Cellular Origin, Life in Extreme Habitats and Astrobiology 14

Joseph Seckbach
Aharon Oren
Editors

Microbial Mats

Modern and Ancient Microorganisms in Stratified Systems

Springer

MICROBIAL MATS

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Volume 14

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Joseph Seckbach
The Hebrew University of Jerusalem, Israel

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Edited by

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Cover illustration: Layered communities of phototrophic prokaryotes in a gypsum crust covering the bottom of a saltern evaporation pond in Eilat, Israel. The crust, whose overlaying water contained about 200 g/l salts, shows an upper layer colored orange-brown by unicellular cyanobacteria (*Aphanothece halophytica*), rich in carotenoids. Below a green layer is found, mainly containing filamentous cyanobacteria (*Phormidium* sp.). The red-purple layer harbors a dense community of purple sulfur bacteria (*Chromatium*-type) that oxidize sulfide produced by sulfate reducing bacteria in the bottom layer. The photograph was taken in February 2006 by Andreas Thywißen, Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e.V., Hans-Knöll-Institut, Abt. Molekulare und Angewandte Mikrobiologie, Jena, Germany. All rights reserved to Andreas Thywißen. See also the chapter on "Mats of filamentous and unicellular cyanobacteria in hypersaline environments" by A. Oren in this volume.

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FOREWORD: MICROBIAL MATS MATTER AS MARVELOUS MANIFESTATIONS OF LIFE

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My good friend and colleague Dick Castenholz and I were talking about microbial diversity some time ago and the phrase "the unseen majority," which was being used to refer to the microbial world, came up. Dick told me "What do they mean by 'unseen,' I see microorganisms all the time." Of course, Dick was referring to the microbial mats he has studied in Yellowstone Park since the 1960s. Indeed, microbial mats are the most remarkable example of the manifestation of microbial life on Earth. Furthermore, mats matter both from evolutionary and ecological standpoints.

1. Mats and Evolution

Not only do mats matter now but they mattered even more in the Precambrian. Evidence from stromatolite formations which are fossilized microbial mats, dates their occurrence well back to almost 3.5 Ga BP in the geological record, or about 1 Ga after Earth was formed. During the Proterozoic period, much of the organic "matter" on Earth was tied up in microbial mats. From a global perspective, microbial mats really mattered then. They are thought to have been extensive in coastal and terrestrial aquatic areas and were arguably the major source of primary production on the planet along with phytoplankton.

Mats persisted as the primary visible evidence for life on Earth for almost 3 Ga. During this period, they would have been the dominant biological feature of life on the planet. It is thought that their prominence gradually began to dissipate as eukaryotic organisms evolved to feed on them and compete. Nonetheless, even today, they persist in their special places such as thermal springs, high salinity environments, and sulfur springs where conditions are too extreme for extensive eukaryotic grazing.

Most microbiologists believe the mats were the ideal settings for evolutionary processes. One of the major areas of evolutionary research centers on the discovery that deeply branching lineages of the tree of life are thermophiles. Members of both the archaea and bacteria occur in thermal settings in hot springs and hydrothermal vents. This fascinating finding has been controversial, but it has helped spur research in all thermal environments. More recently astrobiologists are

interested in thermal environments, particularly hydrothermal vents, because some believe they were the setting for early evolution on Earth as well as other planets such as Mars.

Scientists still do not know which microbial group was the first to become photosynthetic. Most believe it was one of the anoxygenic photosynthetic groups. If so, was it the purple bacteria (Proteobacteria), the green sulfur bacteria (Chlorobi), the heliobacteria group (Firmicutes), or the green filamentous bacteria (Chloroflexi)?

Most doubt that the cyanobacteria were the first, because it is believed that oxygenic photosynthesis, which is more complex in that it requires both photosystems I and II, was derived from that of other photosynthetic groups. But guess what? Even when the genomes of the different types of photosynthetic bacteria are compared, it is not yet possible to discern which group was ancestral. Why might this be? The microbial mat may be the explanation. In the mat setting, these photosynthetic organisms are in continuous contact and interaction. Because of the close proximity of organisms, DNA can be readily transferred in mats from one organism to another by various horizontal gene transfer mechanisms.

So, in a way, in these complex communities that contain a great variety of microorganisms, a species may pick up and incorporate many genes from the environment and retain those that are most suitable for their livelihood. What remains remarkable is that the end result has not produced a single phylum that "does it all." However, the explanation that none of the five different phyla containing photosynthetic bacteria has lost the ability for photosynthesis may be because no single phylogenetic group can accomplish all of the things that enable it to fill all of the microbial niches for photosynthesis. Although the persistence of seemingly less efficient life forms on Earth seems puzzling, it is reassuring to those who are concerned about loss of microbial biodiversity. Each type has remained distinct in many respects, in particular its ability to capture its part of the radiation spectrum that satisfies its energy requirements. Apparently major energy yielding processes survive as individual organisms become extinct.

The mat setting may also have served not only as a training ground for eukaryotic predation, but as a place where other microbial groups have evolved. In particular, hot springs as well as hydrothermal vents are major areas for the evolution of thermophiles. As another example, Dave Stahl recently mentioned in a lecture, that the mat setting may have been the place where spirochetes evolved. This group is unique in its type of motility with its endoflagella that are located inside the outer membrane of their cells. As a consequence spirochetes move like corkscrews through viscous tissues in humans and other animals. Perhaps this unique feature of the bacteria in this phylum was selected in dense microbial mats where organisms are held together by extracellular polymers.

2. Microbial Mats for Field Research

Interestingly for many years microbial mats were considered as odd environments for research. They were not seen to be important ecosystems for study, but more as a curiosity. Also, most of them were not in close proximity to research institutions so it was not easy to investigate them. However, as microbiologists began to study them seriously in the 1960s they realized how important they really are. Thermal environments exemplified the extreme limit for life at high temperatures and the studies of thermophile diversity and ecology were particularly fascinating. Determining the upper temperature limits for various processes such as photosynthesis, chemosynthesis, nitrogen fixation, etc. have been fruitful areas of research.

Ecological research, as illustrated by several chapters in this book, has emerged as a major area of study. Studies of the carbon, sulfur and nitrogen cycles are bearing fruit. For example, high temperature activities in nitrification, nitrogen fixation are suggesting that perhaps a complete nitrogen cycle exists in thermal springs and mats. The mat environment is ideally suited for community studies. A sample of the community can be readily cut out of the community and studied intact or dissected. This is accomplished by taking a vertical core with a cork-borer. Each vertical layer, which represents a particular part of the community, can then be carefully subsampled and studied separately if desired.

One of the most important and largely unexplored areas in microbial ecology is that of biogeography. Thermal mat communities represent "island" communities that are separated from one another by thousands of kilometers. For this reason, it is not surprising that some of the earliest work on microbial endemism has been carried out in hot springs from the pioneering work by Dick Castenholz's and Dave Ward's labs. The more recent work of Rachel Whitaker on the distribution of emerging species of the thermoacidophilic archaeon, *Sulfolobus islandicus* at globally separate hot springs is particularly informative. For the first time, there is solid evidence that speciation in Archaea and Bacteria is affected by geography.

This book contains chapters that cover in much greater detail all of the foregoing comments. This book illustrates the vibrant, exciting, and diverse nature of ongoing mat research today.

3. Archaea and Bacteria

Of course, nothing remains static. The physical and chemical conditions on Earth led to the formation of life. As those conditions change they will have a dramatic impact on the planet's life.

What is the future of microbial mats if Earth's life survives the threat from bolide impact or some other disastrous fate? In another billion years or so, as the

sun's temperature increases, conditions for Earth's plants and animals will be too hot for their survival unless evolutionary processes lead to some very dramatic changes. Barring that, Earth's microbial life will continue for perhaps another 2 Ga. The last remaining living organisms will likely be those that inhabit hot springs and thermal mat communities. As life on Earth began, so it may end.

4. Acknowledgement

The author appreciates the helpful comments made by Richard Castenholz.

Biodata of **James Staley**, author of foreword ***"Microbial Mats as Marvelous Manifestations of Life"***

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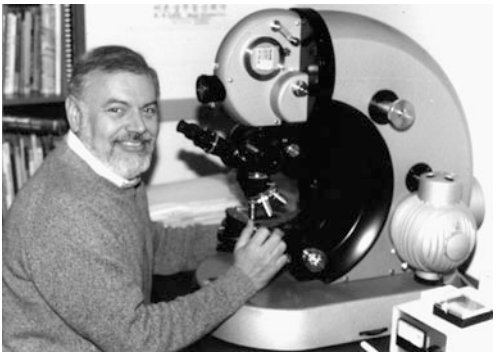

PREFACE

Microbial mats are multilayered sheets of microorganisms, generally composed of both Prokaryotes and Eukaryotes, growing at interfaces. They can reach a thickness of a few centimeters. Microbial activities cause the formation of steep concentration gradients of different chemicals, and the components of the microbial communities arrange themselves so that each finds its optimal conditions for life. In shallow aquatic environments where sunlight is available, the uppermost layers are generally dominated by aerobic photosynthesizing cyanobacteria, diatoms, and other oxygenic phototrophs, while the lowest layers are usually dominated by different types of anaerobic bacteria. In moist conditions the mats are held together by slimy extracellular polymeric substances secreted by the microorganisms. In many cases, filamentous microorganisms form tangled webs that add to the coherence of the mats.

Prior to the evolution of algae and higher plants on early Earth, photosynthetic microbial mats probably were major forms of life on our planet. Microbial mats are therefore extensively found in the fossil record as early as 3.5 billion years ago, and they are the earliest form of life on Earth for which there is good fossil evidence. Stromatolites are petrified microbial mats and these often show structures similar to those found in recent stratified microbial mats. In a later stage in the geological history, after oxygenic photosynthesis had originated, the mats started releasing oxygen. Later when plants and animal evolved, extensive microbial mats became rarer, but they still are present in many ecosystems and have a great impact on the global biogeochemical cycles.

Today extensive microbial mats only exist in special places where they have little competition from plants or grazing organisms. They may be found in salt marshes, beaches rich in carbonates, as well as in extreme environments such as in high temperature areas and in hypersaline environments, where competition by higher organisms is absent and grazers are excluded.

In view of the importance of microbial mats for the understanding of life on Earth under extreme conditions, both current and in the past, we decided to compile this volume to spread knowledge of these mat complexes. This volume is a new book in the *Cellular Origin, Life in Extreme Habitats and Astrobiology* series, published by Springer (www.springer.com/series/5775). This book focuses on the study of microbial mats in a variety of environments. The 30 chapters were written by 72 authors and coauthors from 15 countries: Australia, Austria, Canada, the Czech Republic, Germany, India, Israel, Italy, Mexico, The Netherlands, Oman, South Africa, Spain, United Kingdom, and the USA. The editors thank all contributors for their chapters and their cooperation during the compilation

of this volume. We extend our appreciation to the reviewers whose comments helped us to ensure the high scientific standards of the chapters. We hope that the readers will enjoy the wealth of information provided in this book on the intriguing world of microbial mats, now and in the past.

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Professor **Joseph Seckbach** is the Founder and Chief Editor of book series ***Cellular Origins, Life in Extreme Habitats and Astrobiology*** ("COLE"). See www.springer.com/series/5775. He is the author of several chapters in this series. Dr. Seckbach earned his Ph.D. from the University of Chicago, Chicago, IL (1965) and spent his postdoctoral years in the Division of Biology at Caltech (Pasadena, CA). Then he headed at the University of California at Los Angeles (UCLA) a team for searching for extraterrestrial life. He has been appointed to the faculty of the Hebrew University (Jerusalem, Israel) and performed algal research and taught biological courses until his retirement. He spent his sabbatical periods in UCLA and Harvard University, and served at Louisiana State University (LSU), (1997/1998) as the first selected occupant chair for the Louisiana Sea Grant and Technology Transfer, and as a visiting professor in the Department of Life Sciences at LSU (Baton Rouge, LA). He obtained two DAAD fellowships (German fellowships for exchange academicians) in Tübingen (1988) and at the Ludwig-Maximilians-Universität in Munich (2006).

Among his publications are books, scientific articles in the lines of phytoferritin, cellular evolution, acidothermophilic algae, and life in extreme environments. He also edited and translated several popular books. Dr. Seckbach is the co-author (with R. Ikan) of the ***Chemistry Lexicon*** (1991, 1999) and a co-editor of ***Proceeding of Endocytobiology VII Conference*** (Freiburg, Germany, 1998) and the ***Proceedings of Algae and Extreme Environments meeting*** (Trebon, Czech Republic, 2000). His new edited volume (with Richard Gordon) entitled ***Divine Action and Natural Selection: Science, Faith, and Evolution*** has been published by World Scientific Publishing Company. His recent interest is in the field of enigmatic microorganisms and life in extreme environments.

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PART 1:
THE NATURE OF MICROBIAL MATS

Gerdes

Oren

Biodata of **Gisela Gerdes**, author of ***"What are Microbial Mats?"***

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WHAT ARE MICROBIAL MATS?

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1. Introduction

Awareness of biofilms and microbial mats is not a modern attribute (see, in this context, Krumbein, 1993, 1994; Krumbein et al., 2003). Slippery surfaces, mucilage-embedded organic films, plaques, or rock patina may have not only stimulated olfactory, tactile, and visual senses, but also initiated questions about reasons. Today, various fields in biology, chemistry, medicine, geology, paleontology, and finally astrobiology share interest in biofilms and microbial mats. Their wealth of scientific questions results in a choice of definitions. In relation to microbial mats, a selection is listed here:

- "Microbial mats are stratified microbial communities that develop in the environmental microgradients established at the interfaces of water and solid substrates. They form a laminated multilayer of biofilms and largely alter the environmental microgradients in this interface as a result of their own communal metabolism" (Cohen, 1989).
- "Microbial mats are laminated benthic systems, which are often built by cyanobacteria, when occurring in the photic zone" (Wachendörfer et al., 1994).
- "In the broadest sense, they are microbial communities, predominantly populated by prokaryotes that colonize surfaces. Implicit in this definition is the understanding that there is intimate interaction between the microbes, the colonized surface, and the surrounding environment" (Stolz, 2000).
- "They commonly occur on the surfaces of sediment and detrital particles and rapidly form on virtually any new surface placed in sediments" (Decho, 2000).
- "Microbial mats are intimately interwoven microbial communities including laminated, concentric and network-like growth patterns, which by their upward directed growth, physical and chemical gradients, barriers and sticky EPS products trap and embed mineral grains, produce new minerals and, ultimately, laminated and spherulitic sedimentary rocks and structures" (Krumbein, 1983; Krumbein et al., 2003).

The selection mentioned already points towards a common denominator of all definitions, as expressed by Neu (1994): "Microbial mats are by definition true

biofilms." Characters unifying both seem to be (i) the physiological cooperativity of organisms (Costerton et al., 1995); (ii) the presence of extracellular polymeric substances that bind cells and other organic and inorganic compounds together and to the substratum (Characklis and Wilderer, 1989); (iii) the adhesiveness of EPS (Neu and Marshall, 1990); (iv) the affinity to interfaces/substrates; (v) microbial aggregation; and (vi) high water contents (a biofilm is also regarded as a layer of immobilized water associated with an interface) (Cooksey, 1992; Krumbein, 1994; Neu, 1994).

Besides the unifying attributes, differentiating aspects should also be considered. A biofilm *sensu strictu* is regarded more or less as a layer of sessile organisms, embedded in their mucilage, immobilized at a substratum (Costerton et al., 1995; Neu et al., 2003). Organisms forming microbial mats, on the other hand, are predominantly motile. Biofilms can develop intrasedimentary where they may contribute to early diagenesis. Microbial mats, on the other hand, are typical bedding surface phenomena. Therefore, it may be acceptable to understand microbial mats in this chapter as advanced biofilm stages forming laminae on bedding surfaces where they reflect gaps in sedimentation, or in other terms, time for growth, biomass condensation, and biological succession. The starting point of a microbial mat in a sedimentary environment may be a scarcely noticed precursor stage of conditioning organic macromolecules, followed by bacterial cell and slime attachment, cell growth, and biotransformation of bulk liquid chemistry (Stoodley et al., 1999). Through further steps, the abundance of motile microorganisms may increase, dominated on sun-lit surfaces by cyanobacteria. Invading eukaryotes, e.g., protoctists, macroalgae, and invertebrates, further increase the complexity of microbial mats. The more the continuous microbial growth and EPS production add to the three-dimensional overlays at sedimentary surfaces, the more the binding capacity for suspended material will increase, and external matter, e.g., detrital particles and organic substances, will be captured. These and internal processes, e.g., EPS-controlled circulation of liquid or gaseous metabolic products, raise the complexity and autarchy of the ecological system. Today, such autarch microbial systems are particularly common in habitats controlled by harsh environmental conditions. Examples are hypersaline settings and marginal-marine zones where prevailing ecological conditions become more and more unpredictable.

Cyanobacteria may be most prominent mat builders, but not always. Moreover, fungi during mycelian growth of intertwined branches can form mats on mineral surfaces (Verrecchia, 2000). Mats of chemolithotrophic gliding bacteria (e.g., of the genus *Thioploca*) are described for oxygen-depleted mud environments (Oschmann, 2000). Similar structures were also found in well-laminated mat-like structures in Kimmeridgian clay, Yorkshire, England (Oschmann, 2000). Sulfide-oxidizing bacteria, such as *Beggiatoa*, are significant mat-builders at continental margins around cold seeps (Thiel et al., 2001; Aharon, 2000).

The main focus of this chapter is on the sedimentological significance of microbial communities creating mats on sedimentary surfaces. Examples are selected from modern cyanobacteria-dominated microbial mats. Cyanobacteria are the oldest oxygen-producing photosynthetic prokaryotes. Their traces of life

and mats were already present far back in time in Archean sediments. Modern cyanobacteria-dominated mats comparable with those in the fossil records are geographically widely spread, particularly where environmental conditions are extreme. No doubt that the early life forms have also met extreme conditions. There is increasing interest in tools for the recognition of signatures of such early life forms. This interest is recently enlarged according to the increasing fields of astrobiology, which are supposed to expect similar signatures of former surface-dwelling microbial systems on other planets that derived their energy principally from sunlight.

As microbial mats and their sedimentological record are not uniform in time and space, first their phenotypical variations will be detailed, using modern records. Sedimentological proxies will be added, which may be indicative of former presence of microbial mats. The final conclusion will briefly take up again the entitled question "What are microbial mats"?

2. Phenotypic Variations of Microbial Mats and Related Sedimentary Structures

A photosynthetic microbial mat is by no means a motionless and stagnant organic layer, but represents a living system able to cope with various environmental conditions that consequently result in a big range of phenotypic variations. This is quite equal to the statement of Neu (1994) that a "biofilm is a surface accumulation which is not necessarily uniform in time and space."

Phenotypic variation of mats is particularly common where benthic cyanobacteria are involved. Cyanobacteria, a monophyletic group within the kingdom of Bacteria, have been and still are morphologically and physiologically diverse. Various studies of fossil and modern deposits record their success in creating a wealth of different sedimentary structures (Eriksson et al., 2007).

This chapter addresses phenotypic variations of microbial mats and their connected sediment structures in relation to (i) morphological properties of dominant microbes controlling mat fabrics, (ii) eco-physiological strategies to gain the most optimal position within environmental gradients, and (iii) microbial "joint venture" characteristics of mature mats.

2.1. LOCAL DOMINANCE OF CERTAIN MORPHOTYPES CONTROL MAT FABRICS

Coccoid and filamentous cyanobacteria significantly contribute to mat fabrics. In coccoids, the mode of cell division and EPS production is of structural importance. Species dividing by binary fission include encapsulated and "naked" unicells of random or regular dispersal or colonial aggregation. Colonial aggregates are preferentially sessile and matrix-enclosed (Costerton et al., 1995). Characteristic are pustular mat surface structures resulting from the local dominance of colony-forming taxa, e.g., *Entophysalis major* (Bauld, 1984). Several taxa, reproducing

by multiple fission form characteristic nodular aggregates associated with poorly laminated fabrics. Examples are thrombolitic and biolaminoid structures. Thrombolites exhibit complex internal fabrics often including clotted micrite and peloids. The clots are interpreted as irregular agglutinated, in situ calcified patches of coccoid-dominated microbial communities (Riding, 2000; Flügel, 2004). Biolaminoid structures comprise less significantly laminated build-ups of sediments including poor or indistinct laminations, irregular rhythmicity, and discontinuous nodular layers with irregular and wide spaces. Biolaminoid structures are common features in the Platy Dolomite of the coastal area of North Poland (Gasiewicz et al., 1987; Brehm et al., 2002). In the Gavish Sabkha (southern Sinai, Egypt), similar structures were most conspicuous at the rim of a hypersaline shallow water lagoon. The sediments in this area were soaked by trickling water flows. Evaporite incrustations evolved frequently and became redissolved again.

Among mat-forming filamentous species are those with or without heterocysts, and also branching types. Robust felty prostrate mats correspond with local dominance of nonheterocystous cyanobacteria, such as *Microcoleus chthonoplastes* (Fig. 1a). The sheaths of this species are particularly rigid (Fig. 1b) and contribute to organic layers of variable thickness enriched in fibrillar biomass (Fig. 1c, d). Characteristic of this species are changes in growth directions. Resulting structures are often arched and curved (Fig. 1a).

A parameter chiefly relevant to the sedimentary record of microbial mats is the taxic diversity of the glycocalyx (EPS). Some species form only amorphous slime layers, others well-shaped capsules. Some glycocalyx layers are organized in a tight matrix (Fig. 1b); other species form flexible and more easily deformed sheaths, such as *Oscillatoria limosa*. This pioneer species is highly motile and responds rapidly to burial. Mats of this type are usually smooth and fragile in comparison with the felty mats of *M. chthonoplastes*.

Networks dominated by ensheathed filamentous cyanobacteria provide a valuable cohesive strength to the sediment surface, which is more efficient than amorphous slime layers only (Stal, 1994). Studies revealed that the sheath material of the cosmopolitan mat builder *M. chthonoplastes* contains uronic acids (Stal, 1994). Experiments conducted by Dade et al. (1990) revealed a significant increase in the critical shear velocity required for the onset of erosion of sand particles when concentrations of polymeric uronic acid increased in the sand during in situ growth of bacteria. Particularly, sand flats dominated by noncharged quartz grains may get profit from filamentous cyanobacteria capable of synthesizing uronic acids or their related substances.

2.2. INDUCED GROWTH PHENOMENA

Sediment surfaces as the actual places of mat growth are usually affected by a variety of physical disturbances such as sedimentation, erosion, cracking, precipitation of evaporites, percolating groundwater seepages, submersion, or

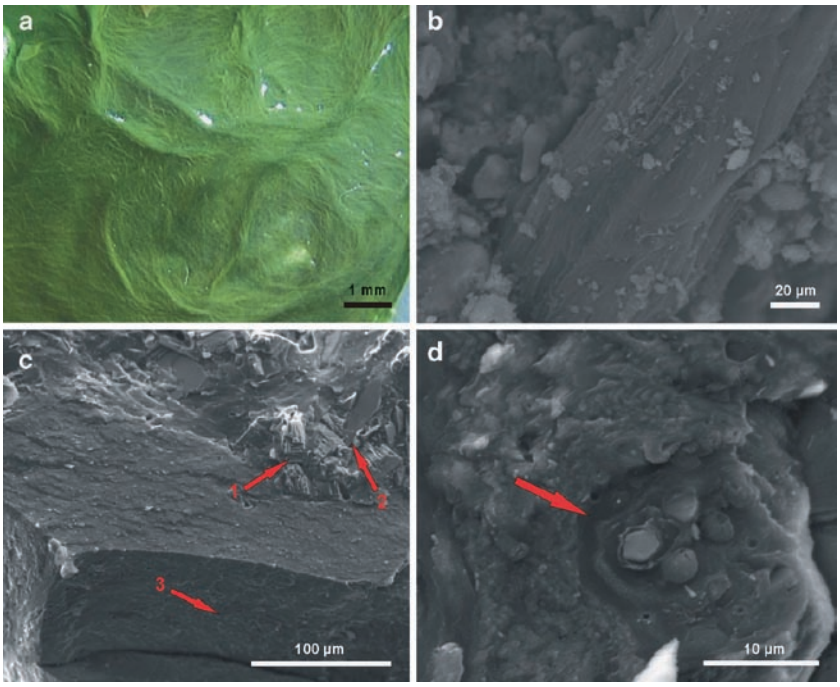

Figure 1. (**a**) Plane view of a mat created by dominance of filamentous cyanobacteria. The arched and curved bulges at the mat surface are made by sheath-enclosed bundles of *Microcoleus chthonoplastes*. Lab-cultured shallow-water mat. (**b**) SEM view of the part of a sheath of *M. chthonoplastes*. Internally enclosed trichomes are indistinctly visible through the surrounding sheath. Detrital particles are bound by extracellular polymers. Clusters of coccoid unicells and rod-shaped bacteria adhere to the tight matrix. Lab-cultured mat. (**c**) SEM view of a compact organic layer derived from a buried microbial mat once produced by high dominance of *M. chthonoplastes*. Oblique plane in the center indicates former mat surface occupied by some authigenic carbonate particles (*arrow 1*) and mica flakes (*arrow 2*). Two mat generations are sandwiched below (*vertical section*). Rounded openings (*arrow 3*) are cross-sections of sheaths of *M. chthonoplastes* (see Fig. 1d). Lab-cultured mat. (**d**) Cut perpendicular to the compact organic layer in Fig. 1c. Note cross-sectioned trichome bundle of *M. chthonoplastes* surrounded by the lamellar sheath (*arrow*).

changing water tables. Of striking importance is the wealth of growth reactions to disturbances carried out by mat-forming cyanobacteria. Various structures can be traced back to such environmentally induced growth (Bouougri et al., 2007). In addition, microbial adhesion and growth continues in the internal mat fabrics. Scanning electron microscopic (SEM) studies revealed that almost all interfaces in the mat's interior are coated. Even capsular and sheath material as well as gas or liquid bubbles derived from metabolic products provide interfaces for microbial adhesion and growth (Gerdes et al., 2000). Proxy structures indicative of "induced growth" phenomena are summarized in Table 1 (for visual references, see Schieber et al., 2007).

Table 1. Proxy structures of active growth responses of microbial mats ("induced growth").

