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PAST, PRESENT, AND FUTURE: MICROBIAL MATS AS MODELS FOR ASTROBIOLOGICAL RESEARCH

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1. Introduction

For over a decade, the emerging field of astrobiology has focused on three broad and far-reaching questions: what is the origin of life? does life exist beyond Earth's biosphere? and finally, what is the future of life? These fundamental questions regarding the past, present, and future of life on Earth and beyond serve as tenets to the multidisciplinary field of astrobiology. The scientific goals that underlie these questions are directly addressed and laid out in the Astrobiology Roadmap (Des Marais et al., 2008). Although the comprehensive nature of astrobiology transcends this single document, these guidelines provide the essential focus and direction to the field for years to come.

To specifically address the scientific goals and questions posed in the roadmap, astrobiological researchers have often relied on model systems. Models, whether they are in situ or in silico, provide the opportunity to experimentally manipulate environmental conditions that mirror modern and ancient ecosystems. One of the most versatile and fecund communities in astrobiological research is microbial mats. Microbial mats are self-sustaining, complex ecosystems that facilitate the cycling of chemical elements and are often driven by oxygenic and anoxygenic photosynthesis. Microbial mats throughout Earth's history have played a substantial role in the evolution and development of the biosphere (Kasting, 2001). Although once ubiquitous on ancient Earth (Awramik, 1984), today modern microbial mats are far more limited in their global occurrence (Bebout et al., 2002). The reduced microbial mat distribution is thought to be in part due to the increase in eukaryotic grazing (Garrett, 1970; Farmer, 1992), competition (Awramik, 1971), or possibly decreases in CO_2 availability (Rothschild and Mancinelli, 1990). Despite the current geographical constraints, the habitats that these modern mat ecosystems occupy represent putative analog environments to those found on ancient Earth such as hypersaline and hyperthermal environments. The theoretical and experimental manipulations of these consortia provide valuable insight into the past, present, and future of Earth's ecosystems.

In this chapter, we focus on the microbes, metabolisms, and molecular mechanisms of model mat ecosystems as they relate to some of the major questions in astrobiology. Specifically, we examine how microbial mat models have been successfully used to elucidate Earth's past and the detection of life's biosignatures. We also look at the role microbial mats play in present day Earth whether it be in global climate change or understanding the boundaries and limits of life on Earth. Finally, we explore the new frontiers of microbial mat models in astrobiology research, and discuss whether delineating the functional genetic complexity of these communities will foster the search for life beyond our own biosphere.

2. Decoding the Past with Microbial Mat Model Ecosystems

2.1. DIAGNOSTIC BIOMARKERS

One of the major challenges in astrobiological research is to understand the evolution of complex microbial communities. Extensive geochemical evidence of ancient microbial communities preserved in the fossil record indicates that these ecosystems date back to over 3 Ga (Beukes and Lowe, 1989). Modern examples of microbial mats have long been regarded as analogs or "living fossils" to these ancient microbial communities (Walter, 1976) with the potential to characterize possible biosignatures of Earth's earliest life. Microbial biosignatures are critical indicators and tools for the study of early life on Earth and the search for life throughout the universe. Biosignatures can take the form of morphological, mineralogical, or chemical fossils (Cady et al., 2003). Although all three categories can be delineated from extinct and extant microbial mats communities (Cady et al., 2003; Simoneit, 2002, 2004), in this section we will focus only on those chemical biomarkers derived from modern microbial mat model communities.

One of the most well-studied examples of modern microbial mat communities is the hypersaline mats of Guerrero Negro (Fig. 1a) located in Baja, California (Des Marais et al., 1992). The mats of Guerrero Negro are ideal model communities as they are biologically diverse (Spear et al., 2003; Omoregie et al., 2004; Ley et al., 2006) and productive microbial consortia (Des Marais, 1995; Jahnke et al., 2008). These microbial mat communities also lend themselves to experimental manipulation in situ and in artificial laboratory growth conditions (Fig. 1b). The search for chemical biosignatures within the Guerrero Negro mats has revealed complex assemblages of microbes that provide critical insight into the potential metabolisms of ancient microbial ecosystems. Of the various chemical biosignatures, lipid biomarkers have proven to be one of the most valuable indicators of microbial ecotypes, physiology, and metabolism (Brocks and Pearson, 2005). The structural characteristics of lipids that render these molecules ideal biomarkers often include cyclic or branched hydrocarbons, which facilitate the molecules to be resistant to microbial degradation during diagenesis (Jahnke et al., 2008), thus facilitating their preservation in the geologic record.

