiol. 7:281-293

Castenholz, RW, and F. Garcia–Pichel. Cyanobacterial responses to UV radiation. In: B. A. Whitton & Potts (Eds.) *Ecology of cyanobacteria*: *Their diversity in time and space* (pp591-611) Dordrecht: Kluwer academy.

Dittrich M, and S Sibler. 2010. Calcium carbonate precipitation by cyanobacterial polysaccharides. Geological Society, London, Special Publications 336: 51-63

Dupraz, C., PT Visscher, 2005. Microbial lithification in marine stromatolites and hypersaline mats. Trends in Microbiology 13: 429-438.

Dupraz, C., R. Reid, O. Braissant, A. Decho, R. Norman and P. Visscher, 2009, Processes of carbonate precipitation in modern microbial mats. Earth-Science Reviews 96: 141–162

González-Muñoz, MT, C. Rodríguez-Navarro, F. Martnez Ruiz, J. M. Arias, M. L. Merroun, and M. Rodríguez-Gallego. 2010. Bacterial biomineralization: new insights from *Myxococcus*-induced mineral precipitation. Geol. Soc. of London, Special publications 336: 31-50

Kahle C.F. 2007.Proposed origin of Aragonite Bahaman and some Pleistocene marine ooids involving bacteria, nannobacteria and biofilms. Carbonates and Evaporites 22: 10-22

Llobet-Brossa, E., R. Rossello-Mora, and R. Amann. 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. Appl. Environ. Microbiol. 64: 2691-2696

Wright, D.T. and D. Wacey, 2005. Precipitation of dolomite using sulphate-reducing bacteria from the Coorong Region, South Australia: significance and implications. *Sedimentology* 52: 987-1008