Categiory	Phenotype	Possible disturbances	References
1. Bedding and lamination fabrics, bedded sequences	Couplets of sand or silt/clay layers and microbial mats	Low rate burial by clastic sediments	Gerdes, 2007; Schieber, 2007
	Biovarvite	Changes in the light gradient	
	Biolaminoid facies	Unpredictable salinity changes	
2. Discontinuity surfaces	Wavy-crinkly laminae	Various reasons (see categories 3–5)	Flügel, 2004; Schieber, 1986
3. Mat surface morphologies	Tufted mats, pinnacle mats	Changes in the light gradient, chemocline migration towards or above surface	Flügel, 2004; Bauld, 1984; Browne et al., 2000
	Reticulate ornaments (complex overgrowth network, elephant skin)	Competitive overriding (space competition?)	Gehling, 1991; Porada and Bouougri, 2007
	Flocculous microbial scums above benthic mat	Submersion, changes in the light gradient, increasing water table, flooding	Browne et al., 2000
4. Bubbles encountered with photosynthesis or mat decay	Blisters (overgrown gas bubbles)	Migrating gas	Stolz, 2000
5. Growth responses to physical mat destruction	Ripple patches, erosion remnants, sharp and smooth transitions at the edges of ripple patches	Obstacle erosion, shear stress	Noffke and Krumbein, 1999; Reineck et al., 1990; Schieber, 2007
	Upturned and overgrown crack margins, convoluted structures	Cracking due to dehydration, desiccation	Bouougri and Porada, 2007
	Healing cracks		
	Petees related to growth, "cabbage heads"	Crystallization of evaporate minerals	Eriksson et al., 2007
	Mat chips	Tearing, cracking, erosion, transport	Schieber, 2007
	Multidirected ripple marks	Currents, sedimentation	Noffke, 2007
	Wrinkle structures	Various controls, physical deformation of microbially bound surfaces	Porada and Bouougri, 2007
6. Features related to mat decay mineralization	Oolitic coatings around nuclei of varied origin; pyrite	New interfaces (intraclasts, bubbles, etc. Providing nuclei for crystal growth)	Gerdes et al., 2000; Schieber, 1986; Flügel, 2004
	Enrichment of minerals and elements in layers "frozen" (carbonate-coated) filaments in life position		
	"Microbial grains"(in-situ origin)		

2.2.1. Growth Induced by Sedimentation Processes

An important environmental trigger of growth processes is light. Changes in the vertical light gradient caused, e.g., by sedimentation, force movements of motile mat-producers by way of gliding. Products of gliding responses in interaction with sedimentation are bedding and lamination structures (Fig. 2a). Further, the proximity of new surfaces for adhesion, enrichment of suitable nutrients, or avoidance of stressful factors may trigger gliding responses (Castenholz et al., 1991).

It has been observed that filamentous cyanobacteria subsequent to gliding re-establish new mats on the newly deposited surface as far as moisture is sufficient (Gerdes and Klenke, 2007). Gaps between sedimentation events support the establishment of new mats. Suspended material including biofilms attached to grains and organisms from the benthic mat after settling increase the taxic, structural, and metabolic diversity of the living surface.

According to their morphology and motility, filamentous cyanobacteria such as *M. chthonoplastes*, *Lyngbya* sp., and *Oscillatoria* sp. are prominent architects of biolaminations in modern clastic tidal flat deposits. Sedimentation events

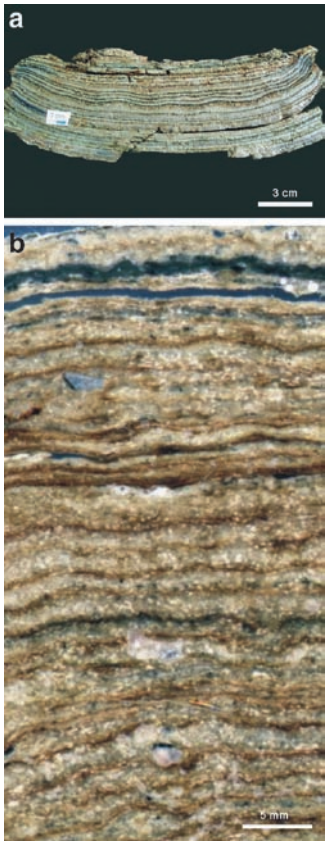

Figure 2. (**a**) Section of a bedded sequence of microbial mats and silt/clay interlayerings. The perpendicular cut reflects growth bedding generated by repeated low-rate sedimentation and re-establishment of mats. Vertical accretion took place at lower supratidal flats adjacent to a tidal channel where overflows of channel margins during high tides repeatedly deposited thin silt/clay layers above the mats. Sample locality: Bhar Alouane sabkha, southern Tunisia. Image courtesy of Hubertus Porada. (**b**) Thin section photograph showing growth bedding of the biovarvite type. Filamentous organisms are dominant in the dark layers (surface populations in winter), and coccoids in the light interlayers (surface populations in summer). Light layers are swollen by large amounts of EPS and filled with authigenic carbonates. Mutual overriding is triggered by seasonal changes of light, salinity, and water level. This vertical section contains 23 couplets of light and dark layers. Sample locality: Solar Lake, Gulf of Aqaba, Egypt. (Modified after a photo published in Gerdes, 2007.)

may represent a serious disturbance; yet low-rate sedimentation stimulates upward-migration of mat-forming organisms. Shading due to burial serves as a trigger mechanism. By gliding, overriding sediments, and escaping from burial, the microbes can multiply into several new mats atop new sediment layers. Re-establishment after burial may happen in a minimal time frame of some days, whereas the intercalated mineral layers indicate burial events possibly acting in a minimal time frame of hours (Gerdes and Klenke, 2007). Unpredictability of sedimentation events in open tidal flats may influence the thickness of the mineral layers between buried mats.

Slimes, immotile cells, dead cells, and sheaths (Fig. 1b) are left behind after burial, while motile trichomes establish their sheaths again at the new surface. The length of sedimentation gaps in which the populations can continue in situ growth is decisive for the thickness and internal condensation of biolaminites.

2.2.2. Trapping/Baffling, Binding

Irregular surface reliefs of microbial mats clotted with EPS may act as sites of trapping and binding (agglutination) of suspended grains and particles. These conditions support the input of allochthonous sediment into mat fabrics. Also, baffling effects may promote the incorporation of suspended matter. Baffling refers to current reduction and gravity-induced dropping of sediment grains to the substrate (Flügel, 2004). Submerged bundles of filamentous cyanobacteria standing perpendicular to the mat surface act sometimes as bafflers. In hypersaline environments, local patches of sucrose carbonates between filamentous build-ups may owe to baffling (Gerdes et al., 2000). A specific binding feature is also lamina-specific mineral enrichment, among others laminae mimicked by heavy mineral grains (Gerdes, 2007, Fig. 2-1-1B). The sticky and slowly vertically accreting surface mats may mediate and preserve these features.

2.2.3. Competitive Overriding: Biovarvites

In the Solar Lake (Egypt), mutual overriding between coccoid and filamentous cyanobacteria in a seasonal cycle creates a stromatolitic carbonate facies (the vertical distance of the biolaminated pile was already more than 120 cm during field study in 1982). A detail of the sequence is shown in Fig. 2b. Important environmental parameters for the development of these so-called biovarvites include rare sedimentation events and quiet hypersaline shallow water settings controlled by a seasonal cycle of higher and lower evaporation rates, water levels, salinity, and light intensities meeting the mat surfaces. These conditions trigger dominance changes between coccoid and filamentous cyanobacteria in the surface mats. Products are varvite-like patterns of alternating dark and light laminae (Fig. 2b). In the dark laminae, filamentous cyanobacteria are dominant (winter populations adapted to lower light intensity). The light layers are created by dominance of coccoid cyanobacteria and particularly high amounts of viscous slime (summer situation). Associated with the light laminae are in situ formed carbonate grains of varying shapes and sizes.

2.2.4. Mat Surface Morphologies

Wavy-crinkly and sometimes wrinkly discontinuity surfaces are common criteria of former mat growth in bedded sequences (Fig. 2a, b). Their mode of development may be diverse, yet in many cases active microbial overgrowth and overriding of basic mats are involved. Some typical structures deriving from overriding behavior of mat-forming cyanobacteria will be detailed in the following.

Microbially overgrown gypsum is characteristic at mat-occupied supratidal flats and sabkhas of arid coasts. As pointed out by Rouchy and Monty (2000), a close interplay between subaerial exposure and submersal is common in these areas. Gypsification takes place mainly during subaerial exposure, whereas during phases of submersal or at least increasing surface moisture, microbial growth is active. Unicellular cyanobacteria dominantly colonize the precipitates.

Tufts are vertically oriented bundles of filamentous organisms that originally contribute to the basic mat. Changes from lateral to vertical growth may be induced by modifying environmental conditions, e.g., changes in the vertical light gradient. Furthermore, the migration of reduced substances from the mat interior to the surface can force the filament bundles to erect tufts above the mat base (Fig. 3a). Sometimes, the tufts provide substrates for other mat inhabitants, e.g., coccoids and diatoms that also try to avoid stressful conditions from the mat's interior. These stabilize the originally soft and flexible tufts in time so that more rigid pinnacles form. Frequently, water-covered mats are strewn with pinnacles of variable height. Some are in the range of millimeters (Fig. 3b); others may reach even several centimeters.

Frequently, coccoidal and filamentous morphotypes thrive in such proximity on the mat surface that mutual overgrowth becomes structurally important. This is reflected by a variety of intersecting bulges and pinnacles, and differently colored spots in between, representing localized populations of coccoids (Fig. 3b). Increasing water cover combined with lower light intensity triggers the overriding behavior of filamentous organisms (Gerdes and Klenke, 2003). Macroscopically, the ornamentic structures resemble "elephant skin" (Gehling, 1991). An ample comparative evidence of "elephant skin" patterns occurs in the fossil record (Hagadorn and Bottjer, 1997).

Also, gas bubbles (alternatively termed blisters; Stolz, 2000) provide surfaces for overgrowth. Blisters frequently accumulate at surfaces as a result of photosynthesis or other metabolic activity of mats underneath. Often, different microbially induced processes are concomitantly involved: (i) gas bubbles produced due to mat metabolism; (ii) surface sediments concomitantly agglutinated and stabilized by microbial films and slimes; (iii) fixation of bubbles by surface mucilage, (iv) bubble-related expansion of the biofilm-stabilized surfaces; and (v) attraction of mat inhabitants to colonize the new surfaces provided moisture or water-enriched gel is present (Fig. 3c). Such a multitude of interacting biological and physical processes creates particularly complex mat surface morphologies (Fig. 3c). After burial, the captured and overgrown bubbles may contribute to open space structures, e.g., birds' eyes (Flügel, 2004).

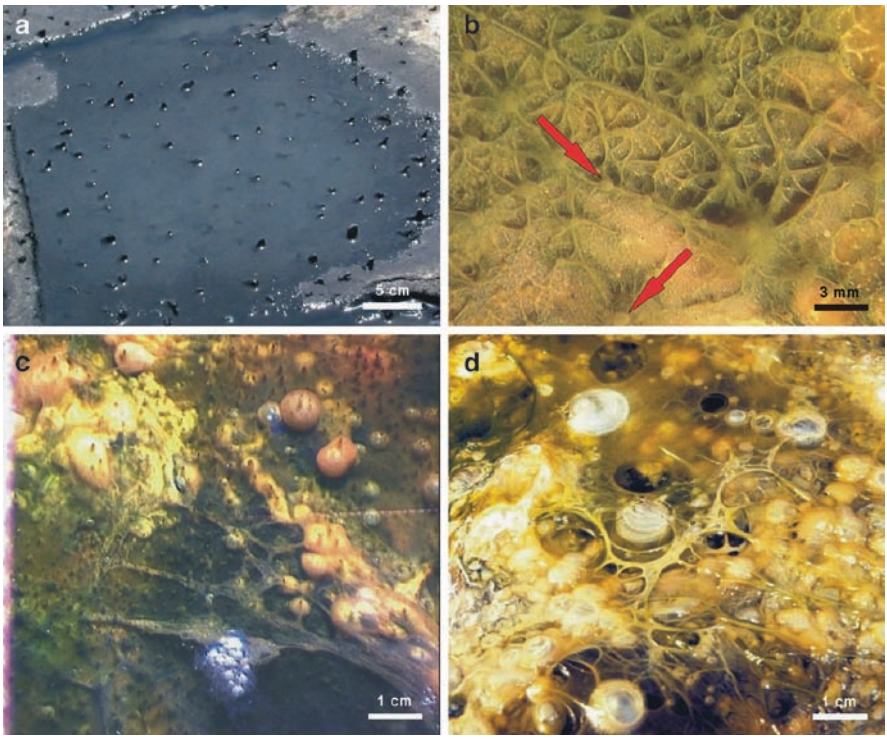

Figure 3. (**a**) *Lyngbya aestuarii* tufts rising 1–2 cm above the sediment surface of a small supratidal puddle. Tufts and sediments are black-colored by iron sulfide imaging sulfide enrichment of sediments below. The chemocline migrated across the sediment surface into a thin superficial water film. Bacterial breakdown of high amounts of organic matter provided by buried mats and stagnant water favored increasing sulfide concentrations. These patterns are signs that the organisms tried to escape the stressful conditions. Locality: Bhar Alouane, southern Tunisia. Image courtesy of Hubertus Porada. (**b**) Complex overgrowth structures: Button-like pinnacles (*arrows*), radial and linear bulges, and smaller reticulated veins are made by dominance of filamentous cyanobacteria (*Lyngbya* sp., *Microcoleus chthonoplastes*, and *Phormidium* spp.). The ornamentic features stretch over yellow-colored basic layers of coccoids embedded in amorphous slime. Lab-cultured mat. (**c**) Complex surface patterns generated by a shallow water-covered mat (water depth about 5 mm). Gas bubbles captured in biofilm-derived amorphous and thread-like slimes offer additional surfaces for microbial adhesion. Note small pinnacles growing on the bubbles and the surface of the basic mat. Coalescing gas bubbles are visible upper left. Lab-cultured mat. Modified after a photo published in Gerdes, 2007). (**d**) Microbial scum floating in quiet surface water on top of a benthic mat (water depth 20 cm). Complex biofilm fabrics and captured bubbles offer a variety of interfaces for concomitant bacterial adhesion and growth. Lab-cultured shallow water mat. (Modified after a photo published in Gerdes, 2007.)

Overgrowth patterns such as tufts, pinnacles, ornamentic patterns, or bubble coatings even may develop in settings where lowest surficial water films occur. Flocculous microbial scums are characteristic of permanently submerged habitats. Costerton et al. (1995) stated that biofilms "constitute a distinct growth phase of

bacteria that is profoundly different from the planktonic growth phase studied so assiduously during the 15 decades following the discoveries of Louis Pasteur." In this statement, planktonic and benthic biofilm phases are juxtaposed to emphasize the higher level of organization and coordinated functions in a benthic biofilm, respectively, microbial mat, compared with mixed bacteria populations floating in liquid media. However, a benthic–pelagic transition of biofilms generated by the benthic mat is not unusual. Results are microbial scums, which in quiet shallow water can be extraordinarily thick (Fig. 3d). Fragile biofilms, slime threads, and amorphous EPS, captured bubbles derived from the benthic mat, provide a variety of interfaces for floating cells. All this make a heterogeneous and complex fabric above the sediment–water interface, which is still controlled by the benthic mat below (Fig. 3d).

As Flügel (2004) stated, microbial mats form on bedding surfaces. Yet, smallest surface disturbances may trigger subsequent vertical growth and, as a consequence, the visual expression of wavy-crinkly and sometimes wrinkly discontinuity surfaces.

2.3. GROWTH RESPONSES TO PHYSICAL MAT DESTRUCTION

A wealth of information is available about sedimentary structures determined by physical mat destruction (for visual references, see Schieber et al., 2007). Possible processes involved include shear stress, erosion, and transport in relation to currents and waves, tearing prevailing over the tensile strength of biostabilized sediments, as well as the widespread cracking of surface mats particularly common in arid peritidal zones because of dehydration and desiccation. Signatures of erosion are ripple patches, erosional remnants, and microbial mat chips, flakes, or similar fragments of microbial mats (Noffke, 2007; Schieber, 2007).

A variety of different crack features occurs in modern peritidal mats (Eriksson et al., 2007). Common are leaf- or spindle-shaped shrinkage cracks, as well as polygonal or reticulate features. Cracks observed on mat surfaces may also reveal sinuously curved, sigmoidal, and circular features. Cracks range from incomplete networks to polygonal patterns. Curled and upturned crack margins are common within polygons. All these features can also be found in ancient sandstone (a selection is shown in Eriksson et al., 2007, their Fig. 4c-2).

Shrinkage and tearing of the living cohesive substrate is connected with a disorder of micro-biotopic conditions (Porada et al., 2007). Once surface moisture and probably nutrient supply is sufficient, the mat community is able to react to these disturbances by growth, the more as upturned crack margins may provide protective micro-niches against stressful insolation. In the immediate vicinity of cracks developed on modern intertidal to lower supratidal flats of southern Tunisia, Porada et al. (2007) observed particularly active growth of microbial mat communities, probably because of the ecological significance of surface-directed groundwater seeps forced by hydraulic pressure (Fig. 4a). Homogeneously thick mats, e.g., occurring at the center of large polygons, may repress surface seepage

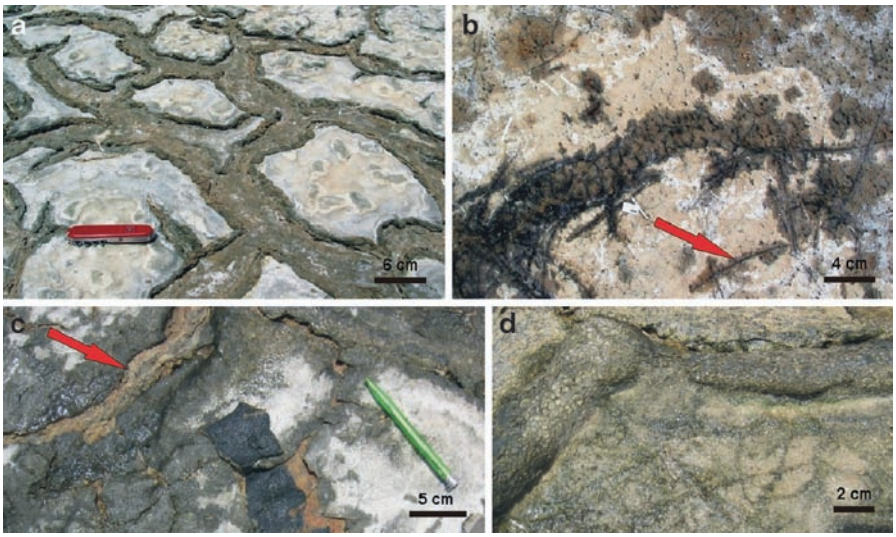

Figure 4. (**a**) Wide polygonal cracks subsequently overgrown and filled by microbial mats. Microbial growth was induced by rising groundwater due to hydraulic "upward pressure" (Porada et al., 2007). Wide shrinkage cracks developed after prolonged subaerial exposure. Crack margins are upturned and locally curled. Upper intertidal zone, El Jellabia; southern Tunisia. Image courtesy of Hubertus Porada. (**b**) Groundwater seepage associated with beginning tearing of a mat. Hydraulic pressure may have forced the upward-directed groundwater flow. Leafs of sea grass (*arrow*) may have interrupted the homogeneous distribution of microbial biomass and through this may have predetermined loci for tearing. Locality: Bhar Alouane supratidal flats, southern Tunisia. Image courtesy of Hubertus Porada. (**c**) Crack between microbial mat polygons completely overgrown by a rehabilitated mat (*arrow*) Transitions between the old polygonal and the new bulging mat and undersurfaces of old mats (*note upside down fragments in center*) are wet from trickling groundwater providing moisture and probably nutrients to the mat community. The new mat filling the cracks is characterized by high taxic diversity and complex surface structures. Locality: Bhar Alouane, southern Tunisia. (**d**) Bulging microbial mats filling the cracks along the margin of a supratidal puddle. Bulbous growth (*top left*) at the termination of one of the puddle margins. Lizard-skin surface texture reflecting structures left behind after bursting of photosynthetic gas bubbles. Curved lines are seagrass ghosts overgrown and imprinted by microbes. Locality: Bhar Alouane supratidal sabkha, southern Tunisia.

of pore water; yet, surrounding cracks provide successful leakages. Impressive patterns of induced mat growth related to leakages of groundwater into cracks were observed at southern Tunisia sabkhas (case study below).

2.3.1. Healing Cracks (Case Study)

In southern Tunisia, field observations were conducted in supratidal shallows joining a lagoon. In this area, various stages of crack development were observed, tearing the mat and leading to the formation of microbial mat polygons (Fig. 4b) and microbial mat polygons occurred. Cracks joining the polygons were completely overgrown by rehabilitated mats (Fig. 4a; close-up in Fig. 4c). Trickling groundwater

was visible at the transitions between the old polygonal and the new crack-filling mats. Considerable micro-topographic irregularities occurring at the surface of the new mats suggested high taxic diversity in closest proximity. This was confirmed by light microscopy (Table 2). Taxic diversity and concomitant structural heterogeneity, particularly of crack-related mats, emphasize ecological importance of crack formation in supratidal sabkhas of arid coasts where hydraulic pressure prevails (Porada et al., 2007). Important ecological parameters related to crack formation are the exposure of sediments formerly below the mats, uprising groundwater that provides moisture and nutrients, and finally light. All this favors the increase in microbial taxic diversity and structural versatility (Fig. 4c, d). Similar studies in other mat-occupied arid settings, allow concluding that the groundwater in these areas is particularly nutrient-enriched (Gerdes et al., 1985). Its availability particularly within the cracks and at crack edges where moisture is creeping on top of the polygon margins enhances increased microbial growth, particularly within the cracks and at the crack edges. This may also explain why bulged crack fillings are particularly characteristic in these settings.

Bulging growth of mats as a reaction to cracking is often associated with convoluted structures. These indicate complex internal patterns related to microbial overgrowth of crack margins, as well triggered by uprising groundwater (Bouougri and Porada, 2007; their Fig. 4f-1). Continuous processes of microbial growth, shrinkage, and overgrowth around crack-edges produce complex rolled-up structures in which microbial layers overgrow themselves and subsequently again undergo shrinkage. This again triggers growth responses. It is possible that a final surface mat completely seals cracks filled by such internal roll-up structures (Bouougri and Porada, 2007, their Fig. 4f).

Table 2. Results of light microscopy of samples taken from the crack-oriented new mat shown in Fig. 4c (study area: Bhar Alouane south, 04.07.06).

Areas sampled on the crack-oriented mat surface		**Results of light microscopy**
(i)	Small bulges	Dominant: *Lyngbya* sp. (*aestuarii*), associated: *Oscillatoria* sp., *Phormidium* sp., *Synechocystis* sp., *Chroococcus* sp., colorless filamentous sulfur bacteria
(ii)	Small depressions	Dominant: *Lyngbya* sp. (*aestuarii*); abundant: bundles of *Microcoleus chthonoplastes* (blue-green colored spots); associated: coccoids (*Synechocystis* sp., *Chroococcus* sp.) as before
(iii)	Yellow-green spots	*Phormidium* sp form felty layers in which sediment particles are bound; *Lyngbya* sp. (*aestuarii*) and *M. chthonoplastes* are associated; at least three other *Phormidium* species are present; aggregates of *Chroococcus* sp. increase in this layer
(iv)	Reticulate surface patterns	Dominant: *Lyngbya* sp. (*aestuarii*), overgrowing a felty layer of *Phormidium* sp. in which sediment particles and cell clusters of *Synechocystis* sp. are bound. Empty capsules of *Chroococcus* sp. up to 40 µm in diameter are abundant in this layer. Mineral particles are mainly in the size of fine-silt

Architects of complexity are the members of the highly diverse community, each growing according to its species peculiarity: *Lyngbya* sp., *M. chthonoplastes*, and *Phormidium* sp. form felty prostrate as well as upward creeping mats due to changes of the growth direction (polarity change; Gerdes et al., 2000), which may be affected by microscale nutritional changes, competition, etc. *Chroococcus* sp. forms colonies of nodular or granular appearance. Finally, the solitary cells of *Synechocystis* sp. in their diffluent mucilaginous envelopes produce the gelatinous matrix, which gives the sediment surfaces a supple appearance (Fig. 4c, d).

In fossil records, similar complex sedimentary structures may mark a concomitant and opportunistic response of a highly diverse microbial community to a complex chain of physical forces.

2.4. MICROBIAL "JOINT VENTURE"

In relation to the combined action of different functional prokaryotic groups, microbial mats are also considered as "joint ventures" (van Gemerden, 1993). Particularly in the mature state of a microbial mat, dominant groups are cyanobacteria, colorless sulfur bacteria, purple sulfur bacteria, and sulfate-reducing bacteria.

In terms of biomass condensation sufficient for the succession towards a joint venture of different metabolic groups, there is a particular phenomenon valuable to be considered. This is the already-mentioned tendency of microbial mats to spread not only parallel to a bedding plane, but also accrete in upward direction. Low sedimentation rates and other triggers stimulate the migration of motile organisms, as far as moisture is present. Even smallest disturbances such as trapped or baffled particles may support upward migration of motile organisms.

As an ideal model, thick three-dimensional piles of internally structured microbial biomass develop because of mutual influences of physical and biotic parameters (Fig. 2a, b). Organic matter becomes constantly deeper buried, and postmortem degradation processes prevail that favor taxic and functional diversity of the joint venture community. As detailed by van Gemerden (1993) and Stal (1994), a cascade of reactions is carried out in which biochemicals are excreted by one group, used and transformed by the other. Fibrillar structures that are embedded in or make up large portions of cyanobacterial sheaths are more resistant to bacterial degradation (Fig. 1b). In quartz-sandy sediments, such remaining fibrillar structures often appear as dark drapes along internal bedding planes (Gerdes and Klenke, 2007). When motile trichomes have left the sheaths or were degraded after death, remaining empty sheaths may provide internally hollow spaces, which may be filled by minute detrital sediments. Such sheath fillings appearing as ghosts of filaments were described by Rouchy and Monty (2000).

The often-described colorful stratification is a very characteristic visual side-effect of upward-directed growth, buried biomass, and increase in metabolic diversity: blue-green cyanobacterial represent the top mats, layers of purple-colored phototrophic sulfur bacteria are in an intermediate position, and black

iron-sulfide is at the base, which results from the activity of sulfate-reducing bacteria. The observation of such a colored stratification in the sandy surface layer of a southern North Sea tidal flat led Schulz (1937) to create the term "Farbstreifen-Sandwatt" (versicolored sandy tidal flats).

Immense biodiversity leads to the interconversion of sulfur compounds together with steep gradients of oxygen and sulfide (Jørgensen and Des Marais, 1986; Neu, 1994). The microbes are associated with a day–night shifting oxic–anoxic interface, which may trigger gliding movement of some species in exploiting shifting gradients within the mat (Fig. 5a, note also Fig. 3a).