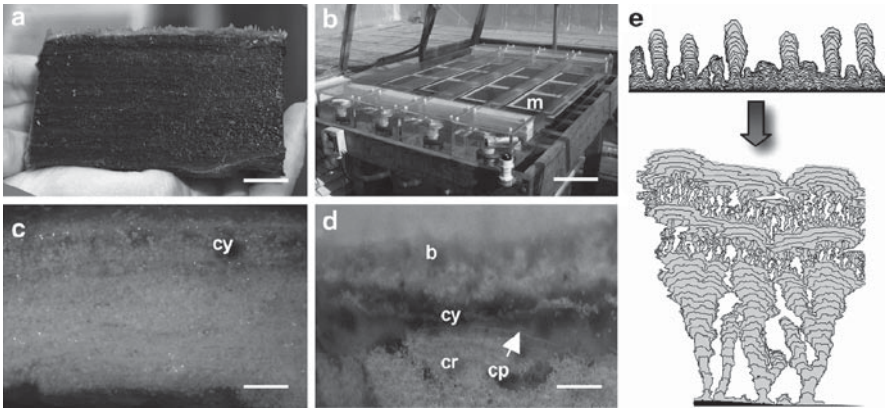

Figure 1. Microbial mat models for astrobiological research. (**a**) Hypersaline microbial mat sampled from Guerrero Negro, Baja California that have been incubated under artificial growth conditions in situ. Bar, 2 cm. (**b**) Recirculating water flume located at the NASA Ames greenhouse facilitates experimental manipulations of Guerrero Negro microbial mats (m). Bar, 10 cm. (**c**) Field collected stromatolites from the Highborne Cay, Bahamas (HBC) with pronounced cyanobacterial layers (cy). Bar, 2 mm. (**d**) Light micrograph of artificial microbialites cultivated in vitro from HBC stromatolites depicting the extensive layering and precipitation of $CaCO_3$ (cp). Layers include an EPS-rich superficial biofilm (b), filamentous and coccoid cyanobacterial layer (cy), and a subsurface micritic crust (cr). Bar, 1 mm. (**e**) Simulated DLA-CA in silico model depicting the alteration of microbialitic fabrics under variable intrinsic and extrinsic parameters. The change in virtual macrostructure reflects manipulation to these variables that stimulate alternations between heterotrophic and autotrophic mat communities. (Modified from Dupraz et al., 2006.)

To examine the presence and diversity of lipid metabolic indicators in the Guerrero Negro mats, vertical core samples have been examined in situ and compared to experimentally manipulated cores maintained artificially at the NASA Ames greenhouse facility (Bebout et al., 2002; Jahnke et al., 2008; Orphan et al., 2008). These microcosm enrichments experiments have successfully facilitated the characterization of several of the key functional groups of hypersaline mats most notably the methanogenic *Archaea*. By manipulating the natural growing conditions of the hypersaline mats, lipid biomarkers derived from potentially underrepresented community members within the Guerrero Negro mats can be detected and analyzed. Specifically, the spatial organization and distribution of methanogenic *Archaea* can be delineated within the microbial mat community (Jahnke et al., 2008; Orphan et al., 2008). These lipid analysis experiments coupled with complementary microbial diversity analyses (i.e., 16S rRNA phylogenetics and in situ hybridization) have provided useful diagnostic biosignatures for methanogens and other archaeal ecotypes (Orphan et al., 2008). For example the spatial distribution of methanogen-specific biomarkers such as 2,6,19,15,19-pentamethylicosane (PMI) coupled with 16S rRNA gene analyses reveal the localization of *Methanolobus*-like methanogenic *Archaea* throughout the mat stratigraphy. PMI has long been considered an

indicator of methanogenic *Archaea* (Brassell et al., 1981) and has been detected from several microbial mat ecosystems where *Methanolobus* spp. are prominent (Wieland et al., 2003; Orphan et al., 2008). Genera-specific and domain-specific lipid biomarkers such as ether-linked isoprenoids (e.g., archaeol, caldarchaeol) can provide a multifaceted examination of the modern and ancient community composition. Through the use of model microcosm enrichment experiments coupled with the studies of the natural in situ communities, diagnostic profiles can be generated for several key functional groups within the microbial mat communities. These diagnostics indicators, such as PMI, can then be used as biomarkers for the presence of target organisms (e.g. *Methanolobus*-like methanogenic *Archaea*) in ancient paleosoils rich in organic materials.

