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Bacterial community of oolitic carbonate sediments of the Bahamas Archipelago

Mara R. Diaz[1,*], Alan M. Piggot[2], Gregor P. Eberli[2], James S. Klaus[2,3]

[1]Marine Biology and Fisheries, and [2]Marine Geology and Geophysics, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida 33149, USA

[3]Department of Geological Sciences, University of Miami, Coral Gables, Florida 33146, USA

ABSTRACT: The present study characterized bacterial communities associated with oolitic carbonate sediments from the Bahamas Archipelago, ranging from high-energy 'active' to lower energy 'non-active' and 'mat-stabilized' environments. Bacterial communities were analyzed using terminal restriction fragment length polymorphisms (TRFLP), clone analyses of the 16S rRNA gene, confocal laser scanning microscopy (CLSM) and the quantitative phenol-sulfuric acid assay for extracellular polymeric substances (EPS). Confocal imaging of oolitic grains stained with cyanine dye-conjugated lectin and EPS quantification demonstrated that all 3 environments harbored attached biofilm communities, but densities increased from the active to the mat-stabilized environment. Bacterial communities associated with all 3 settings were highly diverse and dominated by *Proteobacteria* (50 to 61%). Analysis of similarity (ANOSIM) and similarity percentages (SIMPER) revealed significant differences among the 3 environments in the relative abundance of *Proteobacteria*, *Planctomycetes*, *Cyanobacteria*, *Chlorobi*, and *Deinococcus-Thermus*. Bacterial primary production in the active shoal environment was associated with *Rhodobacteraceae*, *Ectothiorhodospiraceae*, and *Chlorobi*, whereas the lower energy environments appear to harbor a more complex consortium of aerobic photoautotrophs and anaerobic/aerobic anoxygenic phototrophs. The ubiquitousness of photosynthetizers, along with the presence of aerobic/anaerobic heterotrophic microbes (e.g. denitrifiers, sulfate-reducers, biofilm producers/degraders) and the gradient increase in biofilm production on ooid grains from active to mat-stabilized environments, support the potential involvement of these communities in biomineralization and carbonate precipitation.

KEY WORDS: Ooids · Carbonate sediment · Carbonate precipitation · 16S rRNA diversity · Bacterial community · Biofilm

INTRODUCTION

Microorganisms are ubiquitous in marine sediments where their presence and metabolic processes can modify the physical and geochemical conditions of their surroundings. The metabolic diversity of microbial communities allows them to efficiently cycle major elements and mediate the degree of carbonate saturation of their surroundings (Dupraz & Visscher 2005, Dittrich & Sibler 2010, González-Muñoz et al. 2010). Mediation of calcium carbonate ($CaCO_3$) precipitation is a widespread phenomenon occurring in different taxonomic groups and in different ecosystems, including marine and lacustrine environments (Ehrlich 1998, Castanier et al. 2000, Dupraz & Visscher 2005). Recently, much attention has been given to carbonate precipitation, as it plays a fundamental role in calcium and carbon biogeochemical cycles. It contributes to atmospheric CO_2 fixation and carbon sequestration, leading to the for-

*Email: mdiaz@rsmas.miami.edu

mation of carbonate sediments and rocks (Riding 2000, Ridgwell & Zeebe 2005). There are a number of ways by which the physical presence or metabolic activity of microbes could either promote or inhibit $CaCO_3$ precipitation (Dupraz & Visscher 2005). Microbial metabolism in the form of photosynthesis, anoxygenic photoautotrophy, sulfate reduction (SR), denitrification, sulfur oxidation, and ammonification can promote calcification by creating a more alkaline environment (Douglas & Beveridge 1998, Castanier et al. 2000, Ries et al. 2008), whereas aerobic respiration, sulfide oxidation and ammonium oxidation can promote dissolution (Visscher & Stolz 2005).

Precipitation of $CaCO_3$ can also be influenced by microbial extracellular polymeric substances (EPS) (Decho 2000, Visscher & Stolz 2005). EPS are the principal structural components of biofilms and facilitate the attachment of microbes to surfaces or to each other. In doing so, EPS provide protection from physical and biological challenges of the environment, including fluctuations in temperature, ion concentration, desiccation, UV radiation, and wave action (Davey & O'Toole 2000). Precipitation of $CaCO_3$ can either be promoted or inhibited by EPS (Dupraz et al. 2004, Visscher & Stolz 2005, Braissant et al. 2009). EPS can act as chelators, inhibiting carbonate precipitation by trapping free divalent cations (i.e. Ca^{2+} and Mg^{2+}) from solution (Kawaguchi & Decho 2002, Braissant et al. 2009). Conversely, the degradation of EPS by heterotrophic bacteria reduces the cation binding capacity of EPS and liberates Ca^{2+} (Dupraz & Visscher 2005, Baumgartner et al. 2006). While the degradation of EPS by sulfate reducing bacteria (SRB) has been shown to be a primary factor in the lithification of stromatolites and other microbial mats of the Bahamas (Reid et al. 2000, Dupraz et al. 2004, Braissant et al. 2007), EPS produced by photosynthetic organisms can promote early carbonate precipitation and further control cortex accretion in freshwater ooids (Pacton et al. 2012).

Ooids are spherical, sand-sized grains with layers of tangentially or radially arranged calcite or aragonite crystals around a nucleus. Vast areas of modern marine ooids are found in the Bahamas (Ball 1967, Harris 1979), the Arabian Gulf (Loreau & Purser 1973), the South Pacific (Rankey & Reeder 2009) and Shark Bay, Australia (Davies 1970). Ooid sands are considered major contributors to global carbonate accumulation, which has been estimated at 3.2 Gt yr^{-1} (Milliman 1993, Milliman and Droxler 1995). In addition, ooids are important paleoclimatic and paleoceanographic indicators and, as such, are used as proxies for warm waters or tropical settings bearing high salinities (Opdyke & Wilkinson 1990). While it is widely recognized that ooid formation requires water supersaturated with respect to calcium carbonate, and an agitated environment that allows for CO_2 degassing, the role of microorganisms has been debated for decades. Previous work has shown concentric coatings to form in the presence of organic material (Davies et al. 1978, Brehm et al. 2006) and that aspartic acid, an amino acid associated with calcifying agents, is present in ooids (Mitterer 1968). More recently, Kahle (2007) proposed microbial induced precipitation in Bahamian ooids by relating crystal forms to bacteria morphology, while Plée et al. (2008, 2010) documented microbially induced Mg^{2+} calcite precipitation on ooids in the freshwater of Lake Geneva, Switzerland.

The Bahamas Archipelago represents a unique environment, one of the few modern shallow carbonate-producing systems where ooid formation is actively occurring. We used a combined approach of confocal laser scanning microscopy (CSLM), quantitative EPS analysis, clone library sequencing and terminal restriction fragment length polymorphysm (TRFLP) analysis of the 16S rRNA gene to assess the bacterial communities associated with ooids, ranging from high-energy 'active' environments to lower energy 'non-active' and 'mat-stabilized' environments of the Bahamas Archipelago. Differences in microbial composition are likely related to differences in the hydrodynamic characteristics of each environment, which may affect the biogeochemistry of inorganic nutrients and organic carbon availability. The goal is to characterize the bacterial communities within these environments and elucidate their potential functional role in the biogeochemical processes that relate to carbonate precipitation.