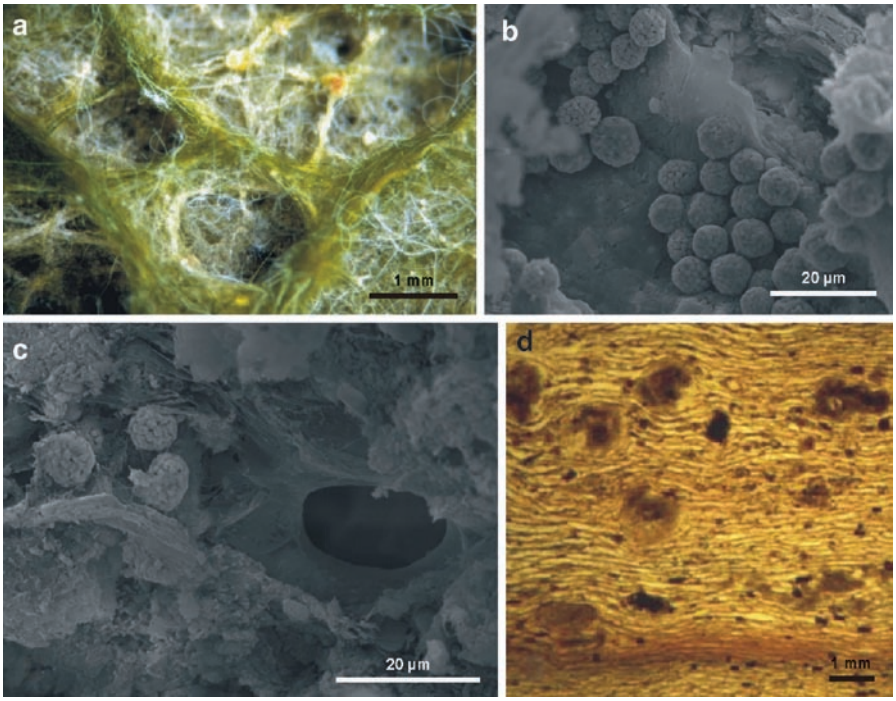

Figure 5. (**a**) White filaments of sulfur-oxidizing bacteria (*Beggiatoa* sp.). The organisms migrated from the inner mat towards the surface of a shallow water mat. The surface is marked by nodular structures and a network of filamentous cyanobacteria (*Lyngbya* sp.). The appearance of the sulfur-oxidizing bacteria at the mat surface marks a shift of the O_2/H_2S chemocline from the inner mat to the mat surface. Lab-cultured mat (modified after a photo published in Gerdes, 2007). (**b**) SEM view of internal sheet-like mat fabrics interspersed with pyrite framboids. Sample locality: Bhar Alouane, southern Tunisia. (**c**) SEM view of internal tangle of filaments, clusters of coccoids, slime threads, amorphous EPS, and pyrite framboids (*upper left*). The small open space structure to the right may result from a gas or liquid bubble. Sample locality: Bhar Alouane, southern Tunisia. (**d**) Thin section view of authigenic carbonate grains dispersed in the filament-dominated fabric of a hypersaline shallow water mat. Probable nuclei for grain formation: single cells or cell clusters, capsules, liquid or gas bubbles, or intraclasts. Sample locality: Lanzarote saltworks, Canary Islands.

Sulfate-reducing bacteria benefit from the continuity of production and degradation of organic matter and the presence of sulfate dissolved in surface and pore water. Products of these processes are: (1) hydrogen sulfide; (2) calcium carbonate, native sulfur, and pyrite (Friedman et al., 1992). Heterogeneous distribution of pyrite framboids (e.g., lamina-specific, clustered, and dispersed) is indicative of the internally complex fabrics of microbial mats (Fig. 5b, c).

Prominent members of the joint venture community are also large sulfur-oxidizing bacteria of the genus *Beggiatoa*. These gliding microorganisms capable of positioning themselves at the oxygen/sulfide interface (Fig. 5a) tend to store sulfur compounds within their cell walls. Sulfur starvation may result in deposition of polyphosphate (Simon, 1984). Phosphatized structures resembling *Beggiatoa* filaments have been observed in laminae occurring in the Monterey Formation, California (Reimers et al., 1990).

Moreover, phototrophic bacteria other than cyanobacteria are common in microbial mat communities. Purple photosynthetic bacteria add to the structural record by their partly rigid capsular sheaths. Recognition of the degradation series and preservation potential of these organisms will increase the likelihood of their identification in the fossil record (Stolz, 1984).

The joint venture community produces a variety of proxies helpful in recognizing former microbial mat presence. Biogeochemical proxies are, e.g., biomarkers, due to pigment lamination (Palmisano et al., 1989), and also characteristic isotope compositions. Element mapping of mat layers compared with mineral interlayers revealed lamina-specific enrichments of metabolically important elements (Kropp et al., 1996). Structural proxies are internal open space structures owing to gas bubbles and gas escape structures immanent of the metabolic processes, which during life are constantly going on in microbial mats (Fig. 5c). Cementation of cavities left after decay of mats; collapse of material within organic tissue of microbial origin, water and gas escape structures in unconsolidated sediments serve for visual structure recognition of former mat presence. Structural proxies are further carbonate coatings around filaments, and also in situ formed coated grains (Fig. 5d). An indication of in situ formation of these “microbial grains” (Flügel, 2004) may be an observed co-existence and proximity of different particle types (ooids, onkoids, peloids, and aggregate grains) within a biolaminated unit (Gerdes et al., 1994). Obviously, the form, size, and internal structures of in situ formed carbonate particles are controlled by mat-immanent heterogeneous micro-textures. Mats are characterized by a multitude of internal surfaces, cell clusters, capsules, sheaths, other tissue remains, intraclasts, and liquid or gaseous bubbles. In conclusion, the often irregular distribution of carbonate particles surrounded by microbially active substrates seems not to be random, but reflect the variety of different nucleation centers due to substrate heterogeneity and biogeochemical diversity offered by the living mat (Fig. 5d).

3. Summary and Conclusion

Today, the manifold importance of microbial mats for global processes is broadly acknowledged, yet it seems almost impossible to find only one single answer to the question: What are microbial mats? Different fields of interest developed their own answers, such as microbial mats being (i) stabilized and well-structured water (Cooksey, 1992; Krumbein, 1994; Neu, 1994); (ii) extremophiles; (iii) mucus-embedded masses of microorganisms; (iv) laminated sediment ecosystems; (v) joint venture (van Gemerden, 1993); (vi) agents of sediment stabilization; (vii) specialized tissues (Krumbein, 1996); (viii) producers of (paleo-) environmental proxies; or (ix) modern analogs of stromatolites representing the oldest known ecosystems on Earth.

Whether questions may be directed to the unifying principle expressed by the term biofilm, or to the wealth of phenotypic variations characteristic of microbial mats in natural environments, one aspect seems to run throughout the various different approaches. This is increasing knowledge that microbial mats are remarkably persistent in space and time in spite of often unpredictable physical disturbances (Zavarzin, 2003). Their emergent properties, namely the chemical species and sedimentary textures that leave remains in sediments, are of primary importance for the search for traces of extraterrestrial life on foreign planets.

Concurrent with their staying power throughout earth history is a broad spectrum of phenotypic variations, since microbial mats have been and still are living surfaces able to cope with various environmental conditions. In addition to carbonate rocks, there is a growing interest in the wide range of mat occurrences and their related structures in siliciclastic depositional environments. An increasing number of findings in sandstones and mudstones indicate the wide scope of microbial interventions in physical, chemical, and biotic characteristics.

Although the discipline of "microbial mat sedimentology" (Schieber et al., 2007) is relatively young, it has already verified remarkable evidence of the excellent preservation potential of microbial mat-associated features in siliciclastic and carbonate deposits. Proxy structures resulting from interaction of microbial mats with sediment are particularly common and well preserved in Precambrian rocks. Multilayered mats are known already from the Archean (Altermann et al., 2006. Bacteria responsible for these structures may only be rarely preserved. However, the proxy structures to a large degree owe their formation to the extracellular polymeric compounds characteristic of biofilms and microbial mats (Schieber et al., 2007). Biogenic microstructures such as capsules, fibrillar features, etc., and also surface patterns relatable to biostabilization in the fossil record show a significant structural correspondence with EPS-related patterns in modern biolaminated deposits. Studying analogous features in modern sediments, the continuum of biofilms and mats through earth history becomes obvious, which may give good reason for the actualistic approach to proxy structures as focused in this chapter.

4. References

Aharon, P. (2000) Microbial processes and products fueled by hydrocarbons at submarine seeps, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 270–281.

Altermann, W., Kazmierczak, J., Oren, A. and Wright, D. (2006) Microbial calcification and its impact on the sedimentary rock record during 3.5 billion years of earth history. Geobiology **1**: 169–178.

Bauld, J. (1984) Microbial mats in marginal marine environments: Shark Bay, Western Australia and Spencer Gulf, South Australia, In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites.* Alan R. Liss, New York, pp. 39–58.

Bouougri, E. and Porada, H. (2007) Complex structures associated with siliciclastic biolaminites, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 111–115.

Bouougri, E., Gerdes, G. and Porada, H. (2007) Inherent problems of terminology: definition of terms frequently used in connection with microbial mats, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 145–151.

Brehm, U., Gasiewicz, A., Gerdes, G. and Krumbein, W.E. (2002) Biolaminoid facies in a peritidal sabkha: Permian Platy Dolomite of northern Poland. Int. J. Earth Sci. **91**: 260–271.

Browne, K.M., Golubic, S. and Seong-Joo, L. (2000) Shallow marine microbial carbonate deposits, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 233–249.

Castenholz, R.W., Jørgensen, B.B., D'Amelio, E. and Bauld, J. (1991) Photosynthetic and behavioural versatility of the cyanobacterium *Oscillatoria boryana* in a sulfide-rich microbial mat. FEMS Microbiol. Ecol. **86**: 43–58.

Characklis, W.G. and Wilderer, P.A. (1989) *Structure and Function of Biofilms.* Dahlem Workshop Reports. Life Sciences Research Report 46. Wiley, Chichester, 386 pp.

Cohen, Y. (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats – Physiological Ecology of Benthic Microbial Communities.* ASM, Washington, DC, pp. 22–36.

Cooksey, K.E. (1992) Extracellular polymers in biofilms, In: L.F. Melo, T.R. Bott, M. Fletcher and B. Capdeville (eds.) *Biofilms – Science and Technology.* Kluwer, Dordrecht, pp. 137–147.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R. and Lappin-Scott, H.M. (1995) Microbial biofilms. Ann. Rev. Microbiol. **49**: 711–745.

Dade, W.B., Davis, J.D., Nichols, P.D., Nowell, A.R.M., Thistle, D., Trexler, M.B. and White, D.C. (1990) Effects of bacterial exopolymer adhesion on the entrainment of sand. Geomicrobiol. J. **8**: 1–16.

Decho, A.W. (2000) Exopolymer microdomains as a structuring agent for heterogeneity within microbial biofilms, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 9–15.

Eriksson, P.G., Schieber, J., Bouougri, E., Gerdes, G., Porada, H., Banerjee, S., Bose, P.K. and Sarkar, S. (2007) Classification of structures left by microbial mats in their host sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 39–52.

Flügel, E. (2004) *Microfacies of Carbonate Rocks.* Springer, Berlin, 976 pp.

Friedman G.M., Sanders J.E. and Kopaska-Merkel, D. (1992) *Principles of Sedimentary Deposits.* MacMillan, New York, 717 pp.

Gasiewicz, A., Gerdes, G. and Krumbein, W.E. (1987) The peritidal sabkha type stromatolites of the Platy Dolomite (Ca3) of the Leba elevation (northern Poland). Lect. Notes Earth Sci. **10**: 253–272.

Gehling, J.G. (1991) The case for Ediacaran fossil roots to the metazoan tree. Geol. Soc. India Mem. **20**: 181–224.

Gerdes, G. (2007) Structures left by modern microbial mats in their host sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 5–38.

Gerdes, G. and Klenke, T. (2003) Geologische Bedeutung ökologischer Zeiträume in biogener Schichtung (Mikrobenmatten, potentielle Stromatolithe). Mitteilung der Gesellschaft für Geologie und Bergbaustudien. Österr. **46**: 35–49.

Gerdes, G. and Klenke, T. (2007) States of biogenic bedding as records of the interplay of ecologic time and environment. Senckenbergiana Maritima **37**: 129–144.

Gerdes, G., Krumbein, W.E. and Holtkamp, E. (1985) Salinity and water activity related zonation of microbial communities and potential stromatolites of the Gavish Sabkha, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems. The Gavish Sabkha.* Springer, Berlin, pp. 238–266.

Gerdes, G., Dunajtschik-Piewak, K., Riege, H., Taher, A.G., Krumbein, W.E. and Reineck, H.E. (1994) Structural diversity of biogenic carbonate particles in microbial mats. Sedimentology **41**: 1273–1294.

Gerdes, G., Klenke, T. and Noffke, N. (2000) Microbial signatures in peritidal siliciclastic sediments, a catalogue. Sedimentology **47**: 279–308.

Hagadorn, J.W. and Bottjer, D.J. (1997) Wrinkle structures: microbially mediated sedimentary structures common in subtidal siliciclastic settings at the Proterozoic–Phanerozoic transition. Geology **25**: 1047–1050.

Jørgensen, B.B. and Des Marais, D.J. (1986) Competition for sulfide among colorless and purple sulfur bacteria in cyanobacterial mats. FEMS Microbiol. Ecol. **38**: 179–186.

Kropp, J., von Bloh, W. and Klenke, T. (1996) Calcite formation in microbial mats: modeling and quantification of inhomogeneous distribution patterns by cellular automation model and multifractal measures. Geol. Rundschau **85**: 857–863.

Krumbein, W.E. (1983) Stromatolites – the challenge of a term in space and time. Precambrian Res. **20**: 493–531.

Krumbein, W.E. (1993) Paracelsus und die mucilaginischen Substanzen – 500 Jahre EPS-Forschung. DGM-Mitteilungen **1993**: 8–14.

Krumbein, W.E. (1994) The year of the slime, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments.* Bibliotheks- und Informationssystem BIS, Oldenburg, pp. 1–7.

Krumbein, W.E. (1996). Geophysiology and parahistology of the interactions of organisms with the environment. P.S.Z.N.I: Mar. Ecol. **17**: 1–21.

Krumbein, W.E., Brehm, U., Gorbushina, A.A., Levit, G. and Palinska, K.A. (2003) Biofilm, biodictyon and biomat – biolaminites, oolites, stromatolites – geophysiology, global mechanism and parahistology, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 1–27.

Neu, T.R. (1994) Biofilms and microbial mats, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments.* Bibliotheks-Informationssystem BIS, Oldenburg, pp. 9–16.

Neu, T.R. and Marshall, K.C. (1990) Bacterial polymers: physicochemical aspects of their interaction at interfaces. J. Biomat. Appl. **5**: 107–133.

Neu, T.R., Eitner, A. and Paje, M.L. (2003) Development and architecture of complex environmental biofilms, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 29–45.

Noffke, N. (2007) Microbially-induced sedimentary structures (MISS) of early and middle Archaean Ages – Moodies Group, Pongola Supergroup, Witwatersrand Supergroup (South Africa), In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 153–157.

Noffke, N. and Krumbein, W.E. (1999) A quantitative approach to sedimentary surface structures contoured by the interplay of microbial colonization and physical dynamics. Sedimentology **46**: 417–426.

Oschmann, W. (2000) Microbes and black shales, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 137–148.

Palmisano, A.C., Cronin, S.E., D'Amelio, E.D., Munoz, E. and Des Marais, D.J. (1989) Distribution and survival of lipophilic pigments in a laminated microbial mat community near Guerrero Negro, Mexico, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats – Physiological Ecology of Bethic Microbial Communities.* ASM, Washington, DC, pp. 138–152.

Porada, H. and Bouougri, E. (2007) Wrinkle structures – a critical review, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 135–144.

Porada, H., Bouougri, E. and Ghergut, J. (2007) Hydraulic conditions and mat-related structures in tidal flats and coastal sabkhas, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 258–265.

Reimers, C.E., Kastner, M. and Garrison, R.E. (1990) The role of bacterial mats in phosphate mineralization with particular reference to the monterey formation, In: W.C. Burnett and S.R. Riggs (eds.) *Phosphate Deposits of the World. Vol. 3. Neogene to Modern Phosphorites.* Cambridge University Press, Cambridge, pp. 300–311.

Reineck, H.-E., Gerdes, G., Claes, M., Dunajtschik, K., Riege, H. and Krumbein, W.E. (1990) Microbial modification of sedimentary surface structures, In: D. Heling, P. Rothe, U. Förstner and P. Stoffers (eds.) *Sediments and Environmental Geochemistry*. Springer, Berlin, pp. 254–276.

Riding, R. (2000) Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. Sedimentology **47**: 179–214.

Rouchy, J.M. and Monty, C. (2000) Gypsum microbial sediments: neogene and modern examples, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 209–216.

Schieber, J. (1986) The possible role of benthic microbial mats during the formation of carbonaceous shales in shallow Proterozoic basins. Sedimentology **33**: 521–536.

Schieber, J. (2007) Flume experiments on the durability of sandy microbial mat fragments during transport, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, pp. 248–257.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (eds.) (2007) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, 311 p.

Schulz, E. (1937) Das Farbstreifen-Sandwatt und seine Fauna, eine ökologisch-biozönotische Untersuchung an der Nordsee. Meereskundl. Arb. Univ. Kiel **1**: 359–378.

Simon, R.D. (1984) Evolution of the microbial mat community: problems and technological solutions, In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites.* Alan R. Liss, New York, pp. 437–447.

Stal, L.J. (1994) Microbial mats: ecophysiological interactions related to biogenic sediment stabilization, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments.* University of Oldenburg, BIS Oldenburg, pp. 41–53.

Stolz, J. (1984) Fine structure of the stratified microbial community at Laguna Figueroa, Baja California, Mexico: II. Transmission electron microscopy as a diagnostic tool in studying microbial communities in situ, In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites.* Alan R. Liss, New York, pp. 23–38.

Stolz, J. (2000) Structure of microbial mats and biofilms, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 1–8.

Stoodley, P., Lewandowski, Z., Boyle, J.D. and Lappin-Scott, H.M. (1999) The formation of migratory ripples in a mixed species bacterial biofilm growing in turbulent flow. Environ. Microbiol. **1**: 447–457.

Thiel, V., Peckmann, J., Richnow, H.H., Luth, U., Reitner, J. and Michaelis, W. (2001) Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat. Mar. Chem. **73**: 97–112.

van Gemerden, H. (1993) Microbial mats: a joint venture, In: R.J. Parkes, P. Westbroek and J.W. de Leeuw (eds.) *Marine Sediments, Burial, Pore Water Chemistry, Microbiology and Diagenesis.* Mar. Geol. **113**: 3–25.

Verrecchia, E.P. (2000) Fungi and sediments, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments.* Springer, Berlin, pp. 68–75.

Wachendörfer, V., Krumbein, W.E. and Schellnhuber, H.-J. (1994) Bacteriogenic porosity of marine sediments – a case of biomorphogenesis of sedimentary rocks, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments.* Bibliotheks-Informationssystem BIS, Oldenburg, pp. 203–220.

Zavarzin, G.A. (2003) Diversity of cyano-bacterial mats, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 141–150.

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PAPER FROM OUTER SPACE – ON "METEORPAPIER" AND MICROBIAL MATS

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1. Introduction

> In einer Zeit, in der einerseits empfindliche Papierkrappheit und andererseits beinahe ausschließlicher Papiergeltumlauf herrschen, in der wir in Ermangelung anderer Rohstoffe unsere Wäsche, Kleider, Decken, Vorhänge und selbst Bindfaden aus Papier herstellen, ist es vielleicht angebracht, einer besonderen Art von Papier zu gedenken, das fast unbekannt ist, von dem aber selbst ernsthaft zu nehmende Leute früherer Zeiten glaubten, daß es vom Himmel gefallen sei, und es deshalb *Meteorpapier* genannt haben.

[In a time in which, on the one hand, paper is in extremely short supply and, on the other, nearly all the money in circulation is in the form of paper banknotes, and in a time in which, because of lack of other raw materials, we manufacture our linen, clothes, blankets, curtains, and even binding rope from paper, it may be appropriate to draw some attention to a special, virtually unknown type of paper, about which in former times even serious people believed that it had fallen from the skies, and therefore had called it meteor paper.][1]

These intriguing sentences were written just after the end of World War I by Dr. Bruno Schröder from Breslau (currently Wrocław, Poland), in a paper entitled "Über Meteorpapier" (Schöder, 1919). Sightings of "Meteorpapier" falling from heaven had first been reported in 1639 and in 1686. Particularly, the latter event aroused considerable interest among contemporary scientists, and many theories were proposed in the course of time on the origin of this paper-like material. Only in 1838 was the enigma of the true nature of the "Meteorpapier" solved; thanks to the observations of the famous botanist Christian Gottfried Ehrenberg (1795–1876) (Fig. 1), who is also remembered as a pioneer of bacterial taxonomy (Ehrenberg, 1838a, b, 1839). He unequivocally demonstrated that the paper-like material originated from desiccated microbial mats that develop on the shores of certain lakes and on flooded grasslands.

[1] Translations from the writings of Schröder, Kersten, Ehrenberg, Cohn and others are by the author.

Figure 1. Portrait gallery of some of the scientists mentioned in the text who had been involved in the study of "Meteorpapier": Simon Pauli the Younger (1603–1680), Ernst Florens Friedrich Chladni (1756–1827), Christian Gottfried Daniel Nees von Esenbeck (1776–1858), Jöns Jakob Berzelius (1779–1848), Freiherr Christian Johann Dietrich Theodor von Grotthuß (1785–1822), Johannes Hieronymus Kniphof (1704–1763), Christian Gottfried Ehrenberg (1795–1876), Heinrich Robert Göppert (1800–1884), Ferdinand Cohn (1828–1898), and Friedrich August Rudolph Kolenati (1812–1864). Biographical data given in the text were derived in part from Schlegel (1999).

The "Meteorpapier" story, which was a popular topic in the scientific writings of the ninteenth century, was nicely summarized in Schröder's above-mentioned paper from 1919, on which much of the information presented here is based. The subject was only rarely mentioned in the more recent literature, a notable exception being the review by Stal (2000) on cyanobacterial mats and stromatolites.

This essay provides an overview of the literature on "Meteorpapier," showing how the concepts about its nature and origin have developed during the past three and a half centuries.

2. Early Reports on "Meteorpapier" and Theories on Its Origin

The first record of "Meteorpapier" probably dates from 1639, when a dense, white mass of paper-like material was found on the fields close to a lake in Norway. It had some resemblance to fine English linen or to Chinese paper. A sample was sent to Simon Pauli, professor of botany at the University of Rostock, but he did not succeed in elucidating its nature.

Far better known became a paper-like mass from Curland (southern Latvia). During a snowstorm in the afternoon of January 31, 1686, the area around a pond near the village of Rauden, north of Memel (currently Klaipėda, Lithuania) became covered with a coal-black, leaf-like, or paper-like substance that had not been there in the morning. Some people had actually seen it falling flake-wise from the air. The rumor spread quickly, and many came to witness this enigmatic phenomenon. Some pieces had the size of a table, and in some places stacks as thick as a finger had accumulated. When wet, the material had a bad smell like rotten seaweed, but in a dry state it lacked any smell. When torn, it appeared to consist of fibrous material, like blotting paper or printing paper. Berzelius (1822), based on earlier published reports, noted that people did not dare to touch the material, fearing that it was a product of witchcraft, but that a poor beggar who was on his way to a nearby estate lifted a piece to show it as a curiosity.

The scientific world at the time became highly excited about this "paper snow." Dr. Johann Georg Weygand, physician in Goldingen (the present-day Kuldīga) in Curland, claimed that it undoubtedly was real paper, washed ashore from a ship wrecked in the Baltic Sea, and having rotten for some time in bales among seaweed on the coast, causing its color and smell. After drying, the material could then have been carried through the air over a long distance by the north-eastern storm. An altogether different, and much more exciting, opinion was expressed by Dr. Philipp Jakob Hartmann, professor of medicine in Königsberg (currently Krolewiec, Poland), who in 1689 published a book entitled "Exercitatio generatione mineralium, vegetabilium et animalium in aëre" [Treatise on the generation of minerals, plants and animals in the air] (Hartmann, 1689). He claimed that the phenomenon had been caused by the activity of a meteor, as the paper-like mass had fallen from the skies in coherent pieces, which were later torn up by the storm. The physicist Ernst Florens Friedrich Chladni (1756–1827), in his 1819 essay about "fire meteors," tentatively classified the masses of paper fallen from heaven as derived from "soft meteors" (whatever that term may mean!). As late as 1825, Nees von Esenbeck, president of the Leopoldinisch-Karolinische Gesellschaft in Halle, considered the paper-like material from Curland as probable "aerophytes" or "plants from the air."

Chemical experts also became interested in the nature of the "Meteorpapier." Theodor von Grotthuß (1785–1822) performed in 1819 a chemical analysis of the Curland paper, a sample of which was present in the estate of his father. He detected three main components: "Kieselerde" (silica), "Kalkerde" (calcium carbonate), and "Bittererde" (magnesium oxide). In addition, he found traces of three components that at the time were considered characteristic of meteors, namely sulfur, nickel, and chromium. Von Grotthuß, therefore, concluded that the material undoubtedly had originated from a meteor. He sent some of the material to the famous chemist Jakob Berzelius in Stockholm to obtain confirmation of its nickel content. However, Berzelius did not detect any traces of nickel in the samples. On renewed analysis, von Grotthuß also failed to find nickel, and he admitted that he probably had mistakenly identified iron sulfide as nickel sulfide.

In the mean time, similar paper-like material had been found in other locations as well. Samples were kept in collections of curiosa from nature, maintained to be admired both by contemporaries and by future generations.

3. The Elucidation of the True Nature of the "Meteorpapier"

Already in the eighteenth century, some investigators began to suspect that the paper-like material may be a product of the plant world. Thus, Johannes Hieronymus Kniphof (1704–1763) wrote an essay entitled "Physikalische Untersuchung des Peltzes, welchen die Natur durch Fäulnis auf einigen Wiesen im Jahre 1752 hervorgebracht hat" [Physical investigation of the coating formed by nature by decay on some grasslands in 1752], and John Strange F.R.S. (1732–1799) wrote in 1764 that water plants, or microscopic filamentous algae ("Conferva" as Pliny had named them) had formed such a paper-like substance.

The true origin of the "Meteorpapier" was disclosed in 1838–1839. The 1839 volume of the "Annalen der Physik" contains two short publications, one by L.M. Kersten, which is followed by a paper by Christian Ehrenberg. Kersten had performed a chemical analysis of a material that had a striking similarity with leather, and that had been formed on grassland on the Drahthammer near Schwarzenberg in the Ore Mountains in Saxony (Kersten, 1839). Kersten described its origin as follows: "On the water that had flooded the grassland, a slimy green material was formed. After the water was slowly drained it settled on the grass, dried out, completely lost its color, and finally it could be removed in large pieces. This natural product resembles on its outer surface soft polished glove leather or smooth fine paper. It is somewhat shiny, soft to the touch, and has the strength of normal white printing paper. On its lower surface, which had been in contact with the water, it has a bright green color. It is still possible to distinguish green leaves from which the leather-like cover originated. A botanist could probably identify the species to which these belong." A detailed account of the chemical analysis of the material then follows, after which Kersten concludes: "The ash of the unknown substance mainly consists of silica and oxides of manganese and iron. The material itself, however, appears to be an aggregate of leaves, from which the chlorophyll as well as the other organic compounds have completely disappeared as a result of an organic process."

Ehrenberg added a postscript to Kersten's article in which he wrote: "Regarding the leather-like material from the Schwarzenberg meadow, microscopic examination clearly shows that it consists of *Conferva capillaris*, *Conferva punctalis*, and *Oscillatoria limosa* [for further information on the names of microorganisms mentioned and their modern equivalents, see Table 1]. Together, these form a dense mat, at the upper surface bleached by the sun, in which a few fallen leaves and blades of grass are incorporated. Dispersed between these microalgae, one finds numerous diatoms, in particular *Fragillaria* species and *Meridion vernale*. I have observed 16 different diatom species belonging to six genera, and in addition three more soft-shelled protists, as well as desiccated specimens of "*Anguillula fluvatilis*" (a nematode), a total of 20 different

Table 1. Names of microorganisms mentioned in the cited documents relating to "Meteorpapier," and their modern equivalents, if applicable. Nomenclature information was derived in part from Geitler (1932) for cyanobacteria and from Fritsch (1956) and Chapman and Chapman (1973) for eukaryotic algae.