2.2. METABOLIC RECONSTRUCTION OF ANCIENT MICROBIAL ECOSYSTEMS

In addition to chemical biosignatures, microbial mats have also served as ideal models to reconstruct the putative metabolisms within past microbial communities. To gain a comprehensive understanding of the metabolic complexity of ancient microbial ecosystems, many researchers have turned to experimental manipulations of modern microbial mats in order to reproduce the putative growth conditions of the early Earth. While it is also important to examine individual metabolisms in culture organisms (i.e., sulfur or nitrogen metabolisms), monocultures often do not delineate the true complexity of the mat ecosystem. For example there are microbial metabolisms that do not occur in cultured organisms such as anaerobic methane oxidation (Reeburgh, 1980; Bebout et al., 2002) and aerobic sulfate reduction (Canfield and Des Marais, 1991). Furthermore, due to the symbiotic nature of microbial communities in which the waste product of one metabolism is the substrate for another (Kolenbrander et al., 2003), and with few axenic microbes currently in culture (Amann et al., 1995; Donachie et al., 2007), experimental manipulations and amendments of in situ and artificial mat communities are critical.

Two of the most amenable modern microbial mat ecosystems for metabolic studies are the mats of Guerrero Negro and Solar Lake (Sinai, Egypt). As mentioned earlier, these hypersaline communities can be easily maintained and manipulated under artificial growth conditions (Bebout et al., 2002; Grötzschel et al., 2002). These mats can also be experimentally manipulated to mimic the nutrient conditions of the ancient oceanic environment. For example an important astrobiological question associated with early life is the biogenic regulation of global climate. Atmospheric greenhouse gases such as CO_2 and methane would have been essential to compensate for the decreased luminosity associated with the "faint young sun problem" (Owen et al., 1979; Walker et al., 1981) that might otherwise have caused a severe global glaciation event. Modeling of the ancient environment suggests that CO_2 alone could not have accounted for the entire

greenhouse effect and that atmospheric methane levels must have been 100–300-fold higher (Pavlov et al., 2000; Kasting, 2001; Bebout et al., 2004). Abiotic methane sources (i.e., mantle oxidation) have been thought to be lower than biogenic sources (Reeburgh, 1996) and consistently degassing overtime; thus it is unlikely that abiotic methane accounted for the significantly elevated methane in the Archean and Proterozoic eons (Delano, 2001). To determine whether photosynthetic microbial mats, which dominated the biological landscape during this part of Earth's history, could account for some, or all, of the discrepancy in methane abundance, modern microbial mats from Guerrero Negro were experimentally manipulated to mimic these ancient conditions. By cultivating the hypersaline mats under low sulfate (SO_4^{2-}) conditions (>200 μM) that mimicked the putative conditions of the ancient Archean ocean (Habicht et al., 2002; Hurtgen et al., 2002), long-term (>1 year) experiments revealed that although methane flux increased daily throughout the manipulations (0.21 μmol m^{-2} h^{-1}) methane comprised less than 0.4% of the total carbon efflux (Bebout et al., 2004). These results suggest that the ecophysiology and spatial organization of the mats prohibited high levels of methanogenesis (Bebout et al., 2004). More importantly, however, these results suggest that the biogenic production of methane in the ancient Archean oceans is far more complicated than previously assumed. Much like modern microbial mat communities, where methanogenesis is not a significant metabolism in mat environments (Oremland and King, 1989; Conrad et al., 1995), ancient oxygen- and sulfate-poor microbial mat communities would have played a more modest role in methane flux, whereas other ecosystems such as water columns or marine sediments (Bebout et al., 2004) may have been a more significant contributor to regulate the global climate of the early Earth through the production of methane.

Experimental manipulations of the Guerrero Negro mat growth conditions have also demonstrated how the environment can influence the metabolic pathways and microbial diversity of key functional groups in microbial mats. In microbial mats treated to low sulfide and salinity conditions there were significant shifts detected in the methanogenic archaeal community. Using the conserved methanogen-specific functional gene (*mrcA*) as a marker of methanogenesis, Smith et al. (2008) detected shifts in the methanogen community. In less than 1 year the community transitioned from being dominated by strict methanotrophs (e.g., *Methanolobus*) in elevated sulfate levels (25–65 mM) to a mixed population consisting of both methanotrophs and hydrogentrophic methanogenic archaea (e.g., *Methanomicrobiales*) under low sulfate conditions (>2 mM). Such manipulations of mat ecosystems increase our understanding of the biogeochemical flux in microbial mats in response to a changing environment.