MATERIALS AND METHODS

Sites and sampling

The Bahamas Archipelago is composed of a series of large isolated carbonate platforms (Fig. 1). Sea surface temperatures range from 23 to 26°C in the northern archipelago, whereas in the south temperatures range from 25 to 29°C. Salinity fluctuates between 36 and 37 ppm. On these platforms, ooid shoal complexes can form margin parallel marine sand belts, tidal bar belts or platform interior blankets that stretch up to 100 km (Ball 1967). The Cat Cay Ooid Shoal forms a narrow margin parallel marine sand belt of approximately 15 km length on the western

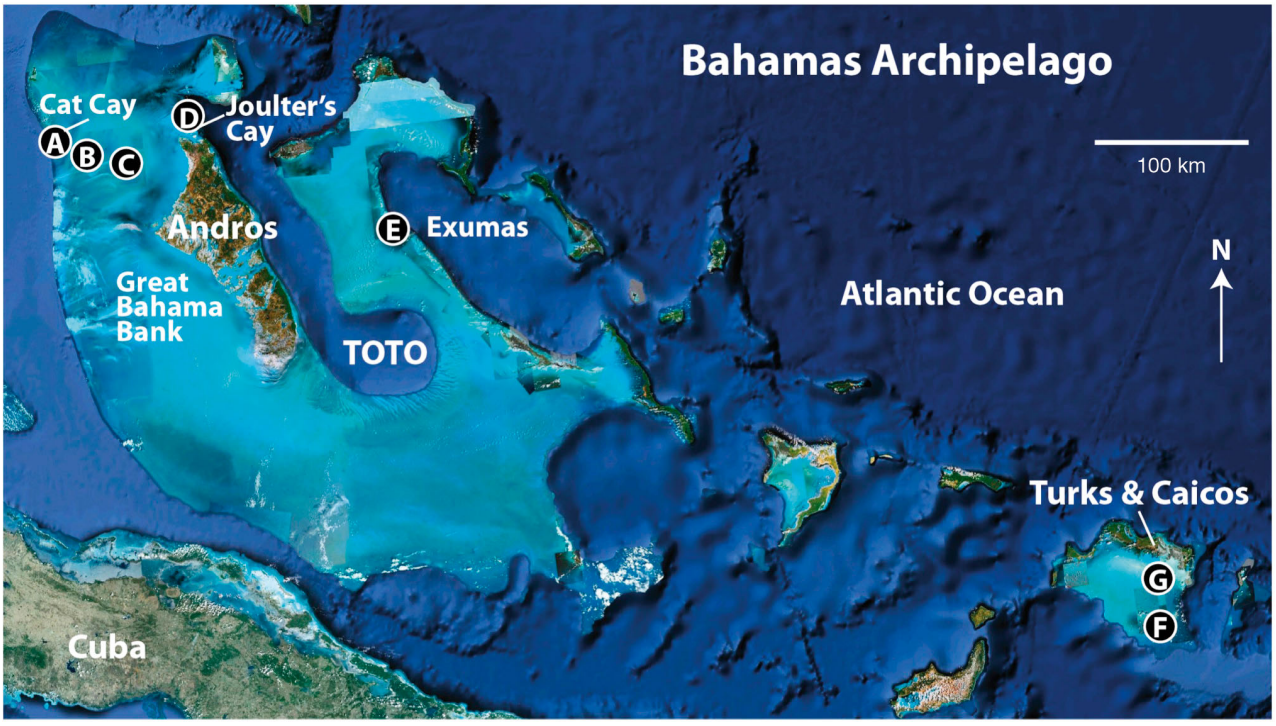

Fig. 1. Sampling sites in the Bahamas Archipelago. (A) Cat Cay ooid shoal (samples GBB-CC-1 through GBB-CC-4), (B) outer bank east of Cat Cay (sample GBB-10-2), (C) inner bank (sample GBB-1Bktop-1), (D) Joulter's Cay (samples GBB-JC-2 through GBB-JC-13), (E) Exumas (samples EX-WW-873, EX-IS-877, EX-HSSC and EX-STF), (F) Ambergris Cay, Turks and Caicos (samples TC-AK-1 and TC-AK-2), (G) Grapestone Shoal, Turks and Caicos (samples TC-GS-1 through TC-GS-3). Map obtained through GoogleEarth. See also Table 1

margin of Great Bahama Bank (Cruz 2008). Maximum tidal range is 119 cm and tidal flow velocities range from 40 to 80 cm s^{-1}. The sediments are moderately well-sorted, medium sand ooid/peloidal grains. The Joulter's Cay shoal complex comprises a 400 km^2 sand flat, cut by tidal channels and fringed by a mobile sand belt (Harris 1979, 1983) in which well-sorted ooids are moved by tidal currents with velocities exceeding 100 cm s^{-1}. The stabilized areas of the sand flat are exposed at spring low tide. The ooid shoals in the Exumas are tidal deltas with maximal tidal velocities of 200 cm s^{-1}. The grains are well-sorted (González & Eberli 1997, Rankey et al. 2006). On the Turks and Caicos Platform the shoals are impacted by cross-platform currents and wind-generated wave action (Wanless & Tedesco 1993). The ooid shoal complexes typically have 3 environments, independent of the shoal morphology. These are herein described as (1) active ooid shoal, (2) non-active ooids and (3) mat-stabilized ooids. In the active portion of the complex, tidal currents or wave action constantly move the ooids. The crests of active ooid shoals are characterized by high hydrodynamic energy with ooid grains colliding and rolling around 5 to 10 cm above the sea floor. There is no vegetation or other components to act as baffles in these areas and ooid grains are typically solitary. The non-active ooid areas flank the shoal crests and lie in deeper waters (~1 to 3 m). The hydrodynamic regime is less intense and can often be characterized as quiescent. Some areas are associated with sea grass. The grains are usually composed of ooids, with some admixtures of peloids, and skeletal grains. In other areas fine microbial mats start to stabilize the ooids without reaching a coherent mat structure.

Replicate samples were collected from each sampling site (Fig. 1, Table 1) during various expeditions aboard the RV 'Coral Reef' from June to July 2010, and consisted of approximately 5 g of ooids transferred into a sterile 50 ml polypropylene tube. Immediately after collection, the seawater was decanted and replaced with either 80% ethanol for DNA analyis, 10% formalin for confocal EPS images or with 0.5 mM ethylene diaminetetraacetic acid (EDTA) for EPS quantification using the phenol-sulfuric acid method. While EPS extractions were performed immediately, all other samples were stored at –20°C until further processing in the laboratory.

Table 1. Location information for the 27 sediment samples analyzed. Letters in parentheses are the sites marked in Fig. 1

Region	Sediment type	Sample	Environment	Latitude (°N)	Longitude (°W)
Great Bahama Bank					
Cat Cay (A)	Oolitic grainstone	GBB-CC-1	Active	25.50027	79.19917
		GBB-CC-2	Mat-stabilized	25.50027	79.19917
		GBB-CC-3	Non-active	25.50027	79.19917
		GBB-CC-4	Non-active	25.50027	79.19917
Outer bank (B)	Oolitic pelletoidal grainstone	GBB-10-2	Non-active	25.29165	78.84259
Inner bank (C)	Pelletoidal packstone	GBB-1Bktop-1	Non-active	25.42896	79.03449
Joulter's Cay (D)	Oolitic grainstone	GBB-JC-2	Non-active	25.29053	78.10764
		GBB-JC-3	Non-active	25.29098	78.10715
		GBB-JC-4	Active	25.27343	78.12161
		GBB-JC-5	Active	25.25856	78.11473
		GBB-JC-7	Mat-stabilized	25.33497	78.14612
		GBB-JC-8	Active	25.33628	78.14311
		GBB-JC-9	Non-active	25.33678	78.14301
		GBB-JC-10	Active	25.33610	78.14508
		GBB-JC-11	Mat-stabilized	25.33566	78.14607
		GBB-JC-12	Mat-stabilized	25.33513	78.14778
		GBB-JC-13	Non-active	25.33446	78.15049
Exumas					
Waderick Wells Cay (E)	Oolitic grainstone	EX-WW-873	Mat-stabilized	24.39048	76.62698
Hockey Stick Shoal (west) (E)	Oolitic grainstone	EX-IS-877	Non-active	24.37545	76.71948
Hockey Stick Shoal (E)	Oolitic grainstone	EX-HSSC	Active	24.37356	76.68262
Shroud Cay (E)	Oolitic grainstone	EX-STF	Mat-stabilized	24.50582	76.77943
Shroud Cay (E)	Oolitic grainstone	EX-SC-11	Mat-stabilized	24.51314	76.77104
Turks and Caicos					
Ambergris Cay (F)	Oolitic grainstone	TC-AK-1	Active	21.29989	71.75354
		TC-AK-2	Mat-stabilized	21.30486	71.68686
Grapestone Shoal (G)	Oolitic grainstone	TC-GS-1	Non-active	21.65315	71.80056
		TC-GS-2	Non-active	21.65315	71.80056
		TC-GS-3	Non-active	21.65315	71.80056

Visualization of sediment biofilm

EPS sediment coatings were visualized by confocal laser scanning microscopy (CLSM) using a cyanine dye-conjugated lectin stain (Burton et al. 2007, Piggot et al. 2009). Sand grains were prepared by incubation with a blocking buffer (5% BSA, Jackson ImmunoResearch and 0.1% Micr-O-protect, Roche Diagnostics, in PBS). Sediments were then rinsed in PBS and incubated with 10 µg ml^{-1} wheat germ agglutinin (WGA) conjugated with Alexa Fluor 647 (Invitrogen) in PBS. After 15 min, the sediments were rinsed 3 times in PBS and imaged using a Leica SP5 confocal microscope.