	Genus and species	Current name
Cyanobacteria		
	Oscillatoria limosa	
	Lyngbya turfosa	?
	Lyngbya sudetica	*Lyngbya sudetica* (Nave) Kirchner ex Hansgirg, 1892
	Nostoc	
Chlorophyceae		
	Conferva capillaris	*Conferva capillaris* **(**Kützing) Rabenhorst 1847 is an illegitimate name; it is currently regarded as a synonym of *Chaetomorpha ligustica* (Kützing)
	Conferva punctalis	An illegitimate name; current equivalent unknown
	Conferva vesicata Agardh	*Oedogonium vesiculatum* Link
	Conferva crispata	*Conferva crispata* Roth 1979 is an illegitimate name. The name is currently regarded as a synonym of *Cladophora glomerata* var. *crassior* (C. Agardh) van den Hoek
	Cladophora fracta var. *viadrina*	*Cladophora fracta* var. *viadrina* (Kützing) Kirchner 1878 is currently regarded as a synonym of *Cladophora fracta* (O.F. Müller ex Vahl) Kützing
	Microspora floccosa	*Microspora floccosa* (Vaucher) Thuret
	Rhizoclonium hieroglyphicum	Rhizoclonium hieroglyphicum (C. Agardh) Kützing
	Cladophora crispa	= ?? *Cladophora crispata* (Roth.) Kützing = ?? *Cladophora glomerata*
	Binuclearia	
	Spheroplea annulina	*Spheroplea annulina* (Roth.) Agardh
Bacillariophyceae		
	Fragillaria	
	Meridion vernale	
Gramineae		
	Glyceria fluitans	(Manna grass)
	Glyceria spectabilis	*Glyceria maxima* (Reed sweet-grass)
Animals		
	Anguillula fluvatilis	Probably a nematode
	Daphnia pulex	The common water flea
	Planorbis	

species." Ehrenberg had also obtained samples of the same material earlier examined by Grotthuß and Berzelius. He did not recognize any recognizable small seeds in these fragments, but he found it to consist of the same microalgae as well as the same types of protists. A piece of yellowish material resembling Chinese silk paper sent by Berzelius, which had been formed (whether recently or in earlier times was not mentioned) on the dried-out shore of a Swedish lake, consisted entirely of yet another

freshwater microalga, *Oedogonium vesiculatum* Link (*Conferva vesicata* Agardh), between which many pollen of spruce trees as well as protists were observed.

Ehrenberg's own article on the true nature of the "Meteorpapier" deserves to be cited in full here:

> XX. Ueber das im Jahre 1686 in Curland vom Himmel gefallene Meterorpapier;
> Von C. G. Ehrenberg.
> Aus den Berichten der K. Preuß. Academie.
>
> Am 31. Januar 1686 fiel beim Dorfe *Rauden* in Curland mit heftigem Schneegestöber eine große Masse einer papierartigen schwarzen Substanz aus der Luft; man sah sie fallen, und fand sie nach Tische an Orten, wo die beschäftigten Arbeiter vor Tische nichts Aehnliches gesehen hatten. Diese 1686 und 1688 umständlich beschriebene und abgebildete Meteorsubstanz was neuerlich von Hrn. v. Grotthuß, nach einer chemischen Analyse, wiederholt für Meteormasse gehalten worden, den angegebenen Nickelgehalt hatte aber Hr. v. Berzelius, der sie ebenfalls analysirte, nicht erkannt, und Hr. v. Grotthuß widerrief ihn dann selbst. In Cladni's Werke über die Meteore ist sie aufgeführt und auch in Nees von Esenbeck's reichem Nachtrage, in R. Brown's bot. Schriften ist sie als Aërophyt angemerkt. Hr. E. untersuchte diese Substanz, von welcher etwas auf dem Königlichen Mineraliencabinet (auch in Chladni's Sammlung) befindlich ist, mikroskopisch. Sie besteht danach völlig deutlich aus dicht verfilzter *Conferva crispata*, Spuren eines *Nostoc* und aus bis 29 wohlerhaltenen Infusorien-Arten, von denen nur 3 in dem größeren Infusorien-Werke noch nicht erwähnt, aber wohl auch schon bei Berlin lebend vorgekommen sind, überdieß auch aus Schaalen der *Daphnia Pulex*. Von den 29 Infusorien-Arten sind nur 8 kieselschaalige, die übrigen weich oder mit häutigem Panzer. Mehrere der ausgezeichnetsten sehr seltenen Bacillarien sind darin häu. Diese Infusorien haben sich nun 152 Jahre erhalten. Die Masse kann durch Sturm aus einer curländischen Niederung abgehoben und nur weggeführt, aber auch aus einer sehr fernen Gegend gekommen seyn, da selbst aus dem Mexicanischen Amerka Hr. Carl Ehrenberg die bei Berlin lebenden Formen eingesandt hat. In der Substanz liegende fremde Saamen, Baumblätter und andere dergl. Dinge würden, bei weiterer Untersuchung größere Mengen, solche Zweifel entscheiden. Die vielen inländischen Infusorien und die Schaalen der gemeinen *Daphnia Pulex* scheinen dafür zu sprechen, daß ihr Vaterland weder die Atmosphäre noch Amerika, sondern Ostpreußen oder Curland war. – Die Substanz und die Abblidungen aller Bestandtheile derselben wurden vorgezeigt.
>
> XX. On the meteor paper that fell from heaven in Curland in the year 1686
> By C. G. Ehrenberg
> From the proceedings of the Royal Prussian Academy
>
> On January 31, 1686, a large amount of a paper-like black substance fell from the skies in the village of Rauden in Curland during a heavy snowstorm. People saw it falling, and workers returning from their lunch found it on places where they had not seen anything of the kind before they had left. This material from heaven, which had been extensively described and illustrated in 1686 and in 1688, was again attributed to meteors by Mr. von Grotthuß, based on chemical analysis. However, Mr. von Berzelius, who also had analyzed the substance, did not detect

> the indicated content of nickel, and this was subsequently withdrawn by Mr. von Grotthuß himself. The material is mentioned in Chladni's works about meteors, and also in Nees von Esenbeck's rich legacy; in the botanical works of R. Brown, it is indicated as an aerophyt. Mr. E[hrenberg] microscopically investigated this material, some of which is present in the Royal Cabinet of Minerals, as well as in Chladni's collection. According to the analysis, it was obvious that it completely consisted of *Conferva crispata* forming a dense mat, traces of a *Nostoc*, and of up to 29 well-preserved species of protists, only three of which have not been previously recorded in the handbooks on protists, and in addition of shells of *Daphnia pulex*. Among the 29 types of protists, only 8 had a siliceous shell, the others are soft-walled or have a skin-like shell. Several notable types of very rare diatom species occur frequently. These protists have been preserved for 152 years. It is possible that the material had been lifted from lowlands in Curland, but it may as well have been transported from a more remote area: Mr. Carl Ehrenberg even has sent forms from Mexico that occur near Berlin. When examining larger quantities, one can identify without any doubt seeds, leaves from trees, and similar objects. The abundance of local protists and the shells of the common *Daphnia pulex* may indicate its origin neither from the atmosphere or America, but from East-Prussia or Curland. The material and illustrations of all its components were presented [at the meeting].

To get hold of additional samples of similar material for a comparative study, Ehrenberg approached the botanist Prof. Heinrich Göppert (1800–1884) in Breslau. The latter had found in the St. Bernhardin library in Breslau four large sheets of a paper-like material, 34 × 2–4 ft in size. It was known as "Oderhaut" ["Skin from the Oder"], and Göppert assumed that it had originated during massive floods caused by the river Oder in 1736. The events that led to its formation had been described by Johann Christian Kundmann (1736), a physician and collector from Breslau (1684–1751), in his book "Rariora naturae et artis" [Curiosites from nature and art]. After the Oder had inundated large areas in Silesia, a tough layer was observed on the grasslands after the water had receded. On being desiccated, it became strong as leather and it could be torn only with difficulty. It was white or yellowish to red-brown in color, and its upper surface was perfectly flat, so that one could write on it. On the lower side, however, it felt like silk. In color and strength, it resembled gray wrapping paper. The upper firm layer could easily be separated from the lower, less dense mat, which was brownish to greenish in color. At the lower side, numerous leaves and roots of grasses (*Glyceria fluitans* and *G. spectabilis*) could be seen, as well as shells of water snails (*Planorbis*). Göppert found the substance to consist almost exclusively of the filamentous green alga *Cladophora fracta* var. *viadrina* together with many small aquatic animals and insects. Ehrenberg detected 19 more species in this material.

In June 1849, Göppert and his student Ferdinand Cohn (1828–1898) made a botanical excursion east of Breslau to the so-called Morgenauer meadow, a humid land tongue between the Oder and the mouth of the Ohle. Flooding of these rivers had caused the formation on pools and puddles in the lower areas, and these

became covered with a dense green floating mat. At the edge around the pools, a dry, yellowish-green or gray, more or less densely woven skin-like material was found on the soil. Parts of it were smooth and thick like course wrapping paper, other parts more loose like sack cloth, linen, or tow. Near the water, it merged with a floating mat of microalgae, and occasionally it was pushed up, perforated, and disrupted by the grass around the pools. Göppert and Cohn immediately remembered the algal mat material examined by Ehrenberg. They concluded that a number of biological and physical conditions must be fulfilled to produce the phenomenon: dense growth of certain types of filamentous algae when the area is flooded, a rapid retreat of the flood water, and a soil that does not retain humidity for long periods but enables desiccation of the algal mat by the heat of the sun before it is degraded. Microscopical analysis showed *Cladophora fracta* var. *vadrina* to be the main component. On and between its filaments lived numerous diatoms, belonging to almost the same species found by Ehrenberg in the "Oderhaut" from St. Bernhardin near Breslau, which had been collected more than a 100 years earlier. Göppert and Cohn commented that these organisms, thus far considered cosmopolitan, may well have their own characteristic geographical distribution, so that it should be possible to compile a flora and a fauna of different countries not only of higher plants and animals, but also of microorganisms. Later in his life did Ferdinand Cohn indeed write such a document for algae and other lower plants, published in 1878 under the title "Kryptogamen-Flora von Schlesien" (Cohn, 1878). In the introduction to this book, he mentioned Göppert's earlier work and noted that the sample collected a century earlier by Kundmann, preserved in the St. Bernardhin library and examined by Göppert in 1840, had been the source of the earliest documented microalgae from Silesia.

There are additional reports from the nineteenth century on findings of similar paper-like algal mats. In October 1854, Ferdinand Cohn found such a dried algal mat on a potato field near Breslau that had previously been inundated by flooding of the Oder. This dark red-colored algal mat was formed by filaments of *Spheroplea annulina*. Similar mats of the same alga had been found near Berlin by Ehrenberg and near Bremen by Ludolph Christian Treviranus (1779–1864). Friedrich August Rudolph Kolenati (1812–1864) from Brno recorded such a thick of reddish to bluish or dark-green layer formed by the cyanobacterium *Lyngbya sudetica* at the Mitteloppa spring on the Leiterberg in the Moravian slope of the Sudeten mountains. Anton Hansgirg (1854–1917) mentioned in his flora of the algae of Bohemia of 1886 mats of *Cladospora fracta* var. *viadrina* at the shore of ponds near Leitmeritz and Lobositz (Litomerice and Lovosice in the Czech Republic). G. von Istvanffy published in 1890 a document in Hungarian about "Meteorpapier" from three additional locations: a *Cladophora fracta* var. *viadrina* mat on river banks near Budapest, similar growth of blue-green paper-like covers of *Lyngbya turfosa* on the banks of Lake Czoba in de High Tatras, and a mat consisting of sterile filaments of *Oedogonium* and of *Microspora floccosa* from peat bogs in Westphalia. On the shore of the Neusiedlersee (Austria), Siegfried

Stockmayer (1868–1938) found such material formed by *Rhizoclonium hieroglyphicum* and *Cladophora crispa*. Then, there is a report on "Meteorpapier"-like material formed on the banks of the Danube near Vienna, allegedly formed by the cyanobacterium *Microcoleus chthonoplastes* (Stockmayer, 1893). [Possibly a misidentification, as this species is not commonly found in freshwater environments; for new insights into the taxonomy of the genus *Microcoleus* see Siegesmund et al., 2008.] Finally, Schöder (1919) repeatedly found "Meteorpapier" on fenland, first in 1900 at the Lanfstuhlmoor near Kaiserslautern in de Rheinpfalz and in 1917 on the Seefeldern near Reinerz (Glatz [Klodzko], Poland) on the Iserwiese in Silesia. In this material, he identified filaments of *Oedogonium*, *Microspora*, *Binuclearia*, and several Zygnemaceae.

4. Final Comments

Although largely forgotten today, it is fascinating to follow the ideas and theories that have existed since the first records of "paper that fell from the skies" in the seventeenth century on the true nature of this material. Although some of the best-known natural scientists of the seventeenth and the eighteenth centuries have occupied themselves with the study of the "Meteorpapier," the true nature of the material was discovered only 200 years after the first record of its sighting in Norway.

This essay opened with the intriguing question by Schöder (1919) whether "Meteorpapier" could be considered as a possible substitute for paper in a time in which the real product was in short supply. He himself gave the answer in the last paragraph of his article:

> So hat sich mit der Erforschung des durch Grün- und Blaualgen gebildeten Meteorpapieres, das sowohl in der Ebene wie auf Bergeshöhen an Flußufern, Teiche und Seen, wie auf Hochmooren in vielerlei Farbentönen vorkommt, die Wissenschaft durch fast drei Jahrhunderte bis in die heutige Zeit mit mehr oder weniger Erfolg beschäftigt, nicht nur die Botanik, sondern auch die Physik, die Chemie und die Geologie. Man gelangte zu den allgemeinen Ergebnis, daß dadurch die Wichtigkeit der mikroskopischen Analyse zuerst zu ihrem Rechte kam, daß die Grundlage zu einer Flora und Fauna niederer Organismen gelegt und die Bildung der Torflachen auf Mooren erklärt wurde, und es ist nur zu bedauern, daß das Meteorpapier unserer heutigen Papiernot nicht abzuhelfen vermag.
>
> [For nearly 300 years until the present time has science – botany as well as physics, chemistry, and geology – thus occupied itself more or less successfully with the examination of the "Meteorpapier." It is formed by green algae and cyanobacteria, and is found in plains as well as high in the mountains, on the banks of rivers, ponds, and lakes as well as on fenland in many different shades of color. The general results were that microscopical analysis first got the importance it deserves, that the basis has been established for a flora and fauna of lower organisms, and that the formation of the bog pools on fens was explained. It is only to be regretted that "Meteorpapier" will not be able to relieve our current scarcity of paper.]

5. References[2]

Berzelius, J. (1822) *Jahres-Bericht über die Fortschritte der physischen Wissenschaften.* Von Jacob Berzelius. Aus dem Schwedischen übersezt von G. G. Gmelin. Erster Jahrgang. Tübingen, bei Heinrich Laupp.

Chapman, V.J. and Chapman, D.J. (1973) *The Algae*, 2nd ed. Macmillan, London.

Cohn, F. (1878) *Kryptogamen-Flora von Schlesien.* Im Namen der Schlesischen Gesellschaft für vaterländische Cultur herausgegeben von Prof. Dr. Ferdinand Cohn. Zweiter Band, Erste Hälfte. Algen. Bearbeitet von Dr. Oskar Kirchner. J.U. Kern's Verlag (Max Müller), Breslau.

Ehrenberg, C.G. (1838a) *Mikroskopische Analyse des curlandischen Meteorpapiers von 1686 und Erlauterung als ein Produkt jetzt lebender Conferven und Infusorien.* Ein Vortrag in der Akademie der Wissenschaften zu Berlin von 6 December 1838. Berlin, 1839.

Ehrenberg, C.G. (1838b) Ueber das im Jahre 1868 in Curland vom Himmel gefallene Meteorpapier und über dessen Zusammensetzung aus Conferven und Infusorien. Abhandlungen der Königlichen Akademie der Wissenschaften Berlin, Physikalische Klasse 44–60.

Ehrenberg, C.G. (1839) Ueber das im Jahre 1686 in Curland vom Himmel gefallene Meteorpapier. Annalen der Physik **122**: 187–188.

Fritsch, F.E. (1956) *The Structure and Reproduction of the Algae*, Vol. I. Cambridge University Press, Cambridge.

Geitler, L. (1932) *Cyanophyceae,* In: *Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz*, Vol. XIV. Leipzig: Akademische Verlag; reprint (1985). Koeltz Scientific Books, Koenigstein.

Hartmann, P.J. (1689) *Exercitatio de generatione mineralium, vegetabilium et animalium in aëre, occasione annonae et telae coelitus delapsorum, anno 1686 in Curonia.* Ephemeridibus Naturae Curiosorum. Nuremberg.

Kersten, L.M. (1839) Ueber eine auf Wiesen gebildete lederartige Substanz. Aus einem Schreiben des Hrn. Prof. L.M. Kersten. Annalen der Physik **11**: 183–186.

Kundmann, J.C. (1736) *Rariora naturae et artis, item in re medica, oder Seltenheiten der Natur und Kunst des Kundmannischen Naturalien-Cabinetts, wie auch in der Artzeney-Wissenschafft I. Examen fossilium & lapidum quorundam rariorum. II. Memorabilia naturae & artis. III. Observationes in re medica singulares.* Breslau and Leipzig, Michael Hubert, 1737.

Schlegel, H.G. (1999) *Geschichte der Mikrobiologie.* Deutsche Akademie der Naturforscher Leopoldina, Halle.

Schöder, B. (1919) Über Meteorpapier. Die Naturwissenschaften **7**: 605–608.

Siegesmund, M.A., Johansen, J.R., Karsten, U. and Friedl, T. (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J. Phycol. **44**: 1572–1585.

Stal, L.J. (2000) Cyanobacterial mats and stromatolites, In: B.A. Whitton and M. Potts (eds.) *Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer, Dordrecht, pp. 61–120.

Stockmayer, S. (1893) Ueber die Bildung des Meteorpapiers und eine aus *Microcoleus chthonoplastes* zusammengesetzte Algenhaut aus dem Inundationsgebiete der Donau bei Wien. Verhandlungen der Kaiserlichen-Königlichen Botanischen Gesellschaft **43** (2): 28–30.*

[2] Documents to which the author did not have access during the writing of this essay are marked with an asterisk.

PART 2:
MICROBIAL MATS IN THE GEOLOGICAL RECORD

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Krumbein
Eriksson
Sarkar
Banerjee
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Callow
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MICROBIAL MATS ON THE EARLY EARTH: THE ARCHEAN ROCK RECORD

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1. Introduction

Fossilized microbial mats are among the most robust records of life on the early Earth; the textural and chemical evidence of microbial colonization of surfaces that they provide is well documented in the rock record of the Archean eon (~3.8–2.5 Ga) (Schopf, 2006; Wacey, 2009; Tice and Lowe, 2006; Walsh and Lowe, 1999; Westall et al., 2006). Stromatolites, accretionary sedimentary structures produced by mat-building phototrophic communities, are perhaps the most commonly recognized macroscopic feature produced by microbes, and have been reported from several Archean locales (Hofmann et al., 1999; Schopf, 2006; Allwood et al., 2007). However, preservation of microbial mat laminae or individual cells is rare in most stromatolites, which are most commonly preserved in carbonate rocks. Diagenetic and metamorphic alteration of carbonate sediments typically result in recrystallization that precludes preservation of fine-scale structures. Furthermore, the origin of some Archean stromatolite-like structures, including the ones preserved in siliceous rocks, may be abiological (Lowe, 1994). This chapter will therefore focus on the best examples of microbial mats formed during the paleo- and meso-Archean eras (2,900–3,600 Ma), ones whose fine-scale textures are preserved in cherts, sedimentary rocks made up almost exclusively of primary or secondary silica. Note that although the term "microbialite" is often used for organosedimentary deposits resulting from microbial activity (Burne and Moore, 1987), in this discussion "fossil microbial mats" will be used because of the focus on the characteristics of the carbonaceous mat material rather than on the surrounding sedimentary matrix.

Fossil mats in Archean cherts are preserved as subparallel wavy or crinkly carbonaceous laminations that commonly make up layers 0.5–2 cm thick, separated by bands of pure microcrystalline quartz or by detrital layers. In some instances, the laminations are buckled or broken or folded over on themselves. The carbonaceous laminations are interpreted to represent the remains of microbial mats and the interlayered silica as a precipitate that was deposited on the surface, and then covered by subsequent mat growth (Schopf, 2006; Wacey, 2009; Tice and Lowe, 2006; Walsh and Lowe, 1999; Westall et al., 2006). Carbonaceous

or lithic particles may be associated with the laminations. The carbonaceous matter is usually identified microscopically by its amorphous texture and low reflectance, with verification through determinations of total organic carbon (TOC) content of representative samples. Although the TOC values are very low (generally <1%), hand samples of carbonaceous cherts typically appear black because of the colorless translucence of the microcrystalline quartz matrix. Carbon isotopic analyses signatures support the biological origin of the carbonaceous matter (Schopf, 2006; Tice and Lowe, 2006; Hofmann and Bolhar, 2007). The microbes that constructed or inhabited the mat are typically not detected; the preservation of individual cells within the laminations is rare and difficult to detect or authenticate (Altermann and Kazmierczak, 2003). However, some structures that resemble individual cells have been recognized, as will be discussed in this chapter.

2. Examples of Archean Microbial Mats

Well-preserved fossil microbial mats have been documented from two Archean complexes, the Kaapvaal Craton in southern Africa and the Pilbara Block in northwestern Australia. In general, preservation of fine-scale structures is better in the Barberton rocks (Altermann and Kazmierczak, 2003); hence, those will be described first.

2.1. KAAPVAAL CRATON

The Barberton Greenstone Belt, located in the eastern part of the Kaapvaal Craton, South Africa, has been the focus of many studies of the early Earth because of the remarkable preservation of many primary fabrics and textures. The three main lithostratigraphic units are the Onverwacht Group (>3.5–3.2 Ga), composed predominantly of volcanic rocks with interbedded sedimentary layers; the Fig Tree Group (~3.2 Ga), a volcaniclastic sedimentary sequence; and the Moodies Group (<3.2 Ga), a primarily shallow marine, quartz- and feldspar-rich sedimentary succession (Lowe and Byerly, 2007). Fossil microbial mats have been found in the Hooggenoeg and Kromberg Formations of the Onverwacht Group and in the Moodies Group. The carbonaceous nature and biologically consistent carbon isotopic signature of the mat-like features have been established through numerous studies of the rocks and, in some cases, individual fossils (Schopf, 2006; Tice and Lowe, 2006; Hofmann and Bolhar, 2007).

The Hooggenoeg Formation is a thick sequence of volcanic rocks and interbedded shallow-water sedimentary units. Black-and-white banded cherts that contain fine carbonaceous laminations resembling microbial mats are found within the sedimentary packages (Walsh and Lowe, 1999). One instance of possible microbial fossils has been reported from carbonaceous layers in a thin chert of the Hooggenoeg (Walsh, 1992). The approximately 2 cm-thick black layer is

made up of fine (~1–5 μm thick) planar carbonaceous laminations separated by thicker (~10–25 μm) laminations of pure microcrystalline quartz. The solid filaments, which have cross-sectional diameters ranging from less than 0.2 to 2.5 μm and lengths up to 200 μm, are composed of kerogen and fine pyrite grains (Walsh, 1992). Although the filamentous morphology suggests that the structures could be microbial remains, they appear solid and brittle and may have formed abiologically.

The Kromberg Formation is a 1,700 m thick sequence of komatiites and basalts with minor interbedded sedimentary rocks, including carbonaceous cherts. Fossil mats have been described from several of the chert layers (Tice and Lowe, 2006; Walsh and Lowe, 1999; Westall et al., 2006). Most of the mats are made up of flat to wavy carbonaceous laminations subparallel to bedding (Fig. 1a), but some are detached and overturned (Fig. 1b, c), suggesting disruption through current activity, possibly preceded by desiccation. An in-depth study of the Buck Reef Chert identified three distinctive mat types: (1) anastomosing laminations that drape underlying detrital grains or form silica-filled lenses, (2) meshes that encompass detrital grains and dark laminations, which loosely drape

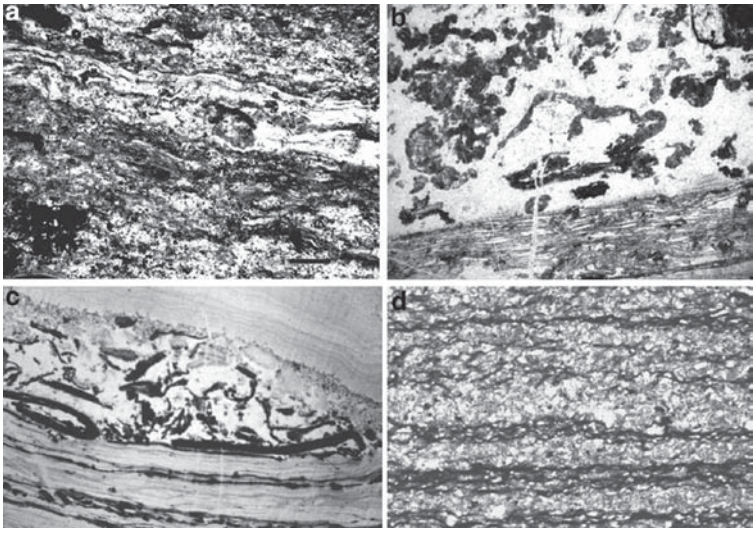

Figure 1. Photomicrographs of carbonaceous laminations in rocks of the Kaapvaal Craton, southern Africa. (**a**) Fine laminations within a fossiliferous black-and-white banded chert of the Kromberg Formation. (**b**) Rolled-up chip of mat (Tice and Lowe, 2006) in a sample from the Buck Reef Chert, Kromberg Formation (photo courtesy of Mike Tice). (**c**) Folded and overturned mat overlain by mat fragments in a sample from the Buck Reef Chert, Kromberg Formation (Walsh and Lowe, 1999). (**d**) Carbonaceous laminations in fine-grained quartz sandstone, a type of microbially induced sedimentary structure (Noffke et al., 2006), from the Moodies Group (photo courtesy of Nora Noffke). Scale bar shown in A = 200 μm in **a**, 300 μm in **b**, 200 μm in **c**, 500 μm in **d**.

coarse detrital grains, and (3) fine, even carbonaceous laminations that tightly drape the underlying detrital grains (Tice and Lowe). Sedimentological evidence indicates all of the fossil mats in the Buck Reef formed in shallow water, suggesting that they were formed by photosynthetic organisms; the differences in mat composition most likely resulted from differences in current energy, and possibly light energy (Tice and Lowe, 2006).