While Smith et al. (2008) demonstrated that changes in methanogenic communities were pronounced, other key groups appeared to be less affected by these experimental manipulations. For example cyanobacterial diversity remains relatively stable under varying salinity and sulfate conditions and is dominated by *Microcoleus chthonoplastes* and *Oscillatoria* spp. (Green et al., 2008). The stability

of cyanobacterial communities under lowered salinity conditions has also been observed in other hypersaline mat communities such as those found in San Salvador, Bahamas (Paerl et al., 2003). Although ecotype diversity remains stable, relative abundances of cyanobacteria increase under lower salinity and sulfate conditions (Green et al., 2008). Several of the cyanobacterial 16S rRNA gene sequences recovered from these treatments share high similarity to other known hypersaline microbial mat communities such as Shark Bay (Australia), Solar Lake (Sinai, Egypt), and the Delta de Ebro (Spain) (Green et al., 2008) suggesting cosmopolitan nature of these dominant cyanobacterial ecotypes. These tolerances to such changes in salinity and sulfate are thought to be a major factor in the dominance of cyanobacteria as the primary producers in hypersaline mat communities (Cohen et al., 1986; Nübel et al., 2000). These results also indicate the malleability of mat communities and suggest that by maintaining ecotype and metabolic diversity the mat community may respond more effectively to changing environmental conditions.

3. Microbial Mats as Modern Environmental Indicators

In addition to elucidating Earth's past, microbial mat model systems amendable to experimental manipulation are critical tools for examining the physiological and molecular limitations to life on present day Earth. Whether it is determining the boundaries of microbial survival and growth on Earth and in the space environment or delineating the genetic and biochemical mechanisms by which microbes cope with extreme environmental duress, understanding how microbes interface and manipulate their surrounding ecosystem is the key to astrobiological research on the modern Earth.

3.1. MICROBIAL MATS AND CARBON SEQUESTRATION

One of the most pressing issues in astrobiological research today has been the role of the carbon cycle in the regulation of Earth's climate. Atmospheric concentrations of carbon dioxide (CO_2) have risen sharply in the past 250 years (Raynaud et al., 1993), from 280 ppmv (parts per million per volume) in the 1850s to 371 ppmv in 2001 (Post et al., 2004). With anthropogenic emissions expected to continue (approximately 6.3×10^{15} g year^{-1}), it is anticipated that approximately half (3.2×10^{15} g) of the CO_2 released will remain in the atmosphere each year (Post et al., 2004). The world's oceans represent the largest carbon sink for atmospheric CO_2 with an approximate net removal of 1.7×10^{15} g of CO_2 annually (Post et al., 2004). Understanding the various biogeochemical and molecular facets to the modern carbon cycle and its link to the regulation of climate over time has been an important component of both astrobiological and environmental research. One important ecosystem amenable to examining the role of microbes in biologically

induced carbon concentration and sequestration is modern marine stromatolites. Stromatolites are organosedimentary structures that are formed via the trapping, binding, and precipitation activities of microbial mats (for review Dupraz and Visscher, 2005). While rare on the modern Earth (Grotzinger and Knoll, 1999), these laminated stromatolitic mat communities offer a unique opportunity to delineate the microbes, metabolisms, and mineralogy associated with biologically induced carbon sequestration.

In stromatolitic mats one of the most significant by products of carbon sequestration is the precipitation of calcium carbonates ($CaCO_3$; Des Marais, 1997; Dupraz et al., 2004). Three principle factors are known to affect $CaCO_3$ precipitation: the saturation index (SI), exopolymeric substances (EPS) (for review see Dupraz and Visscher, 2005), and pH (for review Hammes and Verstraete, 2002). Increases in pH, due to bacterial activity such as sulfate reduction, increases the SI and therefore may influence the extent of carbonate precipitation. In addition to the SI, EPS material also plays a critical role in stromatolitic mat precipitation (Kawaguchi and Decho, 2000, 2002) where it serves as a nucleation site and chelator for cations (for review Decho, 2000). A predominant producer of EPS material in microbialites are cyanobacteria (Foster et al., 2009). Cyanobacterial EPS has been shown to bind metal ions such as Ca^{2+} to key functional groups of the sugars and amino acids that comprise the EPS matrix (Beech et al., 1999; Braissant et al., 2007). The characteristics of the EPS material (e.g., acidic polysaccharides, negatively charged uronic acids, proteins) control the type and quantity of the calcium carbonate minerals produced in the stromatolitic mats (Kawaguchi and Decho, 2000, 2002). The extent of EPS production by cyanobacteria in the Highborne Cay stromatolitic mats appears to be species-dependent and light-induced (Foster et al., 2009). Cyanobacterial EPS has also been shown to serve as a structural component of microbial mats including the stromatolitic mats (Decho, 2000). Physical stabilization of these communities is critical in the high-energy environment (i.e., wave impact) of the marine subtidal zone.