EPS quantification

EPS was extracted using a modified protocol of Decho et al. (2005). Approximately 2 g fresh sediment was collected in duplicate at active, non-active, and mat-stabilized sites of Joulter's Cay. Each sample was incubated with 0.5 mM EDTA for 15 min at 40°C and gently shaken 3 times every 5 min. After each treatment, samples were centrifuged at 8000 × *g* and the supernatant was pooled. The supernatant was mixed with cold (4°C) ethanol (final concentration of 70%) and settled for 8 h. Quantification of the EPS followed the phenol-sulfuric acid method as described by Piggot et al. (2012). Absorbance was measured at 490 nm using a spectrophotometer (BioPhotometer plus). The amount of carbohydrate present was determined by comparison with a calibration curve using D-glucose. The sediments were washed with deionized water to remove salts and were dried to measure EPS g^{-1} dry weight (DW).

DNA extraction and PCR amplification

DNA was extracted from 500 mg (wet weight) of each sample (Table 1), following the manufacturer's protocol of the FastDNA-spin Kit for soil (Q-BIOgene). Amplified products of the 16S rRNA gene

were obtained through PCR with bacterial forward primer U9F (5′-GAG TTT GAT YMT GGC TC) and bacterial reverse primer U1509R (5′-GYT ACC TTG TTA CGA CTT) (Integrated DNA Technologies). For the TRFLP analyses U9F was labeled at the 5′ end with phosphoramidite fluoro-chrome 6-carboxyfluorescein (6-FAM).

Targets were amplified in microtubes using Phire Hot Start II DNA Polymerase (New England Biolabs) in final volumes of 25 or 50 µl. The master mix contained 1.5 mM $MgCl_2$, 1.0 µM of forward and reverse primer pairs, 1.0 U Phire Hot Start II DNA Polymerase, and 200 µM each of dGTP, dCTP, dTTP and dATP. PCR reactions were incubated at 98°C for 30 s followed by 32 cycles consisting of 5 s of denaturating at 98°C, 5 s of annealing at 55°C and a 25 s extension at 72°C. A final extension step was carried out at 72°C for 1 min. PCR reactions were done in quadruplet, and pooled replicates were cleaned using a QIAquick PCR purification kit (QIAGEN). To ensure complete removal of primer-dimers, the bound DNA product was washed with 750 µl of a 35% guanidine hydrochloride solution.

Clone library sequencing and phylogenetic analysis

Purified amplicons were ligated with the pGEM-T Easy Vector (Promega). Recombinant vectors were transformed into chemically competent *E. coli* JM 109 cells. Subsamples of the transformed cells were transferred into Luria-Bertani (LB) agar plates supplemented with 100 µg ml^{-1} ampicillin, 0.5 mM IPTG and 80 µg ml^{-1} X-gal. Following overnight incubation at 37°C an average of 96 white clones per library were picked and screened for the presence of a forward orientated insert via PCR using plasmid primer T7 and U1509R. Positive inserts were unidirectionally sequenced on an ABI 3730xl sequencer (Applied Biosystems) using T7 and BigDye Terminator v3.1 Kit (Applied Biosystems) as per the manufacturer's protocol.

Sequences were checked for chimeras with Chimera Check v2.7 on the Ribosomal Database Project II (RDP II) website. Reads with high quality were assembled into consensus sequences by the program MegAlign (DNAStar Inc. v6). Sequences were trimmed to remove vector sequences prior to being deposited in GenBank under the accession numbers JQ917486 to JQ917719. Phylogenetic trees were constructed using select GenBank reference sequences to elucidate taxonomic assignment of phylotypes. Phylogenetic trees were generated with PAUP*4.0b10 (parsimony analysis, stepwise addition, random addition sequence, nearest neighbor interchange, 100 maximum tree). The robustness of the phylogenetic trees was tested by bootstrap analysis based on 1000 samplings. Coverage values of the clone libraries were calculated by the formula of Good (1953). Rarefaction calculations were done with the software Analytic Rarefaction v1.3. To determine the significance of differences between clone libraries, both UniFrac and phylogenetic (P)-test analyses were employed using the UniFrac web interface (http://bmf.colorado.edu/unifrac). Differences between bacterial assemblages were further assessed using lineage-specific analysis (http://bmf. colorado. edu/unifrac).

TRFLP analysis

Restriction digests of purified PCR products were performed independently using the tetrameric FAST Digest Restriction Enzymes, *Hha*I, *Msp*I, and *Rsa*I (Thermo Scientific), in accordance with the manufacturer's protocol. Digests were further purified using standard ethanol precipitation. One µl of the purified DNA digest was mixed with 10 ml of Hi-Di formamide solution (Applied Biosystems) and 0.15 ml of MapMarker1000 ROX-labeled sizing standard (Bioventures). TRFLP profiles were obtained using an ABI 3730xl capillary sequencer (Applied Biosystems) and the electropherograms were analyzed with GeneScan software. To eliminate primers and uncertainties associated with fragment size determination, peaks smaller than 65 bp and larger than 1000 bp were culled from the data set. Replicates representing 10% of the screened samples were analyzed in order to assess the variability associated with DNA extraction, PCR and restriction digest steps. To allow comparison of the clone library data with the TRFLP analyses, an *in silico* terminal restriction digestion was performed on the clone library sequences to generate fragment patterns that could be compared with the TRFLP patterns. Further theoretical digest comparisons were made with both the RDP II and an in-house database comprising bacterial sequences from coral reef environments (Frias-Lopez et al. 2002, Klaus et al. 2007). The *in silico* digests were undertaken using the TRFLP-Tap tool of the RDP II.

Similarity percentage analyses (SIMPER) were undertaken to establish TRFLP peak contributions to the average dissimilarity of samples between different groups. Multidimensional scaling (MDS) ordinations were performed to visually assess the related-

ness of samples, and the analysis of similarities test (ANOSIM) was performed using PRIMER v6 software. The Shannon-Wiener diversity index (*H'*) was calculated using PRIMER v6 software.

RESULTS

CLSM and EPS quantification

Confocal image analysis of ooid grains stained with cyanine dye-conjugated lectin demonstrated a clear progression in the amount of EPS coating from the active to the mat-stabilized environments (Fig. 2). The mat-stabilized sample displayed a more even distribution of the EPS coating, while representative samples from active and non-active environments displayed a spotted EPS distribution. These results were confirmed by phenol-sulfuric acid quantification of ooid EPS levels. Active ooids contained 3.9 ± 0.9 µg EPS g^{-1} DW, non-active ooids 11.6 ±7.2 µg EPS g^{-1} DW, and mat-stabilized ooids 31.4 ± 6.7 µg EPS g^{-1} DW.