Filamentous structures that are more convincingly biological than those described from the Hooggenoeg Formation are found in a fossil mat in the Kromberg (Walsh and Lowe, 1985; Walsh, 1992). Most of the filaments are oriented subparallel to bedding (Fig. 2a), but some extend between layers, or radiate from a

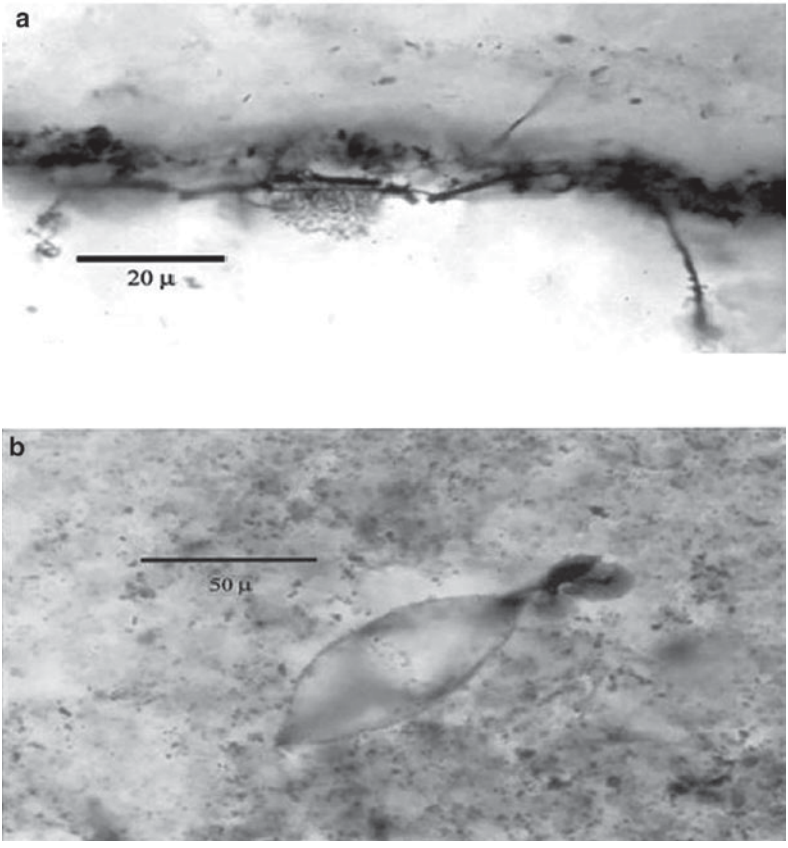

Figure 2. Photomicrographs of possible microbial fossils in the Kromberg Formation of the Kaapvaal Craton (Walsh, 1992). (**a**) Filament within fine carbonaceous lamination in black-and-white banded chert formation. (**b**) Spindle-shaped structure with hollow double core in carbonaceous chert with evaporative textures.

tangle of filaments. The filamentous microbes may represent only a portion of the original community, but seem to have had a role in the construction of the mats. The hollow cylindrical filaments, composed of carbonaceous matter with scattered very fine pyrite, range from 1.4 to 1.2 μm in diameter and 10 to 150 μm in length and solid thread-like filaments range from less than 0.2 to 2.5 μm and lengths up to 200 μm. Most are nonseptate, with a few exhibiting slight constrictions at intervals of approximately 1 μm, or breakage at intervals of several micrometers.

Within other fossil mats in the Kromberg Formation are rare occurrences of other structures that may be of biological origin (Walsh, 1992). Rod-shaped and spheroidal structures whose simple shapes preclude definitive identification as fossils have been described from several locations (Schopf, 2006). Diaphanous spindle-shaped structures ranging from 13 to 135 μm in longest dimension and containing spheroidal cores (Fig. 2b) exhibit a higher level of complexity. They may be the remains of resistant envelopes that surrounded microbial colonies or spores. Similar structures have been reported from the Pilbara (Sugitani et al., 2007), as will be discussed next. However, the association of the spindles with evaporative textures suggests that the structures might be coatings of carbonaceous matter around crystal ghosts of lenticular gypsum or silica-filled structures similar to "birds-eye" fenestrae, which form in evaporitic sediments.

Evidence of microbial mats has been described in fine-grained sandstones in the Moodies Formation (Noffke et al., 2006; Noffke, 2008). The microbially induced sedimentary structures (MISS) include wavy laminations (Fig. 1d), rolled-up packages of carbonaceous laminations, wrinkled surfaces, and desiccation cracks. The features are comparable with structures formed in modern tidal sand deposits in which microbial mat growth on bottom sediments is interrupted by exposure and erosion of the mats, as well as deposition of fine sand. Although the subtlety of the MISS has caused them to be overlooked or dismissed, several lines of reasoning support their biological origin, including their carbonaceous composition, carbon isotopic values, and their exclusive association with very fine-grains, which characterizes modern occurrences of shallow-water mats in quartzose tidal deposits (Noffke et al., 2006; Noffke, 2008).

2.2. PILBARA CRATON

The Pilbara Craton in northwestern Australia contains numerous volcanosedimentary sequences comparable in age and origin to those in the Kaapval Craton. There have been several reports of microbial fossils in carbonaceous cherts in several of the terranes (Schopf, 2006; Wacey, 2009). This discussion will focus only on well-documented examples of microbial mat structures.

The Dixon Island Formation (3.2 Ga) in the Cleaverville Group of the Coastal Pilbara terrane is an approximately 350 m sequence of rhytolitic tuff and cherts. Within a laminated black chert bed are 5–20 cm thick packages of planar, wavy carbonaceous material interpreted to represent "biomats," possibly of

cyanobacterial origin (Kiyokawa et al., 2006). The environment of deposition is hypothesized to be hydrothermal, at a depth of 500–2,000 m. Total organic carbon analyses support the organic nature of the dark material, and carbon isotope values (−27‰ to ~ −13‰ $\delta^{13}C$) are consistent with the biological origin of the material (Kiyokawa et al., 2006). Several types of possible microbial fossils are found within the carbonaceous cherts, including spirals, rods, spheroids, and stalked filaments. Cross-sectional diameters of the structures range from 1 to 20 μm. Some of the filamentous structures are associated with the mat-like layers, commonly extending upward from swirled laminations (Kiyokawa et al., 2006).

Younger (>2.97 Ga) example of fossil microbial mats are present in the Farrel Quartzite of the Gorge Creek Group of the Pilbara Craton (Sugitani et al., 2007). The formation is made up predominantly of quartz-rich sandstone with interbedded conglomerates, mafic tuff, and thin black chert layers. Sedimentary features and stratigraphic relationships indicate that the cherts are sedimentary, deposited in a shallow basin subject to partial evaporation. Within the black cherts are the fossil mats – flat fine carbonaceous laminations with associated detrital carbonaceous particles, very similar to those in the Hooggenoeg and Kromberg Formations (Fig. 1a–c; cf. Fig. 4d in Sugitani et al., 2007). Higher in the sequence, in cherts lacking distinct laminations, but rather made up of diffuse carbonaceous matter in a microcrystalline quartz matrix, are possible microfossils of diverse morphologies: thread-like, film-like, spheroidal, and spindle-like shapes (Sugitani et al., 2007). The film-like structures might represent fragments of eroded and transported mats (Sugitani et al., 2007). The thread-like and spheroidal structures, like similar structures reported from Archean rocks, morphologically resemble bacterial or cyanobacterial fossils, but their simple shape, which could be produced abiologically, precludes a definitive interpretation. The spindle-like structures, which closely resemble possible microfossils reported from the Kromberg Formation (Fig. 2b), are not easily explained by invoking abiological processes, as their orientation and distribution are not consistent with the formation by gas bubbles or as accumulations along mineral boundaries (Sugitani et al., 2007).

3. Discussion

The biogenicity of Archean microstructures continues to be debated (Brasier et al., 2002; Hofmann, 2004; Garcia-Ruiz et al., 2009), with the origin of the carbonaceous matter often central to the debate. The production and distribution of organic material by hydrothermal processes has been suggested as the dominant scenario in the Archean (Brasier et al., 2002). The use of petrographic and geochemical evidence to document fossil microbial mats is an approach that can supply additional information on the biogenicity and environment of formation of some microstructures. It should be emphasized, however, that the lack of microbial mat-like features does not rule out a biological origin; not all microbes would be expected to inhabit or be preserved within mats.

The common characteristics of microbial mats in the rock record include (1) layers of flat or wavy fine (less than 1 mm, but commonly as fine as a few micrometers) laminations, (2) carbonaceous composition, usually determined both through reflected light microscopy and total organic carbon analyses, and (3) carbon isotopic signatures consistent with biological production of the carbonaceous matter. Individual microbes are rarely preserved, but when present, add further evidence for the biological origins of the carbonaceous laminations.

Expanded approaches and emerging analytical techniques show promise for more rigorous scrutiny of both possible microfossils and microbial mat-like features. One of the steps in studying possible fossils or mats should be understanding the geological context, i.e. the tectonic and sedimentary environment in which the samples in question formed (Ohmoto et al., 2008); microscopic and chemical analysis of rocks done in isolation may lead to misinterpretations that become apparent when the larger setting is viewed. The recognition of features of mats and microbes that result from life functions and postmortem degradation, including their cell division patterns, microbial community structure, lysed cells, and pigmentation gradients, may prove invaluable in identifying fossil communities (Hofmann, 2004). The use of a multipronged analytical approach that includes new high-resolution techniques can provide complementary morphological and chemical information that can further refine the discrimination between biological and abiological signals (Glikson et al., 2008; Ohmoto et al., 2008). Laser Raman spectroscopy, nano-SIMS (secondary ion mass spectrometry), high-resolution transmission electron microscopy, and confocal laser scanning microscopy have been used successfully in characterizing microstructures and their surroundings (Oehler et al., 2006; Glikson et al., 2008; Ohmoto et al., 2008).

4. Conclusions

The recognition of fossil microbial mats contributes to our understanding of life on the Archean Earth as well as our ability to recognize biosignatures elsewhere in the universe. Fossil mats provide a microenvironmental context for individual microbial fossils and a record of microbial activity in the absence of such structures. Examining the mats with a combination of traditional petrographic and chemical techniques and new high-resolution analyses will result in a more confident and detailed understanding of the record of life on Earth.

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6. References

Allwood, A.C., Walter, M.R., Burch, I.W. and Kamber, B.S. (2007) 3.43 billion-year-old stromatolite reef from the Pilbara Craton of Western Australia: ecosystem-scale insights to early life on Earth. Precambrian Res. **158**: 198–227.

Altermann, W. and Kazmierczak, J. (2003) Archean microfossils: a reappraisal of early life on Earth. Res. Microbiol. **154**: 611–617.

Brasier, M.D., Green, O.W., Jephcoat, A.P., Kleppe, A.K., van Kranendonk, M.J., Lindsay, J.F., Steele, A. and Grassineau, N.V. (2002) Questioning the evidence for Earth's oldest fossils. Nature **247**: 76–81.

Burne, R.V. and Moore. L.S. (1987) Microbialites: organosedimentary deposits of benthic microbial communities. Palaios **2**: 241–254.

Garcia-Ruiz, J.M., Melero-Garcia, E. and Hyde, S.T. (2009) Morphogenesis of self-assembled nanocrystalline materials of barium carbonate and silica. Science **323**: 362–365.

Glikson, M., Duck, L.J., Golding, S.D., Hofmann, A., Bolhar, R., Webb, R., Baiano, J.C.F. and Sly, L.I. (2008) Microbial remains in some earliest Earth rocks: comparison with a potential modern analogue. Precambrian Res. **164**: 187–200.

Hofmann, H.J. (2004) Archean microfossils and abiomorphs. Astrobiology **4**: 135–136.

Hofmann, A. and Bolhar, R. (2007) Carbonaceous cherts in the Barberton Greenstone Belt and their significance for the study of early life in the Archean record. Astrobiology **7**: 355–388.

Hofmann, H.J., Grey, K., Hickman, A.H. and Thorpe. R.I. (1999) Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. GSA Bull. **111**: 1256–1262.

Kiyokawa, S., Ito, T., Ikehara, M. and Kitajima. F. (2006) Middle Archean volcano-hydrothermal sequence: bacterial microfossil-bearing 3.2 Ga Dixon Island Formation, coastal Pilbara terrane, Australia. Geol. Soc. Am. Bull. **118**: 3–22.

Lowe, D.R. (1994) Abiotic origin of described stromatolites older than 3.2 Ga. Geology **22**: 387–390.

Lowe, D.R. and Byerly, G.R. (2007) An overview of the geology of the Barberton Greenstone Belt and vicinity: implications for early crustal development, In: H.S. Martin, J. van Kranendonk and V.C. Bennett (eds.) *Earth's Oldest Rocks*. Elsevier, New York, pp. 481–526.

Noffke, N. (2008) Turbulent lifestyle: microbial mats on Earth's sandy beaches – today and 3 billion years ago. GSA Today **18**: 4–9.

Noffke, N., Eriksson, K.A., Hazen, R.M. and Simpson, E.L. (2006) A new window into Early Archean life: microbial mats in Earth's oldest siliciclastic tidal deposits (3.2 Ga Moodiess Group, South Africa). Geology **34**: 253–256.

Oehler, D.Z., Robert, F., Mostefaoui, S., Meibom, A., Selo, M. and McKay, D.S. (2006) Chemical mapping of Proterozoic organic matter at submicron spatial resolution. Astrobiology **6**: 838–850.

Ohmoto, H., Runnegar, B., Kump, L.R., Fogel, M.L., Kamber, B., Anbar, A.D., Knauth, P.L., Lowe, D.R., Sumner, D.Y. and Watanabe, Y. (2008) Biosignatures in ancient rocks: a summary of discussions at a field workshop on biosignatures in ancient rocks. Astrobiology **6**: 883–907.

Schopf, J.W. (2006) Fossil evidence of Archaean life. Philos. Trans. R. Soc. Lond. B Biol. Sci. **361**: 869–885.

Sugitani, K., Grey, K., Allwood, A., Nagaoka, T., Mimura, K., Minami, M., Marshall, C.P., Van Kranendonk, M.J. and Walter, M.R. (2007) Diverse microstructures from Archaean chert from the Mount Goldsworthy-Mount Grant area, Pilbara Craton, Western Australia: microfossils, dubiofossils, or pseudofossils? Precambrian Res. **158**: 228–262.

Tice, M.M. and Lowe, D.R. (2006) The origin of carbonaceous matter in pre-3.0 Ga greenstone terrains: a review and new evidence from the 3.42 Ga Buck Reef Chert. Earth-Sci. Rev. **76**: 259–300.

Wacey, D. (2009) *Early Life on Earth: A Practical Guide.* Springer, Dordrecht, 274 pp.

Walsh, M. (1992) Microfossils and possible microfossils from the Early Archean Onverwacht Group, Barberton Mountain Land, South Africa. Precambrian Res. **54**: 271–293.

Walsh, M.M. and Lowe, D.R. (1985) Filamentous microfossils from the 3,500-Myr-old Onverwacht Group, Baberton Mountain Land, South Africa. Nature (London) **314**: 530–532.

Walsh, M.M. and Lowe, D.R. (1999) Modes of accumulation of carbonaceous matter in the Early Archean: a petrographic and geochemical study of the carbonaceous cherts of the Swaziland Supergroup, In: D.R. Lowe and G. Byerly (eds.) *Geologic Evolution of the Barberton Greenstone Belt, South Africa.* Geological Society of America, Boulder, CO, pp. 115–132.

Westall, F., de Vries, S.T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesisi, D. and Severine, A. (2006) The 3.466 Ga "Kitty's Gap Chert," an Early Archean microbial ecosystem, In: *Processes on Early Earth.* Geological Society of America Special Paper 405. Boulder, CO, pp. 105–131.

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GUNFLINT CHERT MICROBIOTA REVISITED – NEITHER STROMATOLITES, NOR CYANOBACTERIA

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1. Introduction

The Gunflint Chert in Canada is the earliest site of Precambrian fossils and biofilms or microbial mats recorded in literature. It consists of several sequences of banded iron formations ranging from Northern Minnesota and western Ontario stretching along the northern shore of Lake Superior. Particularly, the black jasperite layers in the sequence contain numerous microfossils and ooids. The best age determination so far hints at 1.88 Ga. Layers are rich in iron oxide. The mineral phase is hematite. Also, magnetite and ilmenite occur, perhaps also schwertmannite. The iron oxide layers alternate with mainly silica layers into which iron oxide encrusted ooids and debris of a rich growth of microorganisms are washed in most probably by silica-rich hot brines. The first Precambrian microfossil sequence was published by an economic geologist and the then-appointed paleobiologist at Harvard (Tyler and Barghoorn, 1954). The finding was not recognized as revolutionary and forgotten by most earth scientists. It took another 10–15 years until new dating techniques based on several radioactive decay products and improved electron microscopy (SEM and TEM) made it clear that living matter influenced earth's geochemistry much earlier than expected before (Barghoorn and Tyler, 1965). Most of the original papers on the Gunflint Chert were published by former students of E.S. Barghoorn. Later P.E. Cloud and collaborators got interested in the materials (Cloud, 1965). Knoll and Barghoorn (1976) published on similar deposits in Australia: 'Two billion year old black chert lenses from the Duck Creek formation, Northwestern Australia, contain abundant organically preserved microorganisms which are morphologically similar to fossils of approximately the same age from the Gunflint formation, Ontario. Entities include: a relatively small (5–15 μm) coccoid taxon morphologically comparable to *Huroniospora* Barghoorn, a larger coccoid form comparable to an apparently planktonic alga from the Gunflint, *Gunflintia* Barghoorn, and *Eoastrion* Barghoorn (*Metallogenium* Perfil'ev). Gunflint-type assemblages had a wide geographic distribution in middle Precambrian times, and these assemblages may eventually prove useful as biostratigraphic indices'. This statement includes fungi, if properly analyzed and compared to the fact that many fungi also exhibit anaerobic metabolism (Nursall, 1959).

Also, Knoll and Barghoorn (1976) and Knoll and Simonson (1981) were debating the stromatolite theory, for some of the penecontemporaneous quartz cementations in Canada and Australia as not really stromatolitic microbial mat- or reef-like constructions. They regard them rather as debris accumulations.

The original microfossil descriptions included bacterial, algal (cyanobacterial) and fungal origin of the structures (Tyler and Barghoorn, 1954; Barghoorn and Tyler, 1965). Only later, the wave of literature on stromatolites and their mostly cyanobacterial origin as well as photosynthetic anoxygenic and oxygenic cyanobacteria (Krumbein and Cohen, 1974) helped to interpret these mostly as cyanobacterial structures. However, Krumbein (1983) hinted at the fact that stromatolitic structures may be produced by a variety of organisms, including fungi and even molluscs. Further, Knoll (2004) correctly interpreted the Gunflint Cherts as non-stromatolitic assemblages of organism debris. His detailed studies of planktic and benthic influences on ancient ecosystems helped a lot in separating different fossiliferous beds of the late Precambrian and early Proterozoic. Gunflint Chert microbiota, however, remained enigmatic.

2. Material and Methods

2.1. GUNFLINT

The main sampling sites of fossiliferous material were near Gunflint Lake and Kakabeka Falls in the surroundings of the city of Schreiber (Fig. 1). All fossils are embedded in chert and cannot be any recent contaminants. The author used the

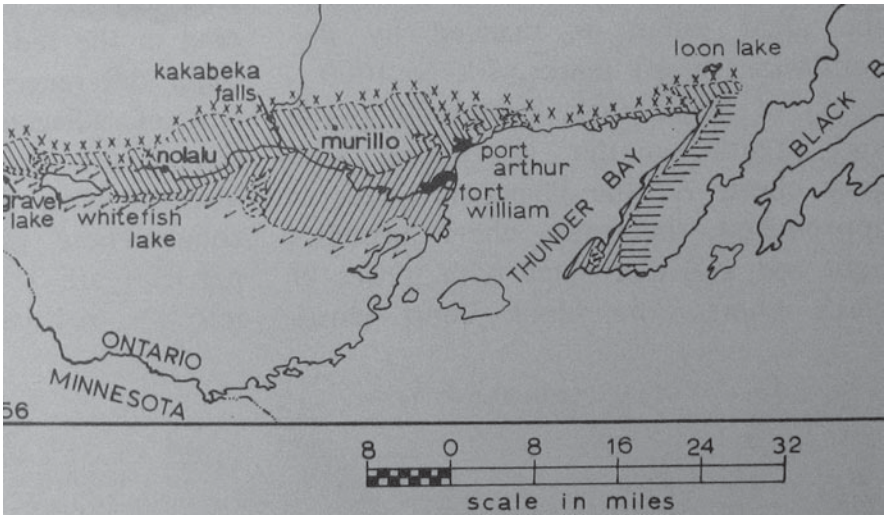

Figure 1. The Gunflint Range, Ontario, Canada. (After Barghoorn and Tyler, 1965.)

thin sections of the collection of E.S. Barghoorn during a sabbatical at Harvard. Work was done and discussed in his office and discussed with him during the last half year of his life. Figures 2–4 shall remind us of these early publications (Tyler and Barghoorn, 1954; Barghoorn and Tyler, 1965).

2.2. FRENCH/SWISS JURASSIC AND WARSTEIN TERTIARY

Jurassic material was collected by Kapila Dahanayake (Dahanayake et al., 1985; Dahanayake and Krumbein, 1986). It consisted mainly of laminated iron ores with inter-bedded pockets of ooids and onkoids. The French material was extensively analysed and will not be demonstrated here. Tertiary samples were collected by Kretzschmar in Tertiary Karst incised into Devonian limestone beds. We received samples, sub-samples, thin sections and material for scanning electron microscopy from him and compared them with own chert materials. Materials were analyzed and published jointly in discussion with Kretzschmar (Dexter-Dyer et al., 1984).

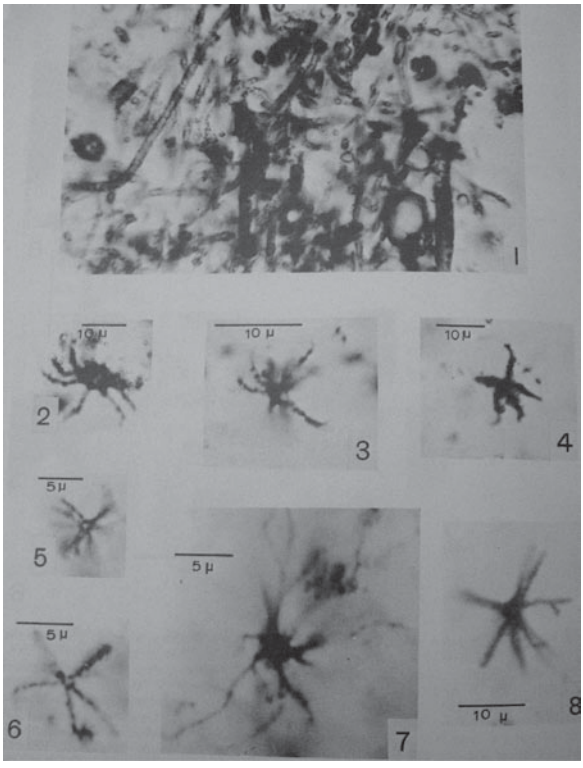

Figure 2. Gunflint microbiota showing the irregular mineralization pattern and many filaments with partial preservation. Numbers 2–8 depict the fine threads very typical for Gunflint and also tertiary fungi. (From the original paper by Barghoorn and Tyler, 1965.)

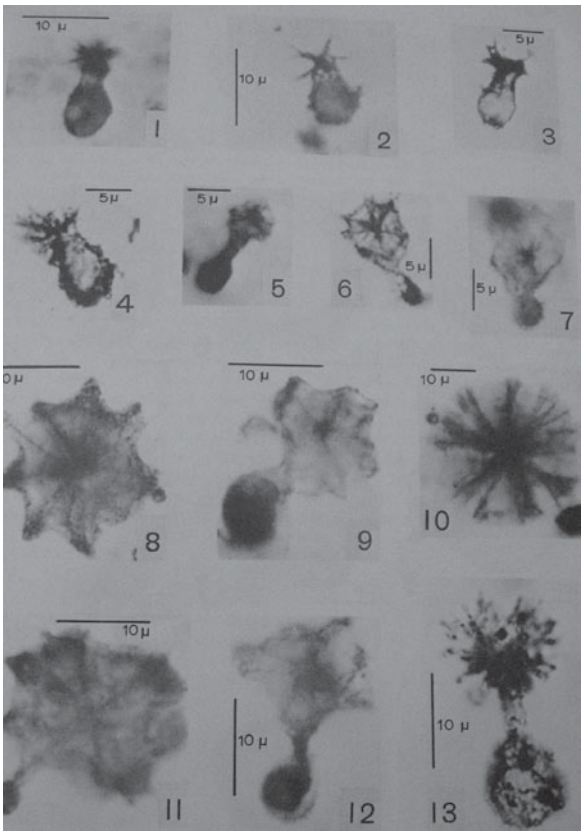

Figure 3. Gunflint microbiota. Number 10 clearly shows the same pattern of *Eoastrion* and *Kakabekia* as the '*Metallogenium symbioticum*' in recent cultures of Dubinina and Krumbein. (Barghoorn and Tyler, 1965.)

2.3. CULTURES

The Geomicrobiology Laboratory of ICBM, University of Oldenburg, isolated cultures of iron and manganese fungi from several different environments, including terrestrial sub-aerial and sub-aquatic biofilms, intertidal sub-aquatic biofilms and samples from the mangrove of the Gulf of Aqaba near Nabq (Egypt). Some of the fungi isolated, exhibited features of the debated microorganism *Metallogenium symbioticum* (Zavarzin, Dubinina). Cultivation was done using a variety of methods, and the techniques were published extensively (Gorbushina, 2007).

Light microscopy, polarizing microscopy, scanning and transmission electron microscopy were used to document morphotypes and morphologies of fossil and recent materials.

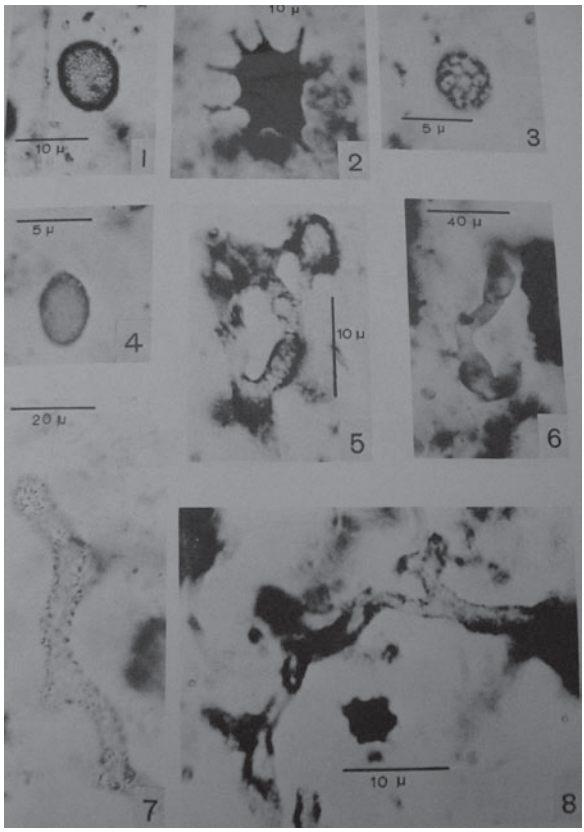

Figure 4. Gunflint biota with clearly branching structures not unlike tertiary and recent iron depositing fungal cultures from Barghoorn and Tyler (1965). (Refer to Tertiary and culture experiment figures.)