The stromatolitic mats of Highborne Cay (Exuma Sound, Bahamas; Fig. 1c) have proven to be amenable to experimental manipulation in both molecular and biogeochemical analyses. Microbial mats derived from the Highborne Cay stromatolites have been successfully cultivated under simulated environmental conditions have been shown to maintain their capacity to form lithified organosedimentary structures in vitro (Havemann and Foster, 2008). These artificial microbialite (AM) models maintain much of the natural stromatolite diversity and develop three principle layers: a superficial EPS-rich biofilm; a pronounced layer of filamentous and coccoid cyanobacteria; and a micritic crust layer containing calcium carbonate (predominately aragonite) precipitate (Fig. 1d). When compared to the natural stromatolitic mat communities the Shannon indices of the artificial model microbialites were similar (artificial, $d = 1.46$; natural, $d = 1.48$; Havemann and Foster, 2008). Sequences representing 18 different phyla were recovered from natural stromatolites with most sequences similar to the *Proteobacteria* (51%) and *Cyanobacteria* (18%). Of these 18 phyla, 17 were also detected in the artificial

microbialites; and again the *Proteobacteria* (42%) and *Cyanobacteria* (19%) were dominant. Only the phylum WS6 had two representative sequences in the natural stromatolites that were not detected in the artificial microbialites (Havemann and Foster, 2008) and the phylum *Verrucomicrobia* was represented in the clone libraries from the artificial microbialites but were not detected in the natural communities. Despite these two differences in phyla the communities were highly similar at the family and genus level (Havemann and Foster, 2008). Similar results have been found in other artificial microbial mat studies, where natural inocula have been successfully cultivated to generate artificial microbial mat communities that mirror the natural communities (Fenchel, 1998a, b, c; Fenchel and Kühl, 2000; Buffan-Dubau et al., 2001; Taton et al., 2003).

Within the artificial microbialites the only region that precipitated carbon as calcium carbonate was the subsurface crust layer. Sequencing of the 16S rRNA genes within the crust revealed several ecotypes that were specific to this layer. Representatives of the *Acidobacteria* and the sulfide-oxidizing *Gammaproteobacteria* order *Thiotrichales* were found only in the crust layer. Additionally sequences with similarity to alkaliphilic *Bacillus halodurans* were also localized to the carbonate precipitates (Havemann and Foster, 2008). While none of these microbialite layers were sequenced to saturation, the spatial organization of the microbialites may provide key information with regard to the ecotypes and metabolic processes associated with the carbonate deposition and development. Future manipulations of the artificial microbialites under variable CO_2, salinity, and sulfate concentrations may detect significant differences in the rates and mechanisms of carbon concentration and sequestration. Additionally, coupling the variable growth conditions with manipulations in ecotype composition of the artificial microbialites may further elucidate whether there is ecotype specificity to the stromatolite lithification process.

In addition to the development of in vitro experimental analogues to the Highborne Cay stromatolites, experimental manipulations of in situ stromatolites have also revealed extensive information into the metabolic processes associated with formation of lithified structure in the marine stromatolites. In laboratory experiments where Highborne Cay stromatolitic mats were examined in situ and under homogenized slurry conditions the potential for aerobic respiration, sulfide oxidation, and sulfate reduction were analyzed (Visscher et al., 1998, 1999). The metabolic potentials of these stromatolitic mats were further characterized by the addition of various substrates such as the addition of thiosulfate in the presence of oxygen to measure rates of aerobic chemolithotrophic respiration. These supplemented communities were then compared to endogenous rates found in the natural unamended stromatolitic mats (Visscher et al., 1998, 1999). The results of such manipulation experiments clearly demonstrated a pronounced spatial and temporal distribution of sulfur cycling. For example, although sulfate reduction was detected throughout the diel cycle, rates were highest at night and in the subsurface (3–5 mm). Nighttime sulfate reduction coupled with a decrease in carbonate dissolution by aerobic heterotrophs results in an increased potential for a net precipitation of carbonate in the stromatolitic mats (Visscher et al., 1998; Dupraz and

Visscher, 2005; Baumgartner et al., 2006). Such manipulations of stromatolitic mat models clearly suggest that sulfur cycling (both sulfate reduction and sulfide oxidation) plays an important and complex in biogenic carbon sequestration.

3.2. MODELING THE BIOLOGICAL AND GEOLOGICAL INTERFACE WITH IN SILICO MICROBIAL MATS

To complement the in vitro and in situ manipulations of modern microbial mat ecosystems, computer-generated in silico models offer the opportunity to examine the long-term impact of intrinsic and extrinsic variables on stromatolite growth and morphology. By simulating the interactions between endogenous cell–cell interactions within mats and exogenous environmental factors, in silico models can emulate the emergence of stromatolitic structures as a direct result of these activities (Fig. 1e).