Phylogenetic diversity

Bacterial 16S rRNA clone libraries were constructed from ooids of active (GBB-JC-4), non-active (GBB-10-2), and mat-stabilized (GBB-JC-12) environments (Table 1). In total, 267 clones were sequenced, among which 172 sequences (≥95% sequence identity) were unique. Based on rarefaction analysis, none of the libraries reached an asymptote (data not shown), suggesting continued sampling would increase species richness. Among the screened clones 57% shared more than 95% similarity to sequences published in GenBank. The total percentage coverage (C) of all 3 clone libraries was 40% at 95% sequence identity and 68% at 90% genera-level cut-off. Based on 95% sequence similarities, individual library coverage values (C) for active, non-active and mat-stabilized libraries were 47, 39, and 38% respectively, whereas sequence similarities to a 90% genera-level cut-off yielded coverage values of 66, 67, and 70%, respectively. Estimation of diversity based on the Shannon-Wiener index (*H'*) for active, non-active and mat-stabilized communities were 5.56, 6.06 and 5.75, respectively. Both UniFrac metric analysis and P-test analysis revealed significant differences in bacterial community composition among all 3 libraries (using 100 iterations; $p < 0.01$ for both tests) and within all pairwise comparisons (using 100 iterations; $p < 0.03$ for all pairwise comparisons for both tests). Based on the UniFrac lineage specific analysis, members within *Parvularculales*, *Sphingomonadales*, *Rhodobacterales*, and *Rhizobiales* made the most significant contribution to documented differences ($p < 0.001$).

All 3 rRNA clone libraries were phylogenetically diverse, represented by no fewer than 12 different phyla (Fig. 3). The active library was characterized by a high incidence of *Alphaproteobacteria* (25.3%), *Gammaproteobacteria* (16.4%), *Acidobacteria* (11%), *Actinobacteria*/*Bacteroidetes* (10%), and *Deltaproteobacteria* (8.8%). A greater percentage of sequences affiliated with *Deltaproteobacteria* (17%),

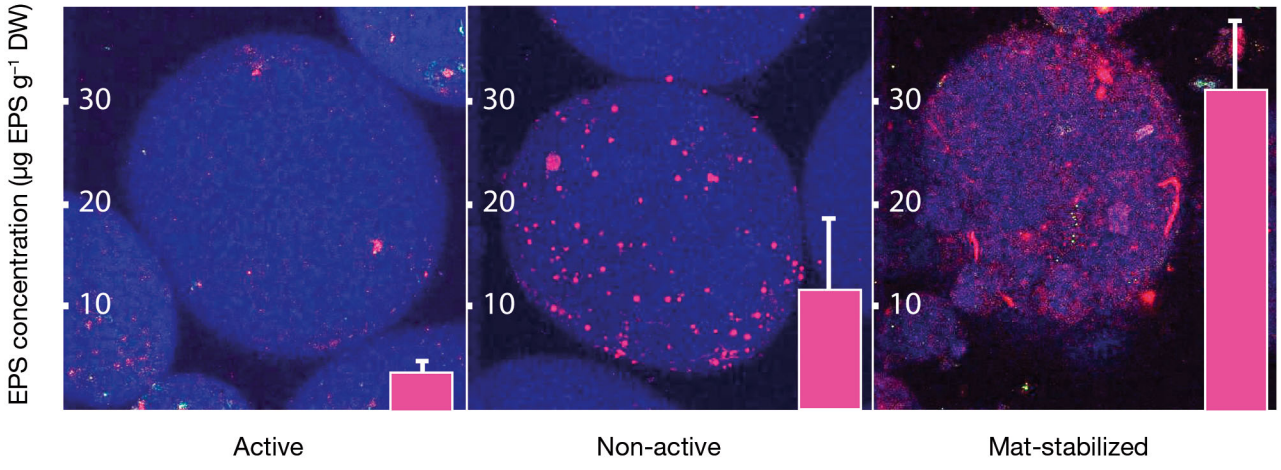

Fig. 2. Confocal laser scanning microscopy (CLSM) images of ooids and measurements of exopolymer secretion (EPS) from active, non-active and mat-stabilized environments of Joulter's Cay, Bahamas. Ooids were stained with a cyanine dye conjugated with lectin wheat germ agglutinin (WGA). Blue zones represent aragonite ooid grains and pink areas denote the EPS that bind the reporter-conjugate molecule. Histogram bars are the average EPS concentrations (µg EPS g^{-1} DW) based on phenol-sulfuric acid quantification. Error bars: SD of 3 sets of samples per environment (for each set n = 2)

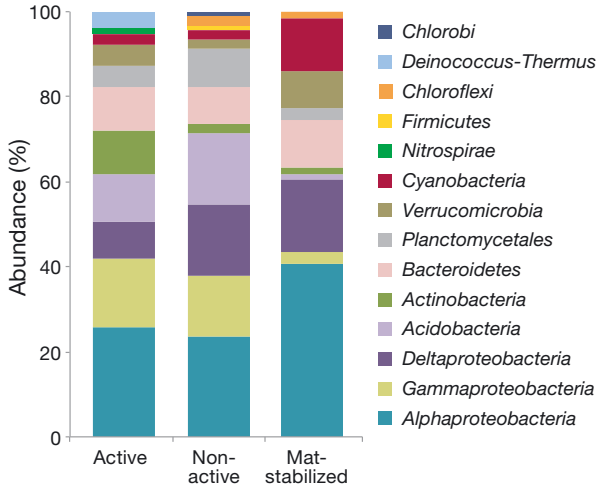

Fig. 3. Bacterial community composition in 16S rRNA clone libraries derived from different carbonate depositional environments. The Phylum *Proteobacteria* was split into the Classes *Alpha-*, *Gamma-*, and *Deltaproteobacteria*

Acidobacteria (17 %) and *Planctomycetes* (9 %) were detected in the non-active environment (Fig. 3). In contrast, the mat-stabilized library contained a much larger percentage of *Alphaproteobacteria* sequences (41 %). Other abundant groups included the *Deltaproteobacteria* (17 %), *Bacteroidetes* (11.3 %), and *Cyanobacteria* (12.7 %). Compared to the active and non-active libraries, *Acidobacteria* (1.4 %) and *Gammaproteobacteria* (2.8 %) were less common in the mat-stabilized libraries (Fig. 3). Members of the *Nitrospirae*, *Firmicutes*, *Chloroflexi* and *Deinococcus-Thermus* were less abundant and represented 1 to 3.8 % of the sequenced clones (Fig. 3).

The majority of phylotypes within the *Alphaproteobacteria* group of the active library belonged to *Hyphomicrobiaceae* and included the biofilm-forming genus *Pedomicrobium* and several phylotypes related to the Orders *Rhodospirillales*, *Rhodobacterales* and *Parvularculales* (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m485p009_supp.pdf). Within the non-active library, the *Alphaproteobacteria* group were dominated by *Rhodobacterales* and *Rhodospirillales*, whereas the mat-stabilized library were mainly dominated by the *Rhodobacterales*, *Sphingomonadales, Parvularculales* and a few members within the *Roseobacter* clade with affiliation to aerobic anoxygenic photosynthetizer species. Some *Sphingomonadales* clones shared 99 % similarities with the anoxygenic phototroph, *Erythrobacter aquimaris* (Fig. S1).

Deltaproteobacteria were the second most encountered group in mat-stabilized and non-active clone libraries, mainly associated with the calcifying biofilm producer *Myxococcales* and sulfate reducing bacteria. The *Desulfobacterales* group comprised 8 clones, 7 of which were from the non-active library. The majority of these clones were distantly related to the sulfate reducer, *Desulfococcus multivorans*. The *Desulfovibrionales*, which were solely present in active and mat libraries, were associated with an uncultured *Desulfomicrobium* sp. (Fig. S2 in the Supplement).