3. Results and Discussion

There are four different sets of information concerning taxonomic relationships of living, fossil and extinguished species. In many cases, it is still extremely difficult to judge the validity of each single set of information. Even worse, when using more and more detailed information, it becomes impossible to define new species and even genera without breaking long established rules. One major example is the case of actinobacteria, where coryneform bacteria, *Geodermatophilus* and *Frankiaceae*, could be merged or separated according to the set of information used (Eppard et al., 1996). These four sets are (1) phenotype (mainly morphology based on pure cultures or single specimen), (2) genotype (based on full or partial sequencing of DNA and RNA), (3) expression of genotype in the proteome (qualitative and quantitative expression of proteins within the specimen in question at a certain moment in its life history), and (4) ecophene (morphology changing under changing environmental conditions and pressure). A fifth set of information

is not related to these data. It is the question of ancientness, i.e., since when is a certain line of information based on the four sets mentioned before documented in earth history.

Doolittle (1980 and later) has expressed this in most elegant wording: 'Each of these former views can now be seriously challenged. Although none of the points I make below is uniquely mine, none can be taken as proven, some remain highly controversial, they deserve to be considered together because together they represent a radical revision of the way in which we think about cellular evolution'. In Fig. 2 of his review, Doolittle (1980) positions the separation line between archaebacteria, eubacteria, chloroplasts, mitochondria and nucleus (the latter three being clearly indicative of eukaryotes including fungi) with a question mark labelled 4–3 billion years BP. At 2 billion years before present, the lineages according to him were already clearly separate. Nothing has changed in the past 25 years, which would contradict the statement of Doolittle (1980) and Doolittle et al. (1996). The whole question prokaryotic/eukaryotic additionally seems simplistic and obsolete in view of the enormous power of symbiotic cooperation and continuous gene exchange of living cells. All eukaryotes are chimaeras (Dyer and Obar, 1985; Margulis et al., 2009).

In this contribution, the case of a miserable and unwanted kingdom is advocated: the kingdom of fungi. Fungal taxonomy, fungal paleontology and evolution seem a topic far below the attraction of anoxygenic and oxygenic phototrophs, dinosaurs, birds and plants. Only medical scientists love fungi, because they cause awful and insisting diseases such as sportive foot infections. Fungi may even finally kill patients suffering from syphilis/AIDS autoimmune deficiencies (Margulis et al., 2009). It is the intention of this chapter to provoke a change in attitude versus fungal evolution and fungal micropaleontology. Both have been neglected in previous studies.

In fungi, what is preserved in the fossil record may be compared with the peak of the iceberg or the butterfly wing changing global climate. Fungi are hidden and often leave behind less than 1% of their living extension as fossil material. Thus, what is left in the fossil record is open to erroneous interpretation. Many cases have been reported, in which communities and single organisms were rapidly eliminated from a growing biofilm or microbial mat (Jones and Renaut, 2007; Krumbein and Cohen, 1974; Krumbein, 2008). This implies that fungi thriving in any ecosystem may be not at all fossilized or if so only in an extremely fragmentary way.

On the background of the notion that the establishment of three of the five kingdoms, namely Prokaryotes, Protoctists and Fungi, took place in the Precambrian at least in the late Proterozoic (2 billion years BP) and was followed by the emergence of the Plant and Animal kingdoms 700 million years ago (Ediacara), we wish to re-examine the Gunflint Chert microbiota, which were the first Precambrian microbial remains presented to the scientific community (Tyler and Barghoorn, 1954; Barghoorn and Tyler, 1965).

Using optical microscopy on examples of thin sections from Precambrium (Gunflint Chert) Jurassic Minette-type iron-rich carbonate beds, tertiary chert

beds and modern laboratory cultures, the notion is established that fungi may have been overlooked and ignored until the beautiful examples of the Devonian Rhynie Chert were documented (Hass et al., 1994; Remy et al., 1994; Taylor et al., 1999). Figures 5–15 give an analogue to what we seek to imply. Selectively mineralized parts of fungal mycelia and typical propagation structures as well as

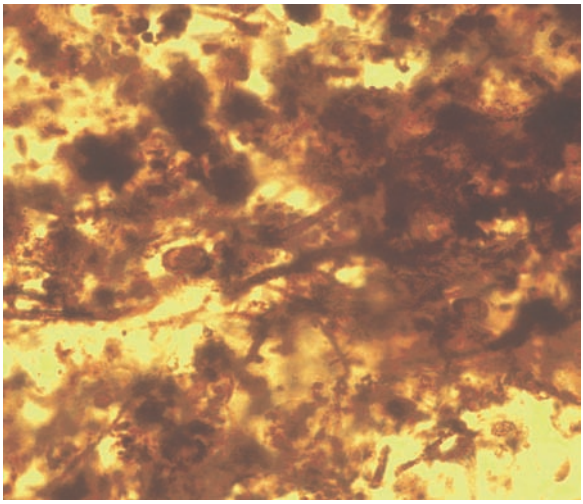

Figure 5. Gunflint filamentous structures. Thin and thick opaque filaments vary, little or no branching visible nodules and blisters are filled in. Only mineralised filaments are visible (or preserved) (Photo Krumbein).

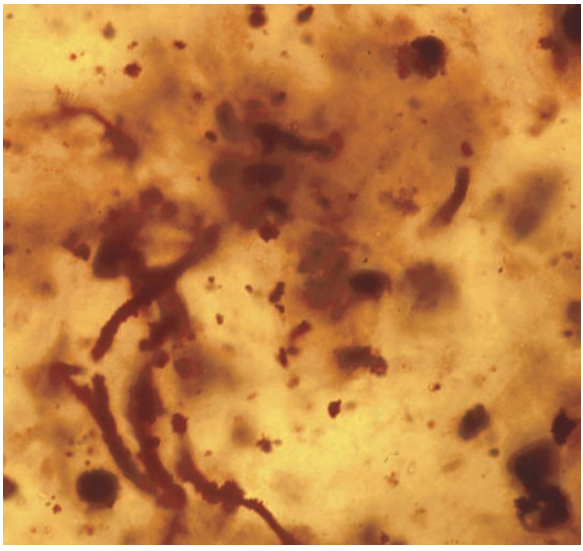

Figure 6. Gunflint selective fossilisation of partially branching filaments, including *Metallogenium*-like radiating structures at some hyphae. Sclerotia-like round structures evenly distributed (Photo Krumbein).

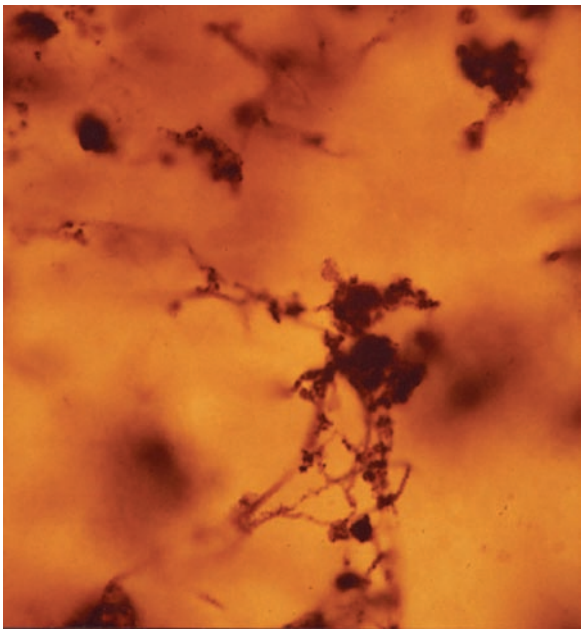

Figure 7. Gunflint filamentous network with typical fungal fine threads and sclerotia-like structures (Photo Krumbein).

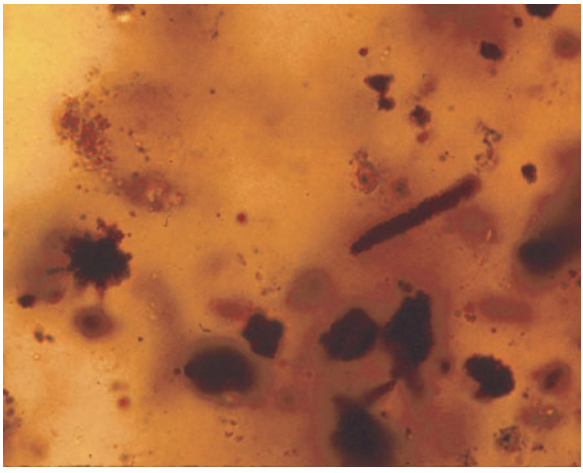

Figure 8. Gunflint filaments and *Eoastreon* (Compare with Figs. 2–4, 11, 12 and 15 for similarity [Photo Krumbein]).

'fractal structures' may explain why fungi cannot be unequivocally identified under certain fossilized circumstances. Much more material of this kind has been published. The most astonishing fact is that most of the so-called moss-agates have fungal mycelia embedded, i.e., decay structures by cave fungi embedded in silica exhalations (Walter and Reissmann, 1994). Fungi often are fossilized only in those parts, which mineralize! It is not necessary to discuss biomineralization

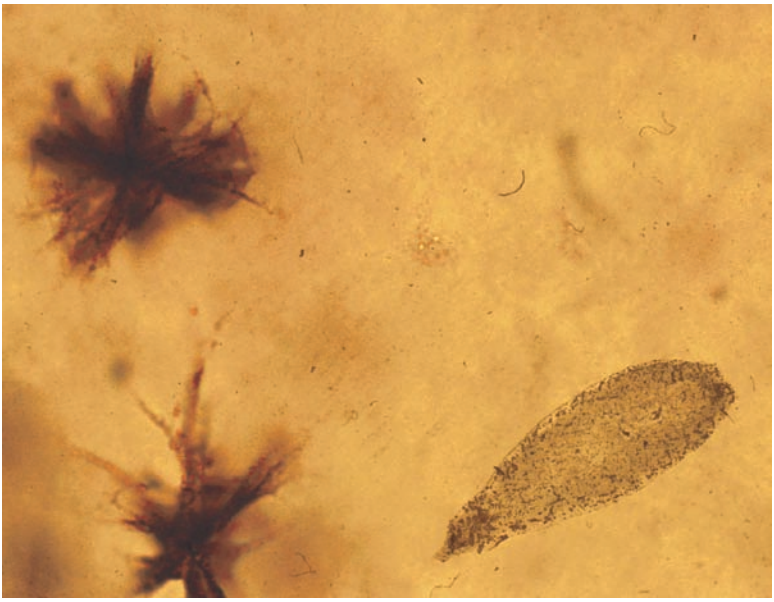

Figure 9. Gunflint *Eoastrion* and surfacing bubble as in recent culture cf. Figs. 11, 14 and 15 (Photo Krumbein).

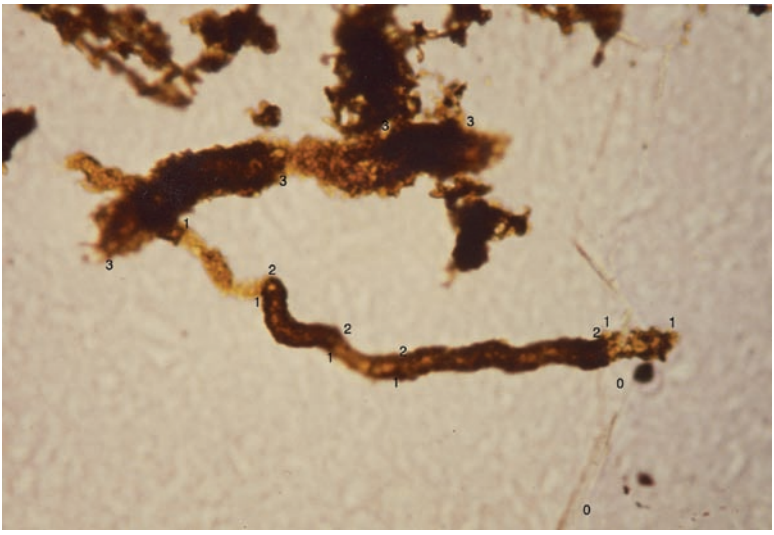

Figure 10. Tertiary selective fossilization. 0–0 is no mineralization; 1–1 is light mineralization; 2–2 is strong mineralization; 3–3 is heavy mineralization of filaments. Branching parts are not fossilized.

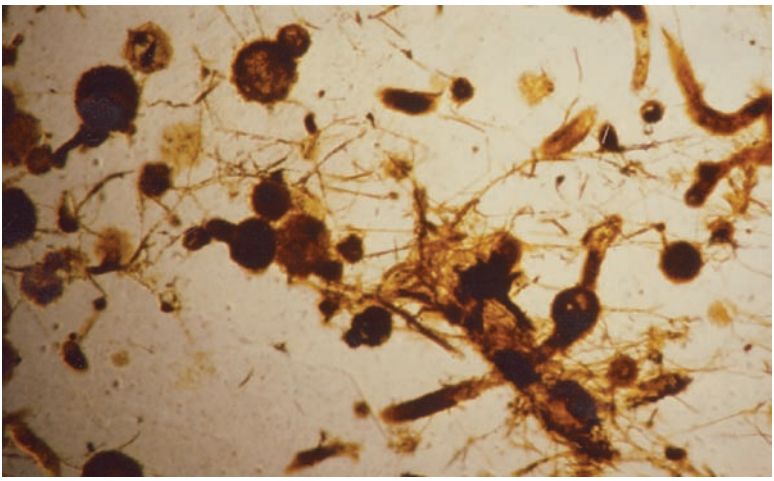

Figure 11. Tertiary selective fossilization, blebs and fine threads; Sclerotia-like round bodies; fine branching threads as in Fig. 8 (Gunflint).

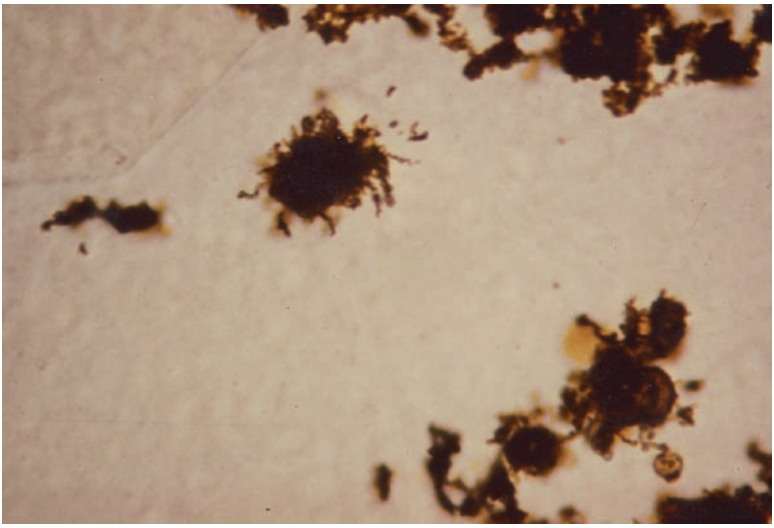

Figure 12. Tertiary *Metallogenium*/*Eoastrion* and sclerotia-like structures.

principles here (Jones and Renaut, 2007). Also, the definition of a stromatolite can be taken elsewhere (e.g. Walter, 1976; Krumbein, 1985). We will not discuss the many possible ways of creating horizontally and spherically laminated structures (Krumbein, 1983; Dahanayake et al., 1985; Brehm et al., 2006). Evidence is sought

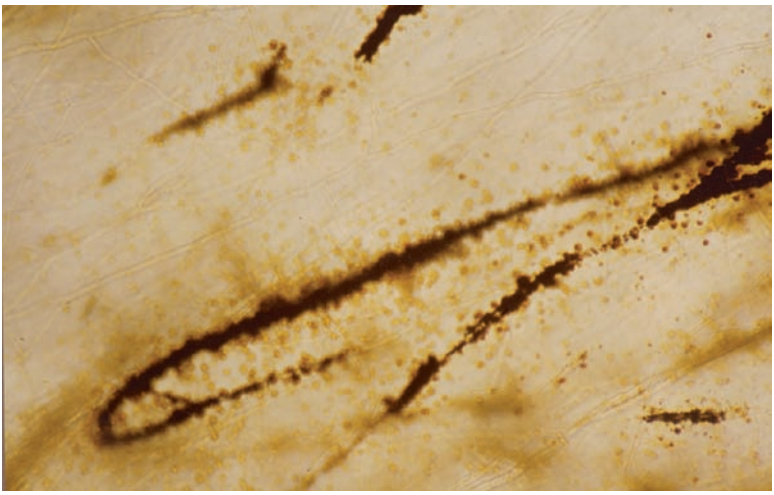

Figure 13. Culture of iron oxidising fungus with typical selective fossilization. Ninety-nine percent of the hyphae are not mineralized and will never get fossilized (Compare to Fig. 11 (Photo Krumbein)).

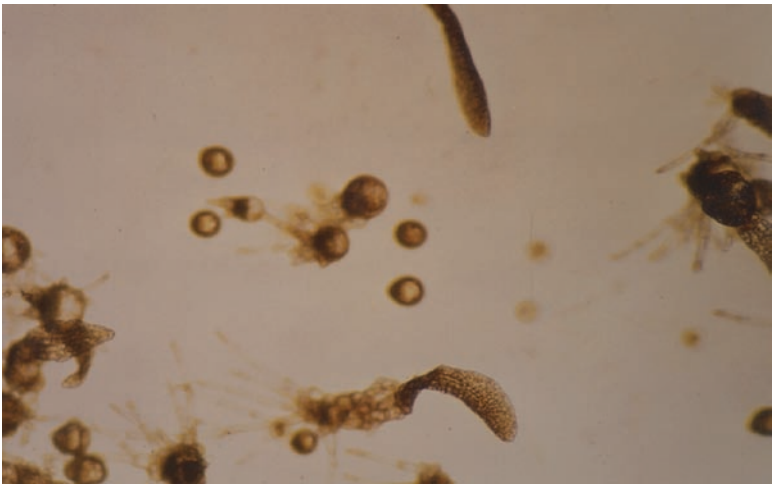

Figure 14. Culture cluster mineralization (Compare to Figs. 8, 10 and 12. Cultures of fungi in semi-liquid media like agar-agar produce similar structures as in the concentrated silica gel solutions embedding Precambrian microbes).

and presented that the first example of a microbial community fossilized in very early periods of earth history, namely the Gunflint Chert microbiota were actually fungi and not cyanobacteria or other ominous microbes (Tyler and Barghoorn, 1954).

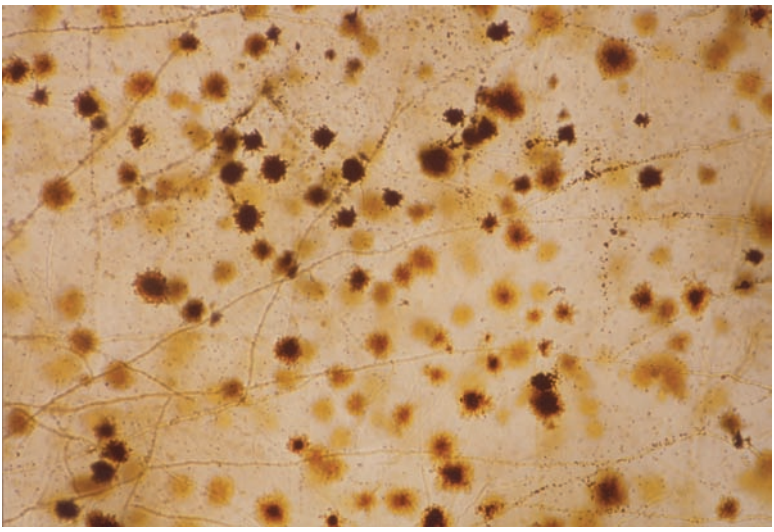

Figure 15. Iron depositing fungus culture exhibiting *Metallogenium symbioticum*/*Eoastrion*-like structures, including *Kakabekia*/*Archaeorestis schreiberensis* characteristics (Barghoorn and Tyler, 1965). All figures are taken at the same magnification. Fungal filaments vary between 1.5 μm and 6 μm, in some heavily encrusted hyphae even more. Only Fig. 15 is presented at 60% lower magnification than Figs. 5–14.

The evidence is circumstantial. It is worth, however, to show the similarities between living and fossilized acidic saprophyte decay environments through earth history from recent back to the Precambrian. Also, Walter and Reissmann (1994) and Walsh and Lowe (1999) have shown structures in cherty quartz preservation, which are strikingly similar to younger and still debatable fungal remains.

Figures 1–4 were the overture of the scenario. The authors at that time used all words available: bacteria, algae, blue-green algae, fungi, etc. (Tyler and Barghoorn, 1954). Figures 5–15 demonstrate the striking similarities of all Gunflint structures to fungal fossil structures throughout earth history and in laboratory cultures. No better evidence can be given. Conclusions have to be taken by the reader. Jones et al. (2000) stated: 'When stromatolites from acidic thermal waters are compared with those from neutral and alkali waters, significant differences in their biota and mineralogy are evident. The biota in stromatolites from neutral and alkaline waters are dominated by prokaryotes (including cyanobacteria), whereas stromatolites from acidic waters are dominated by eukaryotic fungi and to a lesser extent, diatoms' (Krumbein and Werner, 1983; Konhauser et al., 2004). Stromatolites in neutral and alkaline thermal waters are formed almost entirely of opaline silica, with calcite laminae present in a few localities. Although stromatolites in the acidic systems also are composed mainly of opaline silica, they contain substantial amounts of kaolinite and, locally, sulfur and/or jarosite.

In ancient thermal deposits, it may be possible to distinguish stromatolites that grew in acidic waters from those that formed in neutral and alkaline systems by considering their preserved biota and mineralogy (Jones et al., 2000). Thus, we conclude that acidic stromatolitic and oolitic environments are dominated by fungi (and usually even more ephemeric diatoms), while neutral to alkaline environments show prevalence of phototrophic cyanobacteria and algae.

Besides ecophene and morphotype (phenotype), genotype also has to be considered in scrutinizing ancient ecosystems. Recently, several different groups have tried to construct evolutionary trees on the basis of arbitrary distance calculations of important organisms and phyla (Redecker et al., 2000; Kollman and Doolittle, 2000; Heckman et al., 2001; Lutzoni et al., 2001, 2004; Redecker, 2002; Stechmann and Cavalier-Smith, 2002; Bruns, 2006; Embley and Martin, 2006; James et al., 2006; Taylor and Berbee, 2006). None of these analysts come closer to correct conclusions than previous statements by Doolittle (1980) and Doolittle et al. (1996), claiming that the three main lines of prokaryotes and eukaryotes have emerged already between 3 and 2 Ga before present.

At this point, one may get puzzled about the restricted view on photosynthetic microbial mats as the main stromatolite builders in the literature of the past 40 years since the book of Malcolm Walter on stromatolites was published (Walter, 1976). In that book, the key word fungus does not occur! Krumbein (1983) and Gerdes and Krumbein (1987) have tried to attract attention to different microbial mat communities and their intimate relationship with oolites. Also, Krumbein et al. (2003) have tried in several ways to introduce new views and fresh arguments into the streamlined discussion of stromatolites being generated by cyanobacteria and microbial mats decaying and creating decay systems of many different kinds, which should not be confused with true stromatolite growth. Fungi are thought to be mainly terrestrial organisms and the predecessors of all higher life on rock and soil under atmospheric conditions.

Gorbushina (2007) has reviewed sub-aerial biofilms/microbial mats as contrasted to sub-aquatic ones, wherein fungi are also regarded as the forerunners and indispensable symbiotic partners of lichens and plants (Retallack, 1994). The view that a lichen thallus represents a symbiosis of a fungus and a phototroph microorganism is trivial. The view that practically all plants are a symbiosis of a fungus and a phototroph microorganism is still lacking attention. In this context, we regard the Gunflint Chert as a terrestrial system, perhaps intertidal, similar to present-day mangrove forests on a lower morphological level. It is certainly not a reef-like or stromatolitic build-up (Aufwuchs-community sensu Gerdes and Krumbein, 1987) rather a saprophytic decay system of cyanobacterial plankton blooms in shallow slightly acid fresh water or brackish water on large continental often submerged plains near a Precambrian Ocean, stranding its products on extended beaches and being mineralized at this place of wave wash by early fungi.

We claim: The Gunflint microbiota in contrast to many ‘true’ phototrophic stromatolites is a saprophytic decay environment at the borderline to the conquering of land represented by terrestrial or sub-aerial biofilm/microbial mat biota

composed mainly of fungal remains (Gerdes and Krumbein, 1987; Gorbushina, 2007; Krumbein et al., 2003).

All figures in this chapter and many other examples through earth history document that fungi and fungal complex growth, propagation and mineralization patterns have been underestimated or even left unnoticed in paleontological literature as well as in the literature on stromatolites and stromatolitic environments. Only recently, the two different sets of information receive more attention.

4. Conclusions

Eukaryotes with fungal (mycorrhiza) characteristics may have emerged in the late Precambrian.

Fossilized fungi often exhibit patchy or restricted fossilization patterns with few exceptions, e.g., Rhynie Chert.

The Gunflint microbiota morphologically may be described as fungi if compared with other chert inclusions in agate druses, sinter caves and culture experiments.

Gunflintia minuta-like and *Kakabekia umbellata*-like structures are detected in many silicified saprophytic environments and in cultures of iron and manganese trapping or depositing fungi.

A major criticism may be oxygen and its rise via switch from anoxygenic to oxygenic photosynthesis in cyanobacteria (Krumbein and Cohen, 1974). However, many eukaryotic microorganisms and metazoans are anaerobic or can live as saprophytes in oxygen poor environments, hereby fitting also to the Gunflint period.

On the basis of morphogenesis and morphology of fossil remains and genetic treeing evidence, we conclude that the Gunflint Chert microbiota were a fungal decay and shallow water (limnic/terrestrial) depositional environment with immediate silicification and do not represent cyanobacterial stromatolites or cyanobacterial plankton communities.

Admittedly, only circumstantial evidence is presented, and no molecular, biomarker or direct morphological proof of a fungal origin can be given considering the enormous difference in time and appreciation of ancient ecosystems.

5. References

Barghoorn, E.S. and Tyler, S.A. (1965) Microorganisms from the Gunflint Chert. Science **147**: 563–577.

Brehm, U., Krumbein, W.E. and Palinska, K.A. (2006) Biomicrospheres generate ooids in the laboratory. Geomicrobiol. J. **23**: 545–550.

Bruns, T. (2006) Evolutionary biology: a kingdom revised. Nature **443**: 758–761.

Cloud, P.E. (1965) Significance of the Gunflint (Precambrian) microflora. Science **148**: 27–35.

Dahanayake, K. and Krumbein, W.E. (1986) Microbial structures in oolitic iron formations: miner. Deposita **21**: 85–94.

Dahanayake, K., Gerdes, G. and Krumbein, W.E. (1985) Stromatolites, oncolites and oolites biogenically formed in situ. Naturwissenschaften **72**: 513–518.