Computer modeling of stromatolites was first implemented in order to correlate micro- and mesoscale morphologies to macromorphology via iterative physiochemical and biological processes (Grotzinger and Rothman, 1986; Grotzinger and Knoll, 1999; Batchelor et al., 2004). These virtual models also provided independent confirmation of the biogenic origin of certain stromatolites structures (Batchelor et al., 2000, 2004). There have been two principal in silico models to examine the biological and geological interface, the Kardar-Parisi-Zhang (KLZ; Kardar et al., 1986) numerical model and the Diffusion Limited Aggregation model (DLA; Witten and Sander, 1981, 1983) coupled with the Cellular Automata (CA; Wolfram, 1984). The KPZ equation represents a local growth model that when modified to integrate certain variables (e.g. surface tension, accretion through precipitation or cell division, sedimentation, and background environmental factors), it can be used to predict stromatolite growth rate (Grotzinger and Knoll, 1999). However, the KPZ model is limited in it can only effectively simulate large stromatolites structures and simplified morphological structures (Dupraz et al., 2006). The DLA-CA model, however, avoids these potential caveats. The DLA model simulates the aggregation of particles undergoing Brownian motion and their interactions with a substrate whereas CA simulates the localized interactions of virtual cells. Coupling these two approaches generates a holistic model that distills the stromatolite ecosystem to a set of intrinsic and extrinsic variables (e.g., motion index, stability distance). Changes in these variables to simulate predominately autotrophic or heterotrophic growth within the community can have a pronounced effect on the virtual stromatolite structures (Dupraz et al., 2006). Simulations have shown that by alternating the intrinsic and extrinsic parameters of the simulation over time simple knob-like structures develop complex macromorphologies in the in silico models (Fig. 1e; Dupraz et al., 2006). These virtual models provide a simplified means to simulate the long-term growth of stromatolite structure under evolving environmental conditions that may not otherwise be accessible in in situ or in vitro microbial mats manipulations.

3.3. MICROBIAL MATS AS BIOINDICATORS

In addition to examining the effects of artificial environments on mat biocomplexity and physiology, microbial mat models have emerged as useful indicators of natural climate and environmental change. Microbial mats make ideal bioindicators as they naturally harbor high levels of microbial diversity, which have long been thought to facilitate ecosystem stability (May, 1973). Evidence has shown that maintaining high levels of ecotype diversity translates into metabolic functional redundancies, which can facilitate community recovery under environmental stress or changing climate conditions (Fernandez et al., 1999; Briones and Raskin, 2003; Yannarell et al., 2007). For example, the overactive hurricane seasons of the past decade have shown an unfortunate, but effective, use of microbial mat communities as biological monitors of ecosystem health. The hypersaline mat communities of Salt Pond, San Salvador, Bahamas showed dramatic shifts in ecotype abundance as well as in CO_2 and nitrogen fixation immediately after the influx of freshwater due to Hurricanes Floyd (1999) and Frances (2004) (Paerl et al., 2003; Yannarell et al., 2007). Monitoring how ecosystems respond to rapid environmental stress or disturbances can further expand our understanding of the interactions between the microbial world and the surrounding environment, an important tenet of astrobiology.

In addition to using natural in situ mat communities to monitor changes in the environment, simplified artificial mat ecosystems can also serve as critical environmental and astrobiological models. The Microbial Assay Technology System (MATS; Fig. 2) includes replicate mat ecosystems that can monitor the

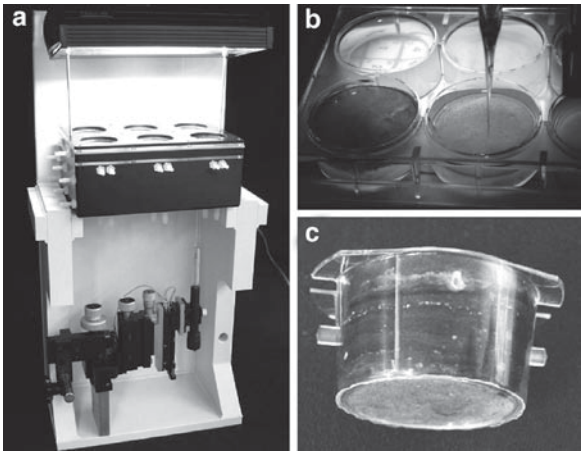

Figure 2. Microbial Assay Technology System (MATS). (**a**) MATS station containing a six chamber module and gas sampling ports. (**b**) Individual chambers can be sampled for chemical profiles (e.g., oxygen microsensor shown here). (**c**) Within each chamber artificial mats are contained within an inner sample container with permeable membranes that can be removed as needed. (Images courtesy of L. Prufert-Bebout.)

physiological responses of the communities to manipulate environmental changes such as temperature, atmosphere, water composition, as well as redox and nutrient levels (Prufert-Bebout et al., 2005). Through biomass and microsensor sampling coupled to an atmospheric and a liquid delivery system, the MATS hardware has the potential to facilitate studies on bioremediation, effects of the space environment (e.g., microgravity, radiation, low pressure), and community gene expression and physiology. To date, this system has successfully monitored CO_2 uptake and nitrogen fixation rates in both natural and laboratory-generated artificial mats (Prufert-Bebout et al., 2005) and has the potential to examine the impact of environmental stresses (e.g., oxidative stress) on ecosystem diversity and functional complexity at the molecular level.