Gammaproteobacteria and *Acidobacteria* were encountered at moderately high levels in both active and non-active libraries, but represented less than 2.7 % of the total clones in the mat-stabilized library (Fig. 3 & Figs. S2 & S3 in the Supplement). *Gammaproteobacteria* in the non-active library included sequences related to the predominantly phototrophic Order *Chromatiales* and the oligotrophic marine group with capacity for aerobic anoxigenic phototrophy, the NOR5/OM60 clade (Fig. S2). The *Chromatiales* group displayed sequences related to the sulfur oxidizing genera *Thiobacillus*, and *Thiovirga* (Fig. S2). The *Gammaproteobacteria* phylotypes in the active-library were diverse and included the denitrifier/nitrogen fixer group, *Pseudomonadales* and 3 phylogenetic groups within the *Chromatiales* group, *Legionellales*. (Fig.S2). *Gammaproteobacteria* were the least diverse group in the mat-stabilized library and recovered a sequence related to an uncultured anoxygenic phototroph, *Ectothiorhodospira* sp. (Fig. S2). *Acidobacteria* sequences were abundant in both the active and non-active libraries and grouped into 3 main clades within the *Halophagales* (Fig. S3). Most sequences related to unclassified environmental bacteria from marine sediments.

Planctomycetes were recovered in all libraries but were less frequent in the mat-stabilized library (Fig. S3 in the Supplement). Some of the *Planctomycetes* clones were associated with known particle-attached species within the Families *Planctomycetaceae* and *Phycisphaeraceae*. The non-active library retrieved sequences associated with the aerobic heterotrophic bacterium, *Pirellula* sp. and the heterotrophic facultative anaerobic bacterium, *Phycisphaera mikurensis,* while the active library retrieved a single clone (Ang C6) distantly related to autotrophic and anaerobic ammonium oxidizers (anammox) (Fig. S3).

Cyanobacteria were abundant in the mat-stabilized library but were minor components in both the active and non-active libraries (Fig. S4 in the Supplement). The active library retrieved a single clone (Ang A10) that branched out from a clade representing known nitrogen fixers within the *Oscillatoriales* group. Similarly, the non-active library recovered a

few clones associated with the *Oscillatoriales* (*Leptolyngbya* sp., *Halomicronema* sp.). Over 40% of the mat-stabilized clones were identified as members of *Oscillatoriales* and shared over 94% similarity with the genus *Leptolyngbya* (Fig. S4). Other taxonomic lineages included the Orders *Pleurocapsales* and *Chroococcales*.

All libraries recovered nearly the same proportion of *Bacteroidetes*, accounting for 10 to 11% of the clones. Despite the polyphyletic distribution of unresolved species (Nakagawa et al. 2002), some clones were related to *Sphingobacteriales*, *Flavobacteriales*, and the predominantly aerobic chemoorganoheterotrophic group, *Cytophagales* (Fig. S4).

Verrucomicrobia (Fig. S4) represented 9, 5 and 2.2% of the total sequenced clones in mat-stabilized, active and non-active libraries, respectively. While most of these sequences displayed over 92% similarity with uncultured bacterium clones, a few clones shared sequence similarity with the aerobic oligotrophic and chemoheterotrophic bacteria, *Verrucomicrobium spinosum* and *Opitutus* sp. (Fig. S4).

Representatives of the *Chloroflexi* group were only retrieved from mat-stabilized and non-active libraries (Fig. S4) and shared 93 to 99% similarities with an uncultured *Chloroflexi* isolate from coastal sediments (Fig. S4). Clones affiliated with *Deinococcus-Thermus* were represented by members of the active library, and included a single clone with 100% similarity to a bacterium associated with the marine sponge, *Discodermia dissoluta* (Fig. S3).

TRFLP analysis

Variation in microbial community composition was also evaluated by comparing theoretical enzyme digests of sequenced clone libraries with TRFLP profiles from 27 sediment samples (7 active, 12 non-active, and 8 mat-stabilized). A combined total of 1938 TRFs (694-*Hha*I, 679-*Msp*I, and 563-*Rsa*I) were identified. Bacterial diversity estimates (Shannon-Wiener's H') based on TRF patterns for active, non-active and mat-stabilized environments were 5.14, 5.17 and 5.27, respectively.

The ANOSIM test for differences in microbial communities associated with active, non-active, and mat-stabilized environments revealed significant differences (Global $R = 0.44$; $p < 0.001$). Furthermore, significant differences were detected in the pairwise comparisons: mat-stabilized versus non-active ($R = 0.58$; $p < 0.001$), and mat-stabilized versus active ($R = 0.53$; $p < 0.002$). In contrast, lower statistical significance levels were documented for active versus non-active ($R = 0.21$; $p < 0.04$). These differences were apparent in the MDS ordination, which showed a gradation from active samples at the far left and mat-stabilized samples scattered to the right (Fig. 4A).

The average dissimilarity between the active and non-active group was 70.9%. Among the TRFs, TRF *Hha*I-88 made the largest contribution to dissimilarities and was likely associated with *Myxococcales,* a phylogenetic group comprising calcifying biofilm producers (Jiménez-López et al. 2011) (Table 2,

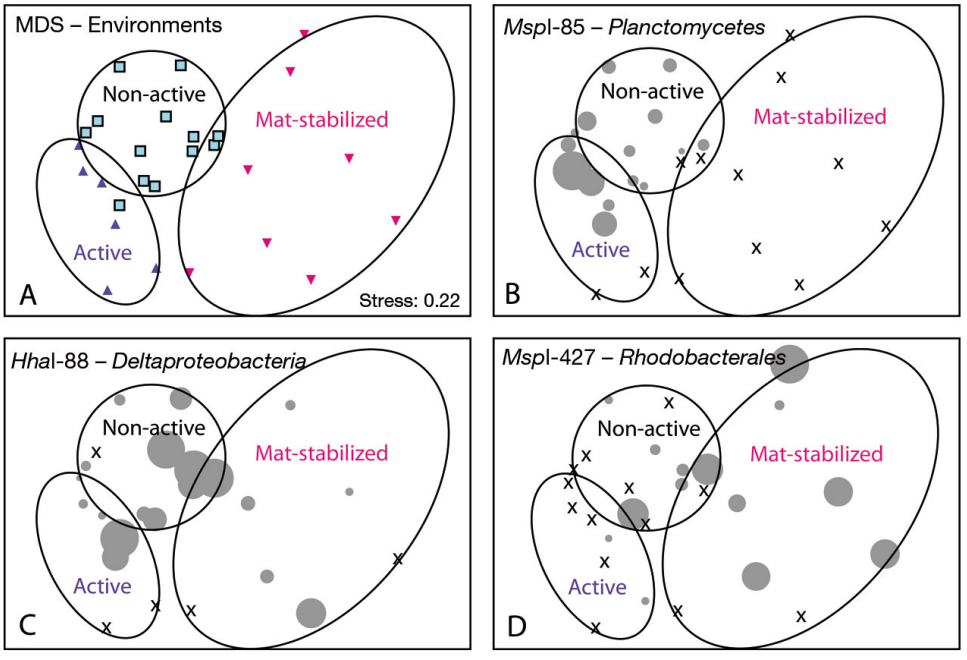

Fig. 4. Bacterial communities associated with oolitic carbonate sediments. Ordination plots were based on the Bray-Curtis similarity coefficient between terminal restriction fragment (TRF) profiles of the 16S rRNA gene digested with the restriction enzymes *Hha*I, *Msp*I, and *Rsa*I. (A) The relative similarity of bacterial communities in the active, non-active and mat-stabilized environments (B), (B–D) the relative abundance of *Planctomycetes* TRF *Msp*I-85, (C) *Deltaproteobacteria* (*Myxococcales*) TRF *Hha*I-88, and (D) *Alphaproteobacteria* (*Rhodobacterales*) TRF *Msp*I-427. Gray shaded bubble symbols represent the relative bacterial abundances between samples

Table 2. Average abundance and taxonomic affiliation of common and representative TRFs. **Bold** text denotes the most probable taxonomic affilation. p-values < 0.05, based on a Kruskal-Wallis nonparametric ANOVA, reflect statistically significant differences between active, non-active and mat-stabilized samples