Dexter-Dyer, B., Kretzschmar, M. and Krumbein, W.E. (1984) Possible microbial pathways playing a role in the formation of Precambrian ore deposits. J. Geol. Soc. **141**: 251–262.

Doolittle, W.F. (1980) Revolutionary concepts in evolutionary cell biology. Trends Biochem. Sci., June, 146–149 (quoted from Dyer, B.D. and Obar, R. (1985) *The Origin of Eukaryotic Cells.* Benchmark papers in systematic and evolutionary biology. Van Nostrand Reynold, New York, 347 pp.)

Doolittle, W.F., Feng, D.-F., Tsang, S., Cho, G. and Little, E. (1996) Determining divergence times of the major kingdoms of living organisms with a protein clock. Science **271**: 470–477.

Dyer, B.D. and Obar, R. (1985) *The Origin of Eukaryotic Cells.* Benchmark papers in systematic and evolutionary biology. Van Nostrand Reinhold, New York, 347 pp.

Embley, T.M. and Martin, W. (2006) Eukaryotic evolution, changes and challenges. Nature **440**: 623–630.

Eppard, M., Krumbein, W.E., Koch, C., Rhiel, E., Staley, J. and Stackebrandt, E. (1996) Morphological, physiological and molecular biological investigation on new isolates similar to the genus *Geodermatophilus* (Actinomycetes). Arch. Microbiol. **166**: 12–22.

Gerdes, G. and Krumbein, W.E. (1987) *Biolaminated Deposits.* Springer, Berlin, 183 pp.

Gorbushina, A.A. (2007) Life on the rocks. Environ. Microbiol. **9**: 1613–1631.

Hass, H., Taylor, T.N. and Remy, W. (1994) Fungi from the Lower Devonian Rhynie chert: mycoparasitism. Am. J. Bot. **81**: 29–37.

Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L. and Hedges, S.B. (2001) Molecular evidence for the early colonization of land by fungi and plants. Science **293**: 1129–1133.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, B., Hofstetter, V., Cox, C.J., Gail Celio, G., Gueidan, C., Fraker, E., Miadlikowsky, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schüler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.W., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lücking, R., Büdel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W. and Vilgalys, R. (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature **443**: 818–822.

Jones, B. and Renaut, R.W. (2007) Selective mineralization of microbes in Fe-rich precipitates (jarosite, hydrous ferric oxides) from acid hot springs in the Waiotapu geothermal area, North Island, New Zealand. Sed. Geol. **194**: 177–198.

Jones, B., Renaut, R.W. and Rosen, R. (2000) Stromatolites forming in acidic hot-spring waters, North Island, New Zealand. Palaios **15**: 450–475.

Knoll A.H. (2004) *Life on a Young Planet.* Princeton University Press, Princeton, NJ, 227 pp.

Knoll, A.H. and Barghoorn, E.S. (1976) A Gunflint-type microbiota from the Duck Creek dolomite, Western Australia. Orig. Life **7**: 417–423.

Knoll, A.H. and Simonson, B. (1981) Early proterozoic microfossils and penecontemporaneous quartz cementation in the Sokoman Iron Formation, Canada. Science **211**: 478–480.

Kollman, J.M. and Doolittle, R.F. (2000) Determining the relative rates of change for prokaryotic and eukaryotic proteins with anciently duplicated paralogs. J. Mol. Evol. **51**: 173–181.

Konhauser, K.O., Jones, B., Reysenbach, A.-L. and Renaut, R.W. (2004) Hot spring sinters: keys to understanding Earth's earliest life forms. Can. J. Earth Sci. **40**: 1713–1724.

Krumbein, W.E. (1983) Stromatolites – the challenge of a term in space and time. Precamb. Res. **20**: 493–531.

Krumbein, W.E. (2008) Biogenerated rock structures. Space Sci. Rev. **135**: 81–94.

Krumbein, W.E. and Cohen, Y. (1974) Biogene, klastische und evaporitische Sedimentation in einem mesothermen monomiktischen ufernahen See (Golf von Aqaba). Geol. Rdsch. **63**: 1035–1065.

Krumbein, W.E. and Werner, D. (1983) The microbial silica cycle, In: W.E. Krumbein (ed.) *Microbial Geochemistry.* Blackwell, Oxford, pp. 125–157.

Krumbein, W.E., Paterson, D.M. and Zavarzin, G.A. (2003) *Fossil and Recent Biofilms – A Natural History of Life on Earth.* Kluwer, Dordrecht, 482 pp.

Lutzoni, F., Pagel, M. and Reeb, V. (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature **411**: 937–940.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A.E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G.H., Lucking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y.W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R. and Vilgalys, R. (2004) Assembling the fungal tree of life: progress, classification and evolution of subcellular traits. Am. J. Bot. **91**: 1446–1480.

Margulis, L., Maniotis, A., MacAllister, J., Scythes, J., Brorson, O., Hall, J., Krumbein, W.E. and Chapman, M.J. (2009) Spirochaete round bodies syphilis, Lyme disease & AIDS: resurgence of "the great imitator"? Symbiosis **47**: 51–58.

Nursall, J.R. (1959) Oxygen as a prerequisite to the origin of the Metazoa. Nature **183**: 1170–1172.

Redecker, D. (2002) New views on fungal evolution based on DNA markers and the fossil record [Review]. Res. Microbiol. **153**: 125–130.

Redecker, D., Kodner, R. and Graham, L.E. (2000) Glomolean fungi from the Ordovician. Science **289**: 1920–1921.

Remy, W., Taylor, T.N. and Hass, H. (1994) Early Devonian fungi: a blastocladalean fungus with sexual reproduction. Am. J. Bot. **81**: 690–702.

Retallack, G.J. (1994) Were the Ediacaran fossils lichens? Paleobiology **20**: 523–544.

Stechmann, A. and Cavalier-Smith, T. (2002) Rooting the eukaryote tree by using a derived gene fusion. Science **297**: 89–91.

Taylor, J.W. and Berbee, M.L. (2006) Dating divergences in the fungal tree of life: review and new analyses. Mycologia **98**: 838–849.

Taylor, T.N., Hass, H. and Kerp, H. (1999) The oldest fossil ascomycetes. Nature **399**: 648.

Tyler, S.A. and Barghoorn, E.S. (1954) Occurrence of structurally preserved plants in Pre-Cambrian Rocks of the Canadian Shield. Science **119**: 606–608.

Walsh, M.M. and Lowe, D.R. (1999) Modes of accumulation of carbonaceous matter in the Early Archaean: a petrographic and geochemical study of the carbonaceous cherts of the Swaziland Supergroup, In: D.R. Lowe and G.R. Byerly (eds.) *Geologic Evolution of the Barberton Greenstone Belt, South Africa.* Geol. Soc. Amer. Special Paper **329**: 115–132.

Walter, M. (1976) *Stromatolites: Developments in Sedimentology 20.* Elsevier, Amsterdam.

Walter, H. and Reissmann, R. (1994) Organische (?) Strukturen in Achatgängen des Osterzgebirges (Sachsen). Paläont. Z. **68**: 5–16.

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PALEOENVIRONMENTAL CONTEXT OF MICROBIAL MAT-RELATED STRUCTURES IN SILICICLASTIC ROCKS

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1. Introduction

The role of biological influences in forming carbonate rocks (e.g., Altermann et al., 2006) is almost universally accepted within geology. In contrast, many see clastic sedimentary rocks as being formed primarily through physical and chemical processes, with biological mediation of their genesis being considered as of relatively minor importance (Schieber et al., 2007a). While sedimentologists and most geologists are familiar with the importance of trace fossils within clastic deposits (cf., the seminal work of Seilacher (1964) and many others since), the role of microbial mats in terrigenous sediment accretion, and in the formation and preservation of a whole host of mat-induced (mi) and mat-related structures within clastic sedimentary rocks, is less well known.

Initial biofilms, comprising clusters of micro-organisms often embedded in extracellular polymeric substances (EPS), develop into tough leathery microbial mats within time, which provide biostabilization of clastic sediment surfaces (Schieber et al., 2007a). Experiments in cultivating modern mats suggest that the time necessary for this transition encompasses several weeks of non-burial (Gerdes and Klenke, 2003, 2007), although a thin mat may develop over beach or tidal flat sands within a matter of only ca. 8 h between a bidiurnal ebb and following flood (Fig. 1). The highly adaptable cyanobacteria, which can essentially grow on any moist clastic sedimentary surface where their energy and nutrition needs are met and where grazing metazoans are either absent or ineffective,

Figure 1. Thin film of microbial mat (patchy in *top left* of photo), which has developed on rippled sand, between the ebb and flood tides, on the modern Chandipur tidal flat, eastern coast of India. Pen for scale.

comprise the most successful mat-builders (Schieber et al., 2007a, c). However, in effect, environmental variability leads to different groups of mat-building bacteria flourishing under different sets of conditions (Schieber et al., 2007c).

Clastic sedimentary deposits influenced by microbial mats have figured in the specialist literature for 4 decades (e.g., Davis, 1968; Krumbein and Cohen, 1977; Reineck, 1979; Horodyski, 1982; Schieber, 1986, 1998, 1999, 2004; Gerdes and Krumbein, 1987; Reineck et al., 1990; Gerdes et al., 1993, 2000; Krumbein et al., 1994; Pflüger and Gresse, 1996; Gehling, 1999; Hagadorn et al., 1999; Noffke and Krumbein, 1999; Riding and Awramik, 2000; Noffke et al., 2001a; Bouougri and Porada, 2002; Porada and Bouougri, 2007a), gradually becoming a part of more mainstream publications. Noffke et al. (2001a) coined the acronym MISS (microbially induced sedimentary structures) and added them as a group to accepted classification schemes for physically formed sedimentary structures. As by no means, all mat-related features are induced directly by microbes, and many are rather related to physical forces acting on biostabilized sediment surfaces, an alternative acronym would be MRS (mat-related structures), possibly reflecting the acceptance of mat-related features within the greater family of siliciclastic sedimentary studies. Schieber (2004) established an essentially process-response classification scheme, which is also followed in the recent compendium written for all geoscientists (Schieber et al., 2007b) and which is used in this chapter. Another classification scheme more related to paleoenvironmental influences (Sarkar et al., 2008) is briefly discussed in one of the case studies in this chapter.

However, it needs to be stressed that the mat-related sedimentary structures in the clastic sedimentary record are commonly subtle and mostly proxy features, reflecting interaction of evolving mats with the loose sediment, where EPS secreted by the various bacteria commonly plays a critical role (e.g., Decho, 1990, 2000). The continuum from EPS and biofilms to fibrous, filamental mats leads to behavior of clastic sediment incompatible with normal physical and chemical controls on sediment deposition, diagenesis and lithification, an example being sediment binding and cohesive behavior of sand (Schieber et al., 2007a). While microbially formed structures and features in mudrocks are much more subtle, within sandy sediments, a range of about 50 features reflects mat growth, mat metabolism, mat destruction, mat decay and diagenetic mat features (Schieber et al., 2007b; various classification strategies are also discussed there).

Evaluations of the paleoenvironmental significance of mat-related structures have been influenced by several factors: (1) such features have been studied most commonly from modern and ancient analogs of shallow marine tidal to supra-tidal settings and hyper-saline lagoons (e.g., Gerdes et al., 1985a, b, c); (2) consequently, some workers regard mat-related structures as at least partially diagnostic of clastic tidal flats lacking storm influences and relate them further to a specific sequence stratigraphic interpretation, as marking transgression–regression cycles (e.g., Noffke et al., 2006b; Noffke, 2007); (3) in contrast, many workers regard them, generically, as non-facies-specific (e.g., Schieber et al., 2007a), a viewpoint supported by widespread occurrence of 'opportunistic mats' (Schieber et al., 2007c). As will be briefly outlined in the next section, microbial mats and their ancient proxies are reported from a very wide range of (paleo)environmental settings. In a sequence stratigraphic context, they can also contribute, at various scales, stacking patterns and resultant architecture to clastic sediment (e.g., Sarkar et al., 2005).

Environmental studies attempting to relate different mat features to specific parts of identified settings have been limited. Gerdes et al. (2000) and Noffke et al. (2001b) discuss this with regard to zones within Pleistocene-modern tidal flats, and Parizot et al. (2005) connect different mat-related structures to inferred water depths within an analogous littoral setting along a Paleoproterozoic epeiric basin margin. We use the term 'littoral' here to mean the inter-tidal zone between normal high- and low-tide marks; supra-littoral implies above the normal high-tide mark and encompasses spring tides. Bose and Chafetz (2009) in a facies-based study of mat-related structures along the modern Texas coast, were able to discriminate six zones within a tidal–supra-tidal setting. In this chapter, we attempt to contribute to this aspect of microbial mat studies, by examining several case studies from the Indian and South African Precambrian clastic record to derive relationships between mat-induced features and sedimentary facies (cf. distinct parts of discrete depositional environments). Precambrian examples have the advantage that metazoan grazing can be largely discounted and this impacts on the application of a uniformitarian approach to MRS when comparing modern, often stressful (e.g., hyper-saline) or low-energy mat-forming environments with potential Precambrian settings.

2. Brief Overview of (Paleo)Environmental Relationships of Mat-Formed Features

The most common setting observed for modern mats growing on clastic substrates is within a continuum of shallow water-tidal marine environments through to supra-tidal and even sabkha settings, but examples are known from lakes and rivers as well (Schieber et al., 2007a; Gerdes, 2007). A second common setting for prolific mat growth is seen in modern mixed clastic-chemical deposits that occur in hyper-saline lagoons (e.g., Gerdes and Krumbein, 1987; Noffke et al., 2001b) or in analogues to such environments within salt works and laboratory situations (e.g., Schneider, 1995; Gerdes and Klenke, 2003). Schieber (1998) inferred the following environmental settings for identifying microbial mat features in the ca. 1,450–850 Ma Belt Supergroup of North America: calm offshore; shallow nearshore; sea-marginal sandflats; shallow nearshore lagoons – all probably within an epicratonic sea connected to the open ocean. Despite the rich assemblages of mat-formed features found in the two noted preferential settings, the highly divergent facies relationships observed within modern and rock record occurrences (e.g., Krumbein et al., 2003; Schieber et al., 2007a) are underscored by well-preserved mat features from the oldest known desert deposits (ca. 1.8 Ga; Eriksson et al., 2000).

Gerdes (2007; her Table 2.1) tabulates modern natural and cultured mats and their resultant features within and upon clastic sediments in a preferential environmental range from permanently inundated shallow lagoons, areas transitional to lagoons, inter-tidal flats and their transitions to supra-tidal conditions, and finally, to supra-tidal areas and sabkhas. However, the inherent importance of low-energy levels and periods of non-burial (and hence low-sedimentation rates) of a number of weeks to even months for hardy mat formation must be borne in mind within any environmental setting or continuum (Gerdes, 2007). The mat-related structures formed during mat metabolism (particularly those related to formation of gases) and post-burial mat decay may equally occur in fully subaqueous settings (Gerdes, 2007).

The formation of a specific mat-formed feature within a particular environmental situation is no guarantee for its final deposition within the same setting: photosynthetic oxygen bubbles enable floating mat fragments (cf. Fagerstrom, 1967), and physical mat destruction by high-energy transporting mediums (cf. mat chips, etc.) can equally remove mat portions from their initial sites of origin (Schieber, 1999; Gerdes, 2007). Research on transport of mat fragments is ongoing, including valuable flume experiments (e.g., Schieber, 2007b for a recent overview).

Schieber et al. (2007c), based partly on ten different stratigraphic units (from Archean to Eocene in age) bearing significant spectra of mat-related features, attempted to tabulate environmental affinities of different groupings of the features. Due to the generally observed relatively weak facies-specific occurrence of these often subtle mat-formed features, they deliberately adopted a robust environmental classification: (1) coastal (= above mean sea level); (2) shallow sea/

lake (= generally agitated shallow water settings below mean sea level); (3) deep sea/lake (= quiet and deep water environments); (4) fluvial and (5) eolian (Schieber et al., 2007c). Although their tabulation for shale-related mat features was essentially almost wholly non-facies-specific, for sandstone-hosted mat features, some differences were apparent: (a) the greatest variety and sheer abundance of mat features was found for the 'coastal' setting; (b) many fewer mat-formed features were ascribed to the 'shallow and deep sea/lake' settings, which also demonstrated an essentially shared group of such features; (c) the 'fluvial' setting had a grouping of mat features resembling that of the 'coastal' environment, but with much reduced variability and abundances – from a bathymetric perspective, the fluvial setting can be compared favourably with the coastal setting, possibly explaining this apparent similarity. The shared features of coastal and fluvial settings mainly comprised surface ornamentation features (e.g., wrinkles), petees and petee ridges, sand cracks and sand chips (Schieber et al., 2007c). However, it is important to emphasize that the Schieber et al. (2007c) summary of possible environmental affinities relies on facies interpretations of the various authors of the case studies used; much greater reliance should thus be placed on modern observations where uncertainties and subtleties of paleoenvironmental differentiation are automatically excluded.

Despite the broad environmental affinity of mat features, both modern (e.g., Gerdes, 2007) and ancient (e.g., Schieber et al., 2007b), detailed processes deduced from careful study of mat features can complement standard techniques of sedimentary facies analysis (Schieber et al., 2007a). The introduction of grazing metazoans in the Phanerozoic decreased the relative importance of mat-related features in many clastic sedimentary settings (except those more marginal ones less conducive to metazoan activities) but has apparently done little to affect the broad environmental adaptability of these mat-related features (Schieber et al., 2007b).

3. The Importance of Microtopography: Influence on Mat Types, Mat Growth and Mat Features in All Settings

Since the classical works of Kendall and Skipwith (1969) on microbial mats in Abu Dhabi (Trucial Coast, UAE) and of Logan et al. (1974) on mat types in Hamelin Pool (Shark Bay, Australia), both areas being favorable for carbonate precipitation, it is known that different types of microbial mats develop according to their position in the supra-tidal to shallow sub-tidal range on tidal flats. Thereby, differences in elevation in the range of centimetres may be decisive in determining which type of mat and mat surface structure will develop. Controlling factors include: (a) frequency of tidal flooding, (b) duration of water cover, (c) frequency and duration of sub-aerial exposure, (d) position of tidal groundwater table, (e) chemistry/salinity of tidal groundwaters, (f) occurrence and rate of mineral precipitation and (g) rate of sediment influx. All these may have a bearing on which cyanobacterial

Table 1. Microbial mats in Hamelin Pool (Australia) and Abu Dhabi area (Trucial Coast, U.A.E.): Correlation of terms and dominant species from the supratidal to the subtidal zone.

Australia	Abu Dhabi	Dominant species	Reference
Film mat	Flat zone Flat mat	*Entophysalis* (coccoid)	Kendall and Skipwith, 1969 Kinsman and Park, 1976
Blister mat	Crinkle zone Blister mat	*Microcoleus, Phormidium* (filamentous) [Australia]	Kendall and Skipwith, 1969 Kinsman and Park, 1976
Tufted mat	–	*Lyngbya, Microcoleus* (filamentous)	–
Pustular mat	Cinder zone Mammilate mat	*Entophysalis* (coccoid) [Australia]	Kendall and Skipwith, 1969 Golubic, 1976
Smooth mat	Polygonal zone Smooth mat	*Microcoleus* [UAE] *Schizothrix* [Australia] (filamentous)	Kendall and Skipwith, 1969 Kinsman and Park, 1976
Gelatinous mat	Polygonal zone (in tidal pond)	*Entophysalis, Aphanocapsa, Aphanothece* (coccoid) [Australia]	Kendall and Skipwith, 1969
Colloform mat	–	–	–

species or combination of species will dominantly build up the mat, and this, in turn, has strong influence on the morphology of the mat surface.

Based largely on mat surface morphological features, Kendall and Skipwith (1969) recognized, in shore-parallel arrangement from the supra-tidal to the lower inter-tidal zone, four 'mat zones', and named them 'flat', 'crinkle', 'cinder' and 'polygonal' zones. Similarly, Logan et al. (1974) distinguished seven morphologically different types of mats in Hamelin Pool and described them as 'film', 'blister', 'tufted', 'pustular', 'smooth', 'colloform' and 'gelatinous'; further descriptive terms were introduced by Kinsman and Park (1976) and Golubic (1976). A comparison of Logan et al.'s (1974) mat types and the 'mat zones' of Abu Dhabi (Table 1), listing also dominant participating cyanobacterial species, reveals that mat types and mat zones are largely comparable, whereas individual types and zones may be biologically different. An explanation for this difference is possibly provided by a diagram, modified after Hoffman (1976), which shows the distribution of mat types in respect of their topographic position and related environmental processes (Fig. 2). From this, it may be concluded that mat types are physically, chemically and biologically controlled adaptations of microbial communities to specific, locally prevailing environmental conditions.

On the large scale of a tidal flat, topographic differences (relief) may result from eolian action (deflation) and tidal currents, mainly ebb currents, which basically are oriented perpendicular to the slope. Ponds produced by local deflation processes in the upper intertidal or supratidal zone may retain tidal water for long periods, whereas 'mat zones' may be interrupted by meandering, shallow depressions in which tidal water remains longer than in adjoining more elevated areas. The distribution of mat types and related structures will thus be much more

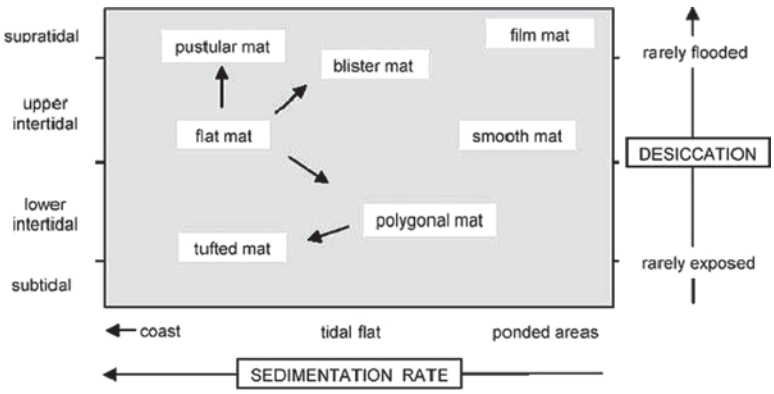

Figure 2. Distribution of mat types on a hypothetical tidal flat, and related environmental processes. Originally designed by Hoffman (1976) for carbonate-precipitating environments, the diagram has been modified to also apply to siliciclastic peritidal systems. *Arrows* indicate development of some mat types as a consequence of biological processes, mainly microbial growth.

irregular than that predicted by the term 'zone'. Additionally, on a small scale, micro-topographic differences are created by the mats themselves, which frequently develop bulges, domes and upturned crack margins on their surfaces. These form small-scale topographic highs and play an important role in the distribution of microbial activity and mat growth dynamics. In the Abu Dhabi area, it has been observed by one of the authors of this chapter (Hubertus Poroda) that 'smooth' or 'polygonal mats' may grade into 'mammilate', 'cinder' or 'pustular', and 'tufted mats' along an evolutionary path (Fig. 3) controlled by preferred growth along bulges and upturned crack margins. When the mat surface is widely covered by tufts and pinnacles, new cracks and polygons form. This seems to indicate that some mat types and related structures are temporary features in the evolution of a complex biological system, subject to small environmental changes and partly induced by the dynamics of the system itself (see also Fig. 2).

In siliciclastic peritidal settings without precipitation of carbonate, basically the same types of mats and mat surface structures may occur, depending on topographic position and locally variable weather conditions like seasonal land-directed winds, or storms and rainfall. Climate thus plays a major role. In both temperate humid and subtropical arid zones, a laminated 'smooth mat', dominated by filamentous cyanobacteria such as *Microcoleus* sp., *Lyngbya* sp. and *Phormidium* sp. is frequently developed in the intermittently emergent, intertidal zone behind a protecting sand shoal or barrier, and in ponded areas elsewhere. Such mats typically develop networks of wide cracks with upturned and overgrown margins and then appear as 'polygonal mats' (Fig. 4a). In regularly flooded areas, mats of this type are quasi-permanent because of their ability to recolonize thin sediment layers deposited on top during storms or by eolian transport.

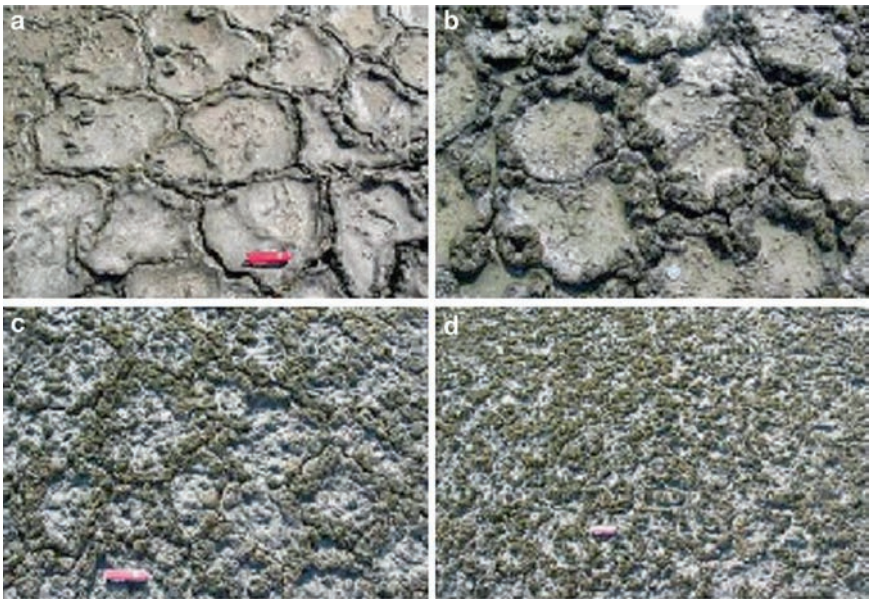

Figure 3. Evolution of a 'polygonal mat' into a 'mammilate' or 'cinder mat' due to preferred microbial growth on small elevations. Trucial Coast, near Abu Dhabi (UAE). (**a**) 'Polygonal mat' with upturned crack margins producing saucer-shaped polygons. Scale (knife) is 8 cm. (**b**) Microbial growth localized along upturned margins. Resulting micro-relief allows trapping of water in the polygons thus supporting microbial growth. Scale (coin) is 24 mm. (**c**) Microbial growth starts to spread from upturned margins over the polygons. Original polygons are still recognizable. Scale (knife) is 8 cm. (**d**) Widespread microbial growth produces 'cinder mat'. Original polygons are locally still recognizable. Scale (knife) is 8 cm.

Alternations of burial and recolonization may lead to vertically aggrading biolaminated deposits ('siliciclastic biolaminites' after Bouougri and Porada, 2007). Upturning of polygonal crack margins usually involves the upper layers of biolaminites (Fig. 4b) thus forming structures of high preservation potential (Park, 1977; Bouougri and Porada, 2007). In temporarily ponded areas, 'smooth mats' will desiccate and shrink with evaporative loss of water and eventually develop shrinkage cracks of various shapes and, locally, in great numbers, frequently with upturned margins. In favorable areas within the 'polygonal mat' zone and around ponds in upper intertidal or supratidal positions, where water cover is in the range of one to a few centimeters over some time, *Lyngbya* sp. filaments may form reticulate growth patterns and micro-scale tufts on the surface mat, thus giving rise to a 'tufted mat' type.