4. The Road Ahead: Future of Microbial Mat Models in Astrobiological Research

4.1. METAGENOMICS IN MICROBIAL MATS

The use of microbial mats as models for astrobiological research has expanded in recent years as new high-throughput gene sequencing techniques have emerged. Elucidating the collective genomes of microbial mat ecosystems via the new field of metagenomics has advanced our understanding of complex microbial consortia at the molecular level (Handelsman, 2004). Metagenomic analyses are proving invaluable to the exploration of gene function and physiology in microbial mats, supplanting the need for ecotype cultivation or isolation. By understanding the functional complexity of Earth's biosphere at the genetic and biochemical level it is then possible to interpret the molecular evolution of life on Earth and to facilitate the search for life elsewhere in the solar system.

To date, metagenomics has been used in two primary approaches, to study the comprehensive microbial diversity and the functional gene complexity in these meta-communities. One of the first successful uses of metagenomics for functional gene analyses in a microbial mat ecosystem was the comparison of two dominant *Synechococcus* spp. isolates from a Yellowstone national park hot spring mat (Bhaya et al., 2007). The monoculture genomes were used as scaffolds for comparing metagenomic sequences from these mats (Bhaya et al., 2007). The results of their metagenomic comparison found that sections of the mats incubated at lower temperature contained greater *Synechococcus* strain diversity than at higher temperatures. This approach also found a *Synechococcus* ecotype that contained genes for iron uptake not found in the isolates genomes that were expressed during mat anoxia (Bhaya et al., 2007).

Metagenomics has also been used on a broader scale to compare the molecular biology of whole mat communities growing in different environments. Breitbart et al. (2009) recently compared the metagenomes of two morphologically distinct microbialite communities from low phosphorus, high nitrate and sulfur geothermal pools found in the Cuatro Cienegas Basin in Northern Mexico.

Their results revealed two distinct, diverse and complex molecular ecosystems (Breitbart et al., 2009). The oncolitic mats from Rio Mesquites were dominated by cyanobacteria with sequence similarity to the *Nostocales* and *Chroococcales*, while the thrombolitic mats from Pozas Azules II were dominated by *Alpha-* and *Gammaproteobacteria*. Both systems, however, contained archaeal, eukaryotic, and phage sequences.

Metagenomic sequencing of the Cuatros Cienegas microbialitic mats also identified key genes associated with phosphate, ammonia, and sulfur metabolisms, as well as the regulation of cellular processes such as photosynthesis, nutrient uptake, and quorum sensing. Breitbart et al. (2009) also identified a number of protein encoding genes associated with EPS biosynthesis and degradation, such as alginate metabolisms (e.g., phosphomannomutase), colanic acid biosynthesis (e.g., GDP-mannose 4,6-dehydratase), and arylsulfatases. All of which are thought to be essential for mat establishment and maintenance (Decho, 2000; Breitbart et al., 2009). A concurrent study of the viral metagenomes from Cuatro Cienegas microbialites and stromatolites found in Highborne Cay, Bahamas indicated that the viral communities differed significantly from each other and had little similarity to other known viral sequences, suggesting that these viral communities have experienced little exchange with the environment (Desnues et al., 2008). Our understanding of the role of viruses in microbial mat evolution and development is extremely limited and such metagenome sequencing efforts may delineate the extent to which viruses have, and continue, to influence the evolution of microbial mats.

The merger between microbial mat model systems and metagenomics has also been used to link physiochemical and genetic gradients in modern microbial mats. The Guerro Negro hypersaline mat metagenome, which consisted of ten separate sequencing efforts on 1-mm thick successive layers (Kunin et al., 2008), clearly demonstrates a genetic gradient throughout the community that mirrors the vertical stratification of key functional groups. The upper oxic 2 mm were dominated by *Cyanobacteria* and *Alphaproteobacteria*, and contained a high number of photosynthetic genes and molecular chaperones (Kunin et al., 2008). Underneath the oxic upper layers, however, the heterotrophic bacterial diversity increases, as do the number of genes involved in anaerobic respiration such as ferrodoxins, sulfatases, methyltransferases, and carbohydrate metabolism. Based on the metagenomic protein sequences, the overall mat ecosystem is enriched in acidic amino acids suggesting that the hypersaline environment has enforced selective pressure on the mat community. Although such results confirm previous biogeochemical and diversity analyses, they do provide the key DNA sequences necessary to characterize specific genes, regulation mechanisms, and protein products associated with the microbial mat metabolisms.