TRF	Average abundance (%)			Kruskal-Wallis p-value	Probable taxonomic affiliation
	Active	Non-active	Mat-stabilized		
*Msp*I-82	1.94	2.99	0.81	0.132	***Planctomycetes* (*Phycisphaeraceae*)**
*Msp*I-85	4.59	2.61	0.02	0.004	***Planctomycetes***
*Msp*I-134	2.60	1.99	0.78	0.014	***Alphaproteobacteria* (*Rhodobacterales*)**
*Msp*I-136	2.50	1.01	0.35	0.001	***Planctomycetes* (*Planctomycetaceae*)**
*Msp*I-150	0.17	0.30	2.45	0.015	***Cyanobacteria* (*Oscillatoriales-Lyngbya* sp.)**
*Msp*I-154	3.83	2.26	1.36	0.172	***Deltaproteobacteria* (unclassified)**/*Gammaproteobacteria* (*Halothiobacillaceae*)
*Msp*I-164	2.61	0.96	0.24	0.013	***Alphaproteobacteria* (unclassified)**/*Actinobacteria*
*Msp*I-427	0.54	2.32	4.48	0.055	***Alphaproteobacteria* (*Roseobacter-Rhodobacteraceae*)**
*Msp*I-479	1.40	2.80	1.99	0.716	***Chlorobi***/*Gammaproteobacteria* (*Chromatiaceae*)
*Rsa*I-80	2.45	1.75	1.06	0.404	***Nitrospirae***
*Rsa*I-109	2.34	2.49	2.63	0.97	***Alphaproteobacteria* (*Parvularculales-Parvularcula* sp.)**
*Rsa*I-111	1.20	2.69	2.81	0.192	***Planctomycetes* (*Phycisphaeraceae*)**
*Rsa*I-112	2.46	1.78	3.34	0.521	***Alphaproteobacteria* (*Sphingomonadales*, *Hyphomicrobiaceae*, *Rhodospirillaceae* & *Rhodobacteraceae*)**
*Rsa*I-121	2.14	1.93	0.56	0.071	***Planctomycetes* (*Planctomycetaceae-Pirellula* sp.)**
*Rsa*I-124	4.04	1.57	0.22	0.012	Unknown TRF
*Rsa*I-859	1.63	2.93	0.36	0.776	Unknown TRF
*Hha*I-88	3.27	8.46	3.84	0.051	***Deltaproteobacteria* (*Myxococcales-Polyangiaceae*)**
*Hha*I-89	1.91	3.04	0.16	0.449	***Alphaproteobacteria* (*Rhodospirillaceae*)**/*Planctomycetes* (*Planctomycetaceae*)
*Hha*I-91	3.13	0.92	0.05	0.255	***Deinococcus-Thermus*/***Gammaproteobacteria*
*Hha*I-201	2.20	2.32	0.13	0.277	***Bacteroidetes***
*Hha*I-202	1.31	3.08	1.16	0.285	***Gammaproteobacteria* (*Pseudomonadales*)**/*Chloroflexi*
*Hha*I-361	0.92	2.81	0.67	0.003	***Planctomycetes* (*Planctomycetaceae*)**/*Bacteroidetes* (*Flavobacteriaceae*)
*Hha*I-500	0.48	1.45	3.02	0.163	***Alphaproteobacteria* (*Parvularculales*)**
*Hha*I-502	0.84	2.50	0.00	0.005	Unknown TRF
*Hha*I-553	0.40	2.35	0.29	0.004	***Gammaproteobacteria* (*Chromatiaceae*)**

Fig. 4C). Other distinguishing TRFs included *Msp*I-85 (Fig. 4B), *Rsa*I-124 and *Msp*I-154, which were all more abundant in the active samples (Table 2). In contrast, *Rsa*I-859, *Hha*I-202, and *Hha*I-89 were more abundant in the non-active samples. Most of these peaks were statistically different from the probable order-level identifications indicated in Table 2.

The non-active and mat-stabilized communities displayed an average dissimilarity of 76.0%, with TRF *Hha*I-88 contributing most to differences in these communities (Table 2). Other TRFs that accounted for differences included *Msp*I-85, *Hha*I-502, and *Msp*I-479, which were more common in non-active environments; and peaks *Rsa*I-112, *Msp*I-427, and *Hha*I-500, which were more abundant in the mat-stabilized environments (Table 2, Fig. 4D). TRF *Rsa*I-112 was identified as an alphaproteobacterium in the Order *Sphingomonadales*, *Msp*I-427 as a *Rhodobacterales* bacterium, *Msp*I-479 as *Chlorobi* and *Hha* I-500 as a *Parvularculales* bacterium.

Active and mat-stabilized communities displayed the highest dissimilarity value (78.4%). The most important TRF contributing to this dissimilarity was TRF *Msp*I-85 with abundance levels of 4.59% in active samples and 0.02% in mat-stabilized samples (Table 2, Fig. 4B). This restriction site, which was identified in clone Ang D7, exhibited 96% similarity with GenBank clone FJ71726 within the *Planctomycetes* Phylum (Fig. S4 in the Supplement). Other TRFs that contributed to the high dissimilarities and were commonly found in the active environment included *Hha*I-91, *Rsa*I-124, *Msp*I-154, and *Msp*I-134 (Table 2). In contrast, *Msp*I-427, *Msp*I-150, and *Rsa*I-112 were abundant in mat-stabilized sediments.

The high average abundance of *Msp*I-427 (4.48%) in the mat-stabilized environment is in sharp contrast to the low average abundance (0.54%) in the active environment (Table 2, Fig. 4D).

DISCUSSION

The 16S rRNA clone analysis of marine ooids from 3 depositional environments in the Bahamas Archipelago revealed diverse microbial assemblages representing phylotypes within 12 major phyla (Fig. 3). Among the represented phyla, *Proteobacteria* was the most diverse followed by *Bacteroidetes*, *Acidobacteria*, and *Planctomycetes*. Over 40% of the sequenced clones shared less than 95% sequence similarity to public 16S rRNA databases. Low sequence homology can be attributed to both high microbial diversity and a paucity of previous microbial surveys conducted on shallow-water carbonate sediments (Hewson & Fuhrman 2006, Uthicke & McGuire 2007, Schöttner et al. 2011). The average Shannon-Wiener diversity indices, as established by TRFLP (H' = 5.19) and 16S rRNA clone analyses (H' = 5.79), surpassed those of permeable coral reef carbonate sands (H' = 4.1) (Schöttner et al. 2011) and stromatolitic mat systems in the Bahamas (H' = 4.96) (Foster & Green 2011), but were similar to that of thrombolytic mat communities (H' = 5.17 to 5.55) in the Bahamas (Mobberley et al. 2012). These estimates suggest that ooid microbial communities are highly diverse and despite modest levels of clone coverage, which at genus level ranged from 66 to 70%, further diversity would have been revealed if a larger population of clones had been screened. Overall, our Shannon-Wiener estimates based on TRFLPs were consistently lower than clone library estimates and exhibited nearly the same level of diversity among all 3 environments, whereas diversity indices and rarefaction curves of the clone libraries suggest the non-active communities are the most diverse. This discrepancy could result from differences in the level of resolution between these 2 methods (Bent et al. 2007). Although TRFLP is a robust method, it has limited sensitivity and low resolution on species sharing similar TRFs (Bent et al. 2007, Orcutt et al. 2009).

Proteobacteria (50 to 61%) from the *Alpha-*, *Delta-* and *Gammaproteobacteria* groups were ubiquitous components in all 3 environments, confirming previous results on bacterial communities in sandy marine sediments (Sørensen et al. 2007, Uthicke & McGuire 2007, Mills et al. 2008, Schöttner et al. 2011) and microbial mat systems in the Bahamas (Baumgartner et al. 2009). Among these, the *Alphaproteobacteria* (25 to 41%) were the most common. This is not surprising given the metabolic diversity of *Alphaproteobacteria* and their common occurrence in oligotrophic waters (Horner-Devine et al. 2003), where they play important roles in carbon, nitrogen and sulfur cycling (Kersters et al. 2006). For example, members of the *Rhodobacterales* group have been shown to utilize both organic and inorganic compounds and play a role in sulfur oxidation, aerobic anoxygenic photosynthesis, nitrate reduction, DMSP breakdown, and production of EPS (Giovannoni & Rappe 2000).