In Tunisian coastal sabkhas (subtropical arid zone), it has been observed (Gerdes et al., 2000, 2008; Noffke et al., 2001b) that towards the upper intertidal zone, the 'polygonal mat' grades into a strongly cohesive 'flat mat' consisting of dominantly coccoid cyanobacteria (e.g., *Synechococcus* sp.) in an upper, orange-

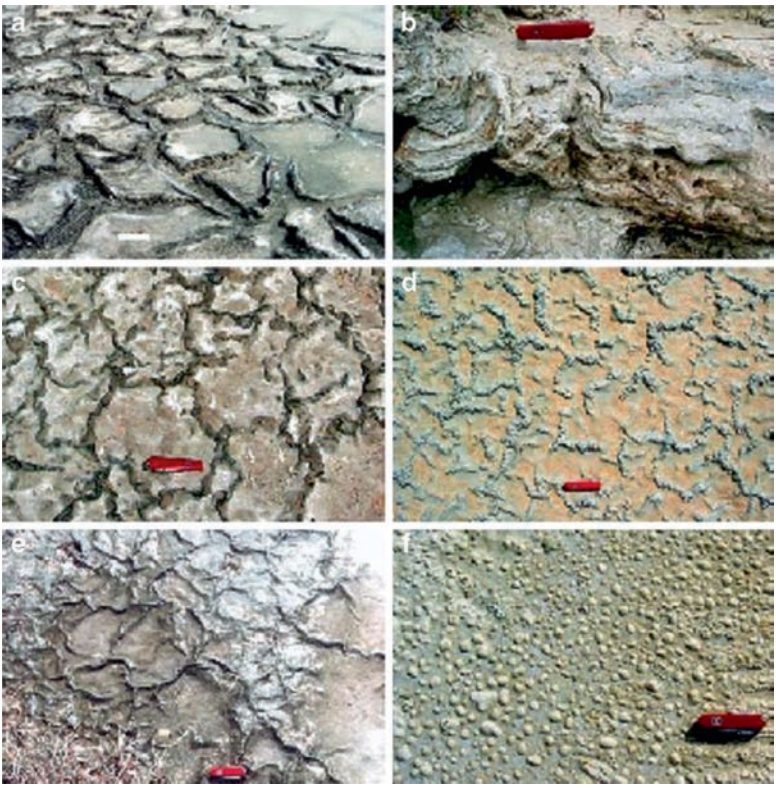

Figure 4. Mat types in siliciclastic peritidal systems, examples from southern Tunisian Bhar Alouane tidal flat and coastal sabkha, and (for **e**) Djerba Island coast intertidal pond. (**a**) 'Polygonal mat' characterized by wide cracks with upturned margins; intertidal zone. Scale bar is 10 cm. (**b**) Section across crack with opposing upturned margins partly involving underlying biolaminites. Scale (knife) is 8 cm. (**c**) Network of 'healed' cracks, overgrown by mats forming bulges spanning the cracks; upper intertidal zone. Scale (knife) is 8 cm. (**d**) Irregular to incomplete polygonal distribution of mat expansion structures ('petees') developed on flat mat of dominantly coccoid cyanobacteria. Note pustular surface of bulges; upper intertidal to lower supratidal zone. Scale (knife) is 8 cm. (**e**) Polygonal network of 'petees' (mat expansion bulges) developed in thin mat of dominantly filamentous cyanobacteria; upper intertidal zone. Scale (knife) is 8 cm. (**f**) 'Blister mat' characterized by overgrown and stabilised 'photosynthetic domes' (PS domes) developed on originally 'flat mat' of dominantly coccoid cyanobacteria; upper intertidal to lower supratidal zone. Scale (knife) is 8 cm.

colored layer that overlies blue-green layers of filamentous species. Isolated small, narrow shrinkage cracks or incomplete networks of such cracks are observed in the upper mat layer. Further upslope, coccoid cyanobacteria increase in dominance and increasingly control the shape of surface morphological features. In this upper intertidal to lower supratidal zone, a variety of mat types are observed, including 'pustular', 'blister' and 'crinkled' mats. They all appear to evolve from originally 'flat mats', as a result of episodic environmental change and biological

reaction, and may be developed, partly as ephemeral features, in a vague zonal arrangement or be merely irregularly distributed.

Marine inundation is rare in this zone, but mats are still supplied with water from below due to capillary water movement and evaporative pumping (Hsü and Siegenthaler, 1969; Porada et al., 2007). Nevertheless, they may shrink and crack or be episodically flooded, and in both cases, bacterial growth will be a reaction. In the case of shrinkage and cracking, groundwater ascending in the cracks will soon induce localized bacterial growth resulting in bulges spanning the cracks (Fig. 4c). Flooding, on the other hand, will cause widespread active growth of the surface mat layer resulting in rapid lateral expansion, and 'if the available space is limited, the blooming mat will dome over the older parts of the structure' (Monty, 1979, p. 219) and develop bulges ('petees') with hollow cavities underneath. In coccoid bacteria-dominated mats, these bulges are generally irregular in distribution and with pustular or cauliflower-like protrusions on the surface (Fig. 4d), due to two- or three-dimensional cell division geometries and resulting botryoidal cell clusters. Mats developing such surface morphological features are readily compared with the 'pustular mat' type of Logan et al. (1974). In contrast, mats dominated by filamentous cyanobacteria whose cells are organized in trichomes surrounded by sheaths, will develop smooth bulges ('petees') forming a more regular, almost polygonal mat surface pattern (Fig. 4e).

A particular feature of the coccoid bacteria-dominated top layer in mats is the richness in extracellular polymeric substances (EPS) that give the layer strong cohesiveness and high elasticity. If filamentous or coccoid cyanobacteria underlying this strong surface film release oxygen as a product of photosynthesis, it will be trapped underneath the elastic surface layer deforming it into small domes ('photosynthetic domes' [PS domes] see discussion of this term in Bouougri et al., 2007), which will soon be stabilized and overgrown by microbes. With continued oxygen production, the domes increase in size or coalesce and eventually may dominate the surface morphology of a then typical 'blister mat' (Fig. 4f).

Although most of the features described earlier may be irregularly distributed on tidal flats, some spatial ordering is recognizable: (1) 'smooth' and 'polygonal mats' overlying 'siliciclastic biolaminites' require regular flooding and are thus preferentially developed in the lower intertidal zone; (2) 'flat mats' with isolated narrow shrinkage cracks or incomplete networks of cracks form in the upper intertidal zone where periodic flooding (spring tides) keeps water supply in balance; (3) 'blister' and 'pustular mats' form in the lower supratidal zone as a reaction to episodic flooding after extended periods of subaerial exposure; (4) abundant shrinkage cracks may occur in temporarily ponded areas where mats can develop and grow for some time but eventually shrink and crack with waning water.

The preservation potential of most of the morphological features described earlier is low, because all the organic material of the mats is strongly compacted during burial and largely decomposed and mineralized. Only if inorganic material was introduced into the system, either chemically by precipitation (e.g., carbonate) or physically by water or wind transport or by hydraulic processes (e.g., silt and sand), will

proxy features possibly become preserved. Furthermore, authigenic clay minerals may form by biogeochemical processes (Krumbein and Werner, 1983), e.g., related to bacterial lysis, and partly replace the organic material during diagenesis.

In siliciclastic peritidal systems without precipitation of carbonate, trapping of silt and sand is the most important process to preserve mat-related structures. This is obvious for shrinkage cracks that are readily filled from above by current- and wind-transported sediment. In a more subtle way, the mats themselves also trap and overgrow silt- and sand-sized sediment grains and thus incorporate them in the layer fabric. They appear in the fossil record within characteristic wavy-crinkly laminae as 'floating grains' set in a matrix of clay minerals or sericite (cf. Schieber, 2007a). Such clayey or sericitic layers may form partings between sandstone beds and allow subtle mat-related structures, such as 'wrinkle structures' to be preserved on sediment surfaces. A specific way to preserve mat surface morphological features, such as petees, domes and overgrown ('healed') cracks, is their filling from below. This may occur if hydraulic pressure in the water-saturated substratum is sufficiently high to lift and move upward individual sediment grains, in a state of potential liquefaction. Usually, the process proceeds very slowly and appears to require additional cyclic energy input, such as exerted by the tides. Upward rise of sediment may, however, be rapid if the sedimentary substratum is liquefied by seismic shock.

4. Case Studies

4.1. MAGALIESBERG FORMATION (CA. 2.1 GA), KAAPVAAL CRATON, SOUTH AFRICA

The ca. 2.1 Ga Magaliesberg Formation, generally ca. 250–300 m thick, is a sandstone-dominated unit within the upper part of the Pretoria Group, the uppermost subdivision of the ca. 2.8–2.1 Ga Transvaal Supergroup (Eriksson et al., 2001 and references therein). In many areas, including the current study area (Fig. 5), it forms the actual floor rocks to the 2,058 ± 0.8 Ma (Buick et al., 2001) Bushveld Complex, the world's largest layered mafic intrusion. Intense contact metamorphism has resulted locally, forming quartzites and minor hornfelses (Bosch and Eriksson, 2008). The Transvaal rocks, including the Magaliesberg, have also been subject to folding and faulting (Bumby et al., 1998; Eriksson et al., 1998), and the sedimentary beds mostly dip towards the centrally located Bushveld Complex. Deposition of the Pretoria Group mudrocks, sandstones and lesser lavas (Fig. 5) is ascribed to two cycles of rifting, followed by thermal subsidence and concomitant epeiric sea formation within an intra-cratonic sag basin on the Kaapvaal craton (Eriksson et al., 2001); the Magaliesberg is interpreted as reflecting a regressive sandy coastline at a high stand to falling stage systems tract transition within the second epeiric sea (Catuneanu and Eriksson, 1999).

Eriksson et al. (1995) identified three architectural elements (cf. Miall, 1985) in the Magaliesberg: (1) medium- to coarse-grained sandstone sheets; (2) fine- to

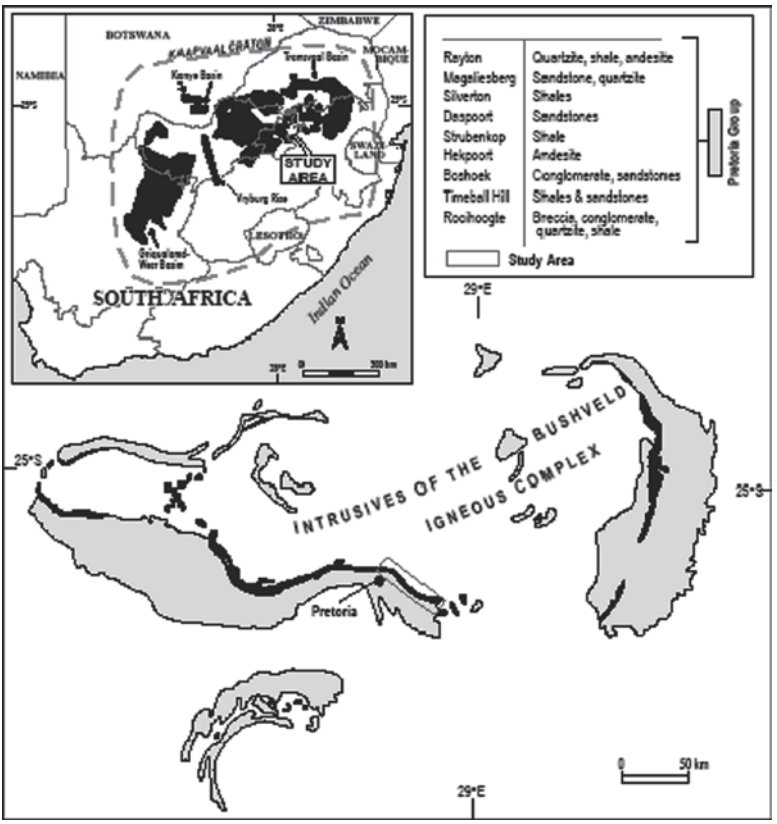

Figure 5. Geological sketch map showing the Pretoria Group and Magaliesberg Formation, as well as a stratigraphic column for the former, applicable to the south-central part of the preserved basin. The study area where microbial mat features were recorded is shown by the box in the Pretoria region and east thereof. (Modified after Bosch and Eriksson, 2008.)

medium-grained sandstone sheets and (3) minor mudrock elements. Characteristic sedimentary structures in the first two elements are horizontal lamination and planar cross-bedding, with subordinate trough cross-bedding, channel-fills and wave ripple marks, and minor double-crested and flat-topped ripples and desiccated mudrock partings (Eriksson et al., 1995). These sedimentary rocks are inferred to have formed through ephemeral braid-delta systems (architectural element #1; palaeocurrent trends are unimodal), which debouched onto high-energy peritidal flats (element # 2; palaeocurrent trends bi- to poly-modal), on the margins of a shallow epeiric sea (Eriksson et al., 2002), where reworking through small waves and macro-tidal action was prevalent (Eriksson et al., 1995). The subordinate mudrocks (element #3) suggest abandonment of braid-delta channels or uppermost tidal flat sedimentation (Bosch and Eriksson, 2008).

Figure 6. Wave ripples on thin (ca. 5 cm) sandstone bed upper surface (Enzelsburg, Marico area, western Pretoria Group basin); note slightly sinuous perfectly preserved ripple crests and bifurcation (open compass for scale). Typically, repeated such rippled thin sandstone beds are widespread throughout the succession of the Magaliesberg Formation (Photo: H. Labuschagne).

The predominant ripple types found on sandstone bed surfaces within the Magaliesberg Formation are wave ripples (Boshoff, 1992; Parizot et al., 2005; Fig. 6); these features occur, with variable crest alignments, on the upper surfaces of many beds within stacked series of element #2 above, with sandstone sheet thickness varying from a few centimeters to several decimeters. These repeated rippled surfaces show some resemblance to palimpsest ripples (cf. Schieber, 2004; Eriksson et al., 2007a), which are defined as preserved sets of ripples on successive sandstone beds where there is an absence of mud between the beds, which are also not amalgamated, and where the ripples themselves have not undergone reworking of ripple crests (Bottjer and Hagadorn, 2007). Biostablization of these repeated rippled sandstone surfaces is implicit, and the inference is that a microbial mat protected the earlier ripples from reworking, upon deposition of a succeeding sand bed (cf. Pflüger, 1999; Bottjer and Hagadorn, 2007). These MRS form part of the mat growth features in the Schieber (2004; see also, Eriksson et al., 2007a) classification scheme. These almost pervasive ripples allow estimation of wave height and water depth at the margins of the epeiric basin, through application of the Tanner (1967, 1971) formulae: water depth varied between 30 and 40 cm and wave heights were mostly about 10 cm (Parizot et al., 2005).

In addition to these very common features, other MRS are known from the Magaliesberg Formation, with cracked sand layers and polygonal petee ridges being relatively common (Bosch and Eriksson, 2008) and ripple-crest cracks, 'elephant skin textures', wrinkle structures and *Manchuriophycus* (sinuous ripple through sand cracks) found only locally (Parizot et al., 2005) (Fig. 7). The latter authors used associated ripple marks to estimate water depths and wave heights for these less common MRS (Fig. 7), with larger wave height and water depth estimates being ascribable to

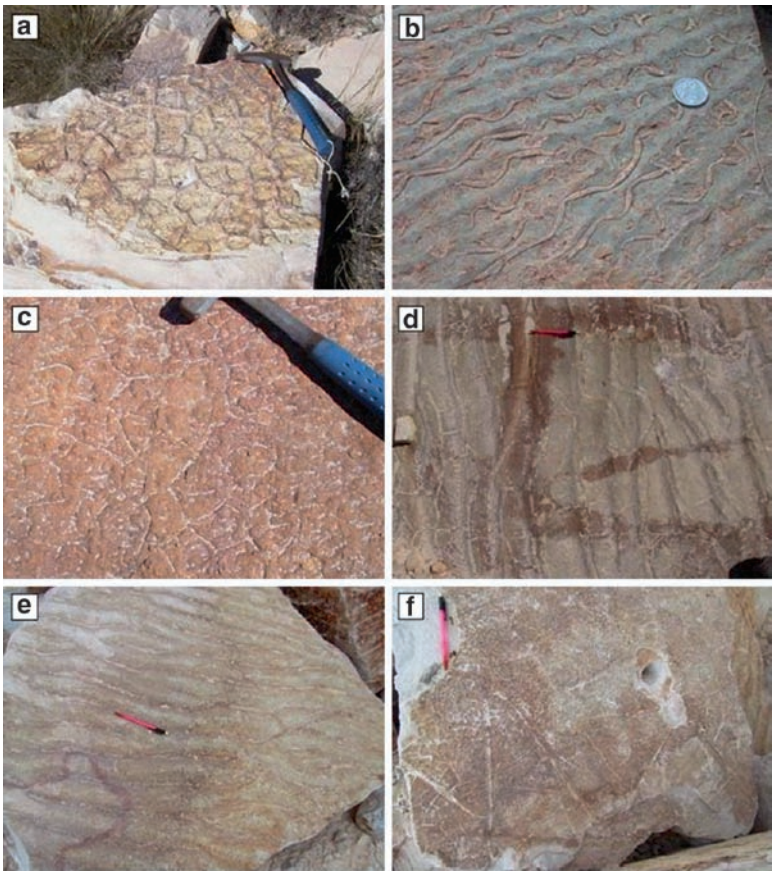

Figure 7. Mat-related structures from the Magaliesberg Formation (study site shown in Fig. 5). (**a**) Polygonal petee ridges, showing second-order features within first-order ridges; (**b**) *Manchuriophycus*, sinuous sand cracks formed within ripple troughs (Photo: Pieter Bosch); (**c**) reticulate crack pattern of 'elephant skin texture', preserved as negative features on the sole of a sandstone bed; (**d**) ripple crest sand cracks; note lesser cracks across ripple troughs and crack bifurcation; (**e**) sand cracks, localized within ripple troughs; compare with sinuous features in (**b**); (**f**) wrinkle structures (*top left* of block) passing into wedge-shaped petee ridges (*bottom right* of block).

a relatively more distal deeper water setting along the Magaliesberg epeiric littoral. In this sense, ripple-crest cracks appear to be relatively more distal than polygonal petee ridges, with *Manchuriophycus* being most proximal (Fig. 8). However, as a caveat, it should be noted that ripple formation and growth of mats with concomitant formation of MRS are almost certainly separated in time, at least to some degree, bearing in mind the periods implicit in mat growth (cf. Gerdes and Klenke, 2003, 2007 – at least several weeks). However, the systematic differences in estimated wave height and water depth parameters shown in Fig. 8 suggest at least a measure of local paleoenvironmental control, although obviously caution should be taken with direct interpretations of the parameters for any specific MRS.

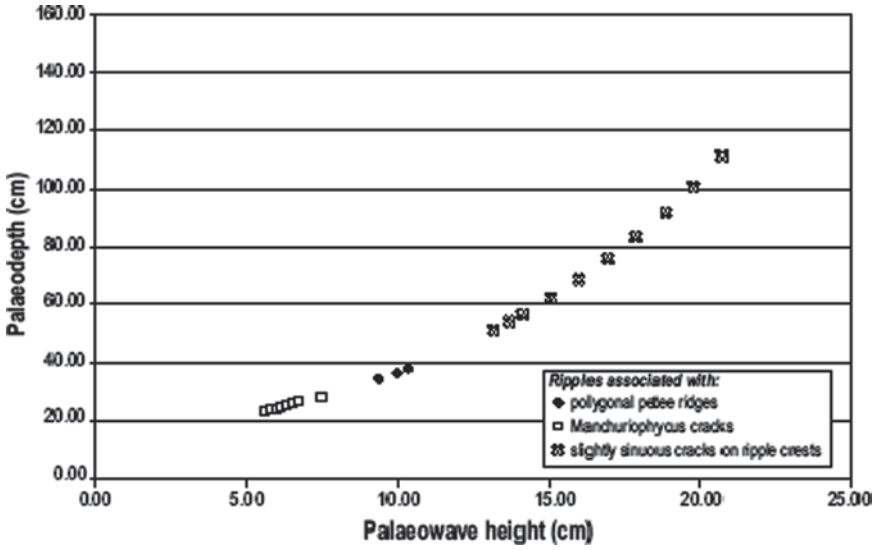

Figure 8. Plot showing estimated water depth against palaeowave height (both calculated from formulae of Tanner, 1971), as determined for the different MRS in the Magaliesberg sandstones from the study area. Note much higher water depth and wave height estimates for ripple crest cracks. (Modified after Parizot et al., 2005.)

Cracked sand layers are inferred to reflect cracks developed in microbial mats growing within the upper sandy surface of a bed, which then may be filled by secondary sand from an outside source, often forming positive features in sandstones once the mat itself decayed and lithification had taken place (cf. Gehling, 1999, 2000). Most of the Magaliesberg features show the positive ridges to have the same sand composition and caliber as the sand in the underlying bed, and may thus be termed petees or petee ridges (e.g., Eriksson et al., 2007b). Petee ridge features may also, in some cases, form through gas related to sub-mat organic matter decay which causes linear bulges in partially loose surface mats, or mats may become disrupted by wind and water currents (Gehling, 1999; Gavish et al., 1985). Linear petee ridges commonly form polygonal networks of 1-3 orders and may also rupture to form cracks (cf. Schieber, 2004; Schieber et al., 2007b) which may also heal due to new mat growth along the ruptures/cracks (as observed by Bosch and Eriksson, 2008, in the Magaliesberg Formation).

Manchuriophycus-type cracks reflect thicker mats developed in ripple troughs, or surviving there after erosion removed mat from the higher lying ripple crests, which were then subject to desiccation and cracking (e.g. Pflüger, 1999; Schieber, 2004). Elephant skin textures are interpreted as the product of mat growth, forming a reticulate pattern of bulges and tufts (cf., Porada and Bouougri, 2007a). The wrinkle structures can be interpreted as reflecting either partial detachment of a mat from its sandy substrate due to traction currents (Hagadorn and Bottjer, 1999; Bouougri and Porada, 2002), or soft sediment deformation of mat-bound sand

surfaces through burial loading or flood-tidal water increases (e.g., Parizot et al., 2005). Ripple crest cracks are not commonly reported in literature, but most likely indicate desiccation of mat with greater tensile shear along ripple crests (Sarkar et al., 2004); in the Magaliesberg examples, the observed relationship with the greatest water depth and wave height estimates led Parizot et al. (2005) to suggest a genesis through mats growing on deeper-lying braid-deltaic channel-floors, subject to later desiccation upon falling water stages following fluvial flood events.

4.2. SONIA SANDSTONE (CA. 0.6 GA), RAJASTHAN, INDIA

The Sonia Sandstone, comprising almost entirely arenitic sediments, is best exposed near Jodhpur City, Rajasthan, India; it rests on a rhyolitic basement and is overlain by the Girbakhar Sandstone containing decimetric shale intervals (Fig. 9a, b). The two formations together comprise the Jodhpur Group (Fig. 9b; Pareek, 1984). Perhaps, the contact between them can be placed most meaningfully, but deviating from previous views (Chauhan et al., 2001; Sarkar et al., 2005, 2008) along the only conglomerate-defined disconformity that is present in the Group (Fig. 9b). The Sonia Sandstone, 150 m-thick, can be termed a sequence *sensu stricto*, divided internally into a basal subaerial interval and an overlying coastal marine interval (Sarkar et al., 2008), both formed in an intra-cratonic setting (Chauhan and Ram, 1999; Sarkar et al., 2005, 2008). The age of the Sonia Sandstone is generally accepted to be Neoproterozoic, close to 600 Ma; recent U-Pb dating of detrital

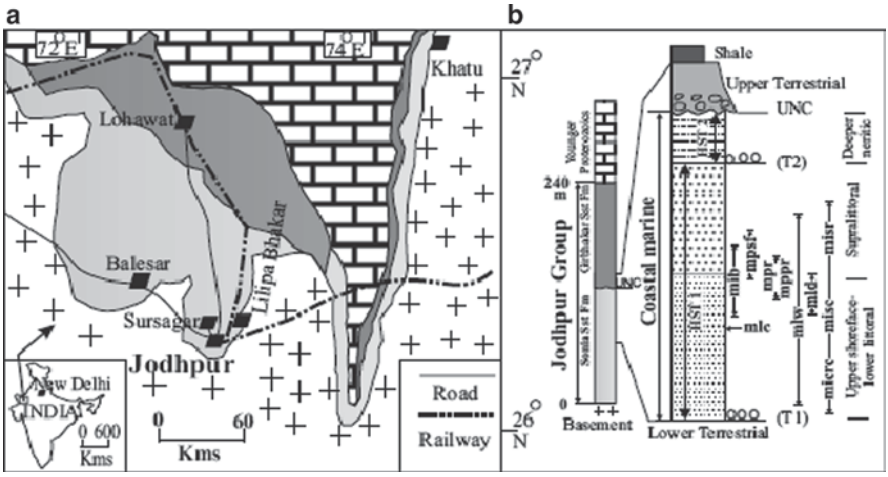

Figure 9. (**a**) Outcrop of Jodhpur Sandstone resting on rhyolitic basement and younger Proterozoic rocks around Jodhpur city, Rajasthan, India. (**b**) Note the paleoenvironmental range of the coastal marine interval of the Sonia Sandstone. Also note that all the MRS (using codes from Sarkar et al., 2008) are described essentially from the lower HST, shown above as HST 1.

zircons from the Jodhpur Group fixes the maximum age of the sedimentation at ca. 800 Ma (Malone et al., 2008).

All the MRS described herein from the Sonia Sandstone are from its younger coastal marine interval, especially from the older of the two high-stand systems tracts (HSTs) that comprise it (Fig. 9b; Sarkar et al., 2005; Catuneanu and Eriksson, 2007). The latter systems tract is progradational and characterized by a coarsening-upward trend. Repeated alternations between sets of cross-strata and planar laminae in its basal part are succeeded by similar structures with uncommon interbedded sandstone sheets with diversely oriented wave ripples, in its medial part; adhesion laminae, translatent strata, grainfall–grainflow cross-strata with occasional wave rippled sheets dominate the upper part of this older high-stand systems tract deposit. The inferred paleoenvironmental range is from shallow subtidal at the base, through a littoral medial portion, to supralittoral at the top. Detrital mica flakes associated with the inferred microbial mat features in the Sonia Sandstone very often show random orientation, or are inclined in a preferred direction, or alternatively, may be arranged in wavy-crinkly laminae; the latter two characteristics argue against simple vertical settling of the mica grains, and support trapping by microbial mats (Fig. 10a, b; cf. Gerdes and Krumbein, 1987; Schieber, 1999, 2004).

The mat-related structures in the basal HST of the coastal marine interval of the Sonia Sandstone can be assigned to three broad classes, using a classification scheme devised by Sarkar et al. (2008): (1) mat layer or mat ground ('ml structures'), (2) mat-induced ('mi structures') and (3) mat-protected ('mp structures'). To avoid confusion, we will here relate these to equivalent groups within the Schieber (2004) classification scheme. Sarkar et al.'s (2008) 'ml' class includes a structure, which is darker in color than the surrounding sediment, non-erosional at its base and corrugated at its top with chains of spindles, concentrically arranged to give rise to a discoidal form, possibly reflecting