Comparative metagenomics has also elucidated key genes associated with essential metabolic and functional gene pathways. Metagenomic sequencing of artificial microbialites cultivated under simulated environmental conditions and natural microbialites isolated from Highborne Cay Bahamas, have shown extensive overlap in function gene complexity (Fig. 3a). Although the metagenomes of

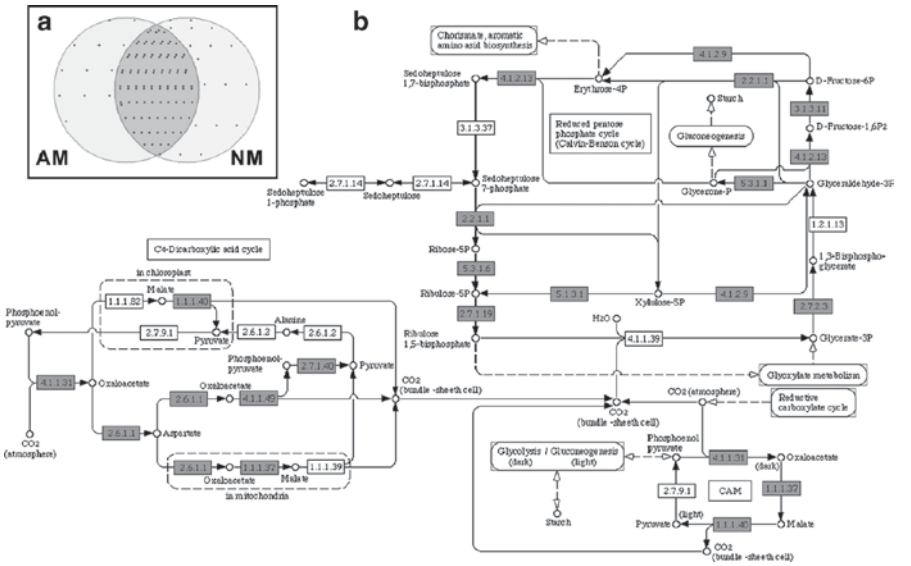

Figure 3. Metagenomics in the modern marine microbialites of Highborne Cay, Bahamas. (**a**) Venn diagram depicting the extent of overlap in the recovered metagenomic sequences in the artificial (AM) and natural (NM) microbialites. (**b**) Carbon fixation pathways in photosynthetic organisms. Rectangles highlighted in gray represent protein-encoding genes recovered from metagenomic sequencing of artificial microbialitic mats.

both communities have yet to be sequenced to saturation, the vast majority of recovered sequences from the artificial and natural microbialites are associated with carbohydrate (22%) and amino acid (21%) metabolisms (Foster, unpublished). These recovered sequences can then be compared to known metagenomic databases (e.g., KEGG, SEED, CAMERA) and detailed metabolic pathways can be characterized within the microbial mats. For example, many of the genes associated with carbon fixation in the artificial Highborne Cay microbialites can be identified and compared to the sequenced genomes of known organisms (Fig. 3b). Delineating the sequences of key genes associated with metabolic pathways (e.g., lipid, nucleotide, xenobiotic degradation metabolisms) will be essential to generate spatial and temporal gene expression patterns within the natural microbial mat community.

Building upon such studies, metagenomics also enables functional gene predictions to be made for these complex mat consortia. To accomplish this goal it will be necessary to first characterize the transcriptional and translational patterns of genes within the multigenome communities. Metatranscriptomics (i.e., the study of global gene expression within a multispecies community) has yet to be applied in complex microbial mats. This nascent field represents an important future direction for microbial mat molecular studies and has been successfully coupled with DNA microarrays in low diversity acidic biofilms (Parro et al., 2007),

mRNA clone libraries for soil (Poretsky et al., 2005), and with high-throughput RNA sequencing of marine microbial communities (Frias-Lopez et al., 2008). Additionally, it will also be important to study the metaproteome of microbial mat communities. Metaproteomics has been used to characterize proteins in low diversity acid mine drainage biofilms (Ram et al., 2005) and from the water column in the Chesapeake Bay (Kan et al., 2005). Together, metagenomics, metatranscriptomics, and metaproteomics will be critical future tools for elucidating the molecular interactions between microbial mats and their environments.

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