Despite commonalities, dissimilarities in the microbial community structure of active, non-active, and mat-stabilized sediments were significant and statistically well-supported by the UniFrac metric ($p < 0.01$; clone libraries), P-test ($p < 0.01$; clone libraries), and ANOSIM analysis ($p < 0.001$; TRFLPs). The difference in microbial community composition among the 3 environments was attributed to phylotypes within *Proteobacteria*, *Planctomycetes*, *Cyanobacteria, Chlorobi*, and *Deinococcus-Thermus*. Differences in microbial composition are likely related to differences in the hydrodynamic characteristics of each environment, which could affect the biogeochemistry of inorganic nutrients and organic carbon availability. For instance, active ooid shoals are exposed to severe hydrodynamic forces that could hamper deposition of sedimentary organic matter. In contrast, non-active and mat-stabilized environments show a certain degree of nutrient and organic matter stratification because hydrodynamic forces are less pronounced. However, the non-active environments can experience macrofauna burrowing activities, which could trigger pore water exchange and disruption of geochemical gradients. Burrowing activities are less pronounced in mat-stabilized sediments where filamentous structures of *Cyanobacteria* and aggregates of bacteria can create a certain degree of stratification and sediment stability. Even though previous studies have hypothesized that hydrodynamic stress contributes to lower bacterial abundance and microbial diversity (Llobet-Brossa et al. 1998, Rusch et al. 2001, Rusch et al. 2003), the present study shows that high-energy environments can support diverse microbial communities associated with sediment particles. The presence of surface colonizing bacteria within the *Alphaproteobacteria* (*Pedomicrobium* sp., *Hyphomicrobium* sp.), *Planctomycetes* (*Pirellula* sp.), and *Bacteroidetes* (*Cytophaga* sp.) (Sly et al. 1988, Crump et al. 1999) in active and non-active environments suggest that these lineages are adapted to sediments undergoing continuous hydrodynamic

flow and/or pore advection processes. Increased numbers of particle-associated bacteria have been reported where there is enhanced exchange of particles between sediments and overlying water (Mills et al. 2008). Under high hydrodynamic conditions, i.e. active ooid shoals, a particle-associated lifestyle may confer some benefit as these communities display higher extracellular enzyme activity (Hoppe 1991) and incorporate substrates at faster rates than their free-living counterparts (Unanue et al. 1992).

Phylogenetic markers based on sequence data can provide important insights on potential functional capabilities of microorganisms, even though they might not always verify whether a microorganism can undertake a given metabolic process or reveal at what rate. Using 16S rRNA as a proxy phylogenetic marker, we found that these communities share functional redundancy for metabolic processes that foster carbonate precipitation (e.g. oxygenic/anoxygenic photosynthesis, denitrification, SR, sulfur oxidation, etc.) (Table 3). However, given the high degree of functional redundancy, it remains difficult to establish differences and the extent to which these communities impact ooid genesis and carbonate precipi-

Table 3. Environmental distribution of taxa associated with various metabolic processes and EPS production. AAnP: aerobic anoxygenic photosynthesis, AnAnP: anaerobic anoxygenic photosynthesis

Photosynthesis	Mat-stabilized	Non-active	Active
Oxygenic – *Cyanobacteria*	*Oscillatoriales* *Chroococcales* *Pleurocapsales*	*Oscillatoriales*	*Oscillatoriales*
AAnP	*Erythrobacter* spp. *Roseobacter* clade *Roseobacter*-like	*Gammaproteobacteria* NOR5/OM60 *Roseobacter*-like *Flavobacteriales*	*Roseobacter*-like *Flavobacteriales*
AnAnP	*Ectothiorhodospiraceae* *Chromatiaceae* *Rhodospirillaceae* *Chlorobi* *Chloroflexi*	*Ectothiorhodospiraceae* *Rhodospirillaceae* *Chlorobi* *Chloroflexi*	*Ectothiorhodospiraceae* *Rhodospirillaceae* (*Rhodospirillum rubrum*) *Chlorobi*
Denitrification	*Hyphomicrobiaceae* (*Devosia* sp.) *Rhodobacteraceae* (*Citreimonas salinaria*) *Pseudomonadales*	*Hyphomicrobiaceae* *Rhodobacteraceae* (*Paracococcus* sp.) *Pseudomonadales* *Rhodospirillaceae* *Sphingobacteriales* (*Flexibacter* sp.)	*Hyphomicrobiaceae* (*Devosia* sp.) *Bacteroidetes* (*Zobellia uliginosa*) *Pseudomonadales* *Rhodospirillum* sp.
Sulfate reduction	*Desulfovibrionales* (*Desulfomicrobium* sp.)	*Desulfobacterales*	*Desulfovibrionales* (*Desulfomicrobium* sp.) *Desulfobacterales* (*Desulfococcus multivorans*)
Sulfur oxidation	*Ectothiorhodospiraceae* *Chromatiaceae*	*Ectothiorhodospiraceae* *Chromatiaceae* (*Candidatus Thiobios zoothamnicoli*) *Halothiobacillaceae* (*Thiovirga sulfuroxidans*)	*Ectothiorhodospiraceae* *Chromatiaceae* (uncultured sulfuroxidizer) *Halothiobacillaceae*
EPS production	*Oscillatoriales* *Chroococcales* *Pleurocapsales* *Myxococcales* *Cytophagales* *Pseudomonadales* *Desulfomicrobium* sp.	*Myxococcales* *Flavobacteriales* *Cytophagales* *Planctomycetaceae* (*Pirelulla* sp.) *Pseudomonadales*	*Flavobacteriales* *Cytophagales* *Hyphomicrobiaceae* (*Pedomicrobium* sp.) *Planctomycetaceae* (*Pirelulla* sp.) *Pseudomonadales* *Desulfomicrobium* sp.

tation. Therefore, we circumscribe our discussion on how the presence of particular lineages may point to specific metabolisms, which could lead to hypotheses about their role in carbonate precipitation. Further studies are warranted to compare metabolic rate processes and functional gene diversity in these communities.

Carbonate precipitation is the result of a balance between metabolic activities that favor precipitation or dissolution, which depend on light availability and on local physico-chemical characteristics of the environment (Dupraz et al. 2009). In the Bahamas, one important metabolism linked to carbonate precipitation is oxygenic photosynthesis, which in microbialite systems is mostly carried out by *Cyanobacteria* and a consortium of bacteria (Dupraz & Visscher 2005). Considering the recognized importance of *Cyanobacteria*, their low occurrence (as supported by both TRFs and clone analysis) in active and non-active environments is rather surprising and suggests that photosynthetic eukaryotes (e.g. diatoms, green algae) could otherwise play an important role in these 2 environments (Freytet & Verrecchia 1998, Plée et al. 2010). In contrast, mat-stabilized environments recovered greater abundance and diversity of *Cyanobacteria* as well as a complex consortium of photosynthetic/diazotrophic bacteria, of which *Leptolyngbya* sp. is the most common. Other commonly occurring phototrophic and/or diazotrophs include clones related to sulfurous or non-sulfurous purple and green bacteria, most of which are ubiquitously present in all environments. The occurrence of photosynthesizers and N_2 fixers is not unforeseen as they are important contributors to carbon (via primary production) and nitrogen (via fixation of N_2) cycles, especially in oligotrophic waters where nutrient availability is scarce (Capone 2001, Zehr 2011).

Apart from *Cyanobacteria*, we documented photosynthetic organisms with capabilities for aerobic anoxygenic photosynthesis (AAnP) and anaerobic anoxygenic photosynthesis (AnAnP). Both processes can lead to calcium carbonate saturation (Bosak et al. 2007). Despite some phylogenetic similarities, we found distinct assemblage compositions. For instance, clones affiliated with known AAnP species (i.e. *Erythrobacter* spp., *Roseobacter* clade spp.) were only present in the mat library, whereas NOR5/OM60 related sequences were unique to the non-active library (Table 3). Although the ecological importance of AAnP remains unclear, these organisms can contribute 4 to 50% of the total bacterial production in oligotrophic waters and can represent 11% of the total microbial communities in marine waters (Koblízek et al. 2001, 2007, Kolber et al. 2001). Their dual phototrophic and respiratory capabilities could lead to a fitness advantage by maximizing their resources under nutrient- and carbon-limited environments such as found in the Bahamas where nutrient levels can range from 0.1 to 17 µg l^{-1} for NH_4-N and 0.3 to 4 µg l^{-1} for TDP-P (Koch & Madden 2001). Anaerobic anoxygenic photosynthetizers such as *Chloroflexi* and *Chlorobi* were also detected mostly in the non-active and mat-stabilized sediments and, based on TRF profiles, *Chlorobi* may be an important member of the anoxygenic photosynthetic community in non-active and mat-stabilized areas (Table 2). Other members include clones related to the purple sulfur bacteria (PSB) (*Chromatiaceae*, *Ectothiorhodospiraceae*) and purple non-sulfur bacteria (PNSB) (*Rhodospirillaceae*), all of which were ubiquitous in all environments. Even though anoxygenic photosynthesis is likely to be constrained due the high oxic conditions in active environments, anaerobic microenvironments associated with particle-attached biofilms could provide oxygen-depleted microniches (Kühl & Jørgensen 1992).

Denitrifiers may play a similar role in carbonate precipitation and, as such, have been implicated in mud flat precipitations (Drew 1911, 1914). The presence of these communities in oolitic environments stresses their importance as they regulate nitrogen availability and ultimately net primary productivity. This study identified some putative denitrifiers, including *Hyphomicrobiaceae*, a family with denitrifying and nitrogen fixing lineages as well as *Paracoccus* sp., and *Citreimonas salinaria* (Fig. S1, Table 3). Several denitrifiers within the *Bacteroidetes* lineage were also identified and included clones with affiliation to *Flexibacter* sp. and *Zobellia uliginosa*. We also detected sequences in the active clone library with affiliation to the heterotrophic denitrifier, *Pseudomonas* sp., while TRFs confirmed the occurrence of denitrifiers in all samples (Table 2). Even though this is mostly an anaerobic process, some *Pseudomonas* spp. and *Paracoccus* spp. can undertake aerobic denitrification (Bonin & Gilewicz 1991, Capone et al. 1992, Takaya et al. 2003, Wang et al. 2011).

Sulfate reduction generates carbonate ions and can potentially lead to carbonate precipitation (Decho et al. 2005, Dupraz & Visscher 2005). This metabolic process, which uses sulfate as a terminal electron acceptor, can account for over 50% of the organic carbon mineralization in marine sediments (Jørgensen 1982). Given their importance it was not unanticipated to find SRB in all 3 environments. SRB within the *Deltaproteobacteria* were represented by

members of the *Desulfobacterales* lineage and were mostly present in the non-active environment, whereas clones related to the *Desulfovibrionales* lineage were recovered only from active and mat-stabilized environments (Fig. S2). The presence of SRB communities in active environments, although not as abundant, suggests SRB have mechanisms to tolerate free radicals and the capacity to sustain high rates of sulfate reduction under oxic conditions (Canfield & Des Marais 1991, Krekeler et al. 1997, 1998, Fournier et al. 2004, Bryukhanov et al. 2011). Within the active ooid shoal, these processes are likely associated with anaerobic microenvironments in the complex polysaccharide matrix of biofilm bacteria, which allows for redox-chemical gradients suitable for sulfate reduction (Kühl & Jørgensen 1992).

We also documented a diverse population of heterotrophic bacteria, including *Proteobacteria, Planctomycetes, Bacteroidetes* and *Acidobacteria*. These organisms, which utilize organic matter to sustain their growth, are key players in energy flow and nutrient cycling in marine ecosystems and constitute major mass components in oligotrophic waters (Fuhrman et al. 1989). Although heterotrophs can induce carbonate dissolution via respiration of organic matter, they can foster carbonate mineralization by liberating cations sequestered by EPS and other macromolecules (Kawaguchi & Decho 2002, Dupraz & Visscher 2005). Notwithstanding, the effect of EPS on $CaCO_3$ precipitation is the net balance between both processes, which, in microbialite systems, is mostly mediated by EPS and heterotrophs (i.e. SRB) (Reid et al. 2000, Decho et al. 2005, Braissant et al. 2007), and in lacustrine systems by EPS and phototrophs (Plée et al. 2008, 2010, Pacton et al. 2012). Both confocal microscopy (cyanine dye-conjugated lectin) and phenol-sulfuric acid methods documented the presence of EPS in ooid grains and showed increasing amounts of EPS from active to mat-stabilized environments. The lower abundance of EPS from ooids in active areas (~4 µg EPS g^{-1} DW) is probably due to shear forces and abrasion that promote detachment of EPS (Zhang et al. 2011, Piggot et al. 2012) or to a rapid consumption of EPS by heterotrophs. In contrast, non-active and mat-stabilized environments allow for higher accumulation of EPS, with average measured values of 11.6 to 31.4 µg EPS g^{-1} DW, respectively. High abundance of EPS, such as in mat-stabilized systems, is most likely due to low hydrodynamic forces and higher diversity and/or abundance of EPS producers i.e. *cyanobacteria* (Table 3).

Although differences in the diversity of the microbial community structure exist, the functionality of these communities suggests that all 3 environments can support microbial mediated carbonate precipitation. However, hydrodynamic conditions could influence where and how the precipitates accumulate. In the active environment, where grains are constantly moving due to tidal currents and/or wave action, precipitation occurs as concentric layers of aragonite crystals, tangentially arranged around the nucleus to form a uniform coating. In non-active and mat-stabilized areas, low hydrodynamic conditions allow for micritic meniscus cementation and microbially induced fibrous aragonite precipitates in the intraparticle spaces of ooid grains, resulting in effective binding of loose oolitic grains (Hillgärtner et al. 2001). In addition, microbial precipitation of lime mud in the Bahamas has been attributed to a community of active denitrifiers within the genus *Pseudomonas,* able to produce copious amounts of aragonite (Drew 1911, 1914, Kellerman & Smith 1914). Based on our findings, these communities could play a role in precipitation processes, since they are abundantly present at all sites (Table 2). Likewise, the occurrence of SRB, especially in the non-active and mat stabilized environments, supports their involvement in carbonate precipitation and supports earlier studies on their prevalence in the Bahamas (McCallum 1970). While previous studies have also implicated SRB in the formation of marine ooids (Monaghan & Lytle 1956, Summons et al. 2010) and microbialite systems (Decho et al. 2005, Braissant et al. 2007), freshwater ooid formation appears to be governed by photosynthetic biofilm communities (Placton et al. 2012).

CONCLUSIONS

The waters of the Bahamas Archipelago are supersaturated with respect to $CaCO_3$ and ideal for precipitation. However, based on our findings of pervasive EPS, as well as microbes with the potential to influence carbonate precipitation (Table 3), abiotic (e.g. temperature, evaporation, CO_2 degassing, supersaturation with respect to carbonate minerals) and biological factors are probably intertwined in the precipitation processes that lead to ooid formation in active environments and cementation processes in non-active and mat-stabilized environments.

Diverse microbial communities inhabit the ooid sands of the Bahamas Archipelago. Assemblages of *Proteobacteria*, exhibiting a wide range of metabolic pathways, are the most diverse and abundant. Despite commonalities in *Proteobacteria* abun-

dance, some differences are evident. The active parts of ooid shoals appear to select for aerobic chemoorganoheterotrophs, some of which are particle-attached and potential primary producers consisting of aerobic/anaerobic photoautotrophs. Non-active and mat-stabilized environments harbor more complex redox-dependent microbial communities consisting of aerobic/anaerobic photoautotrophs, aerobic/anaerobic heterotrophs and aerobic/anaerobic chemoautotrophs. Differences in bacterial communities are likely associated with contrasting hydrodynamic forces, which could influence nutrient recycling and redox conditions. The presence of redox-dependent microbial communities suggests these organisms can exploit a range of redox conditions in the tidal realm where fluctuating oxic and anoxic conditions are common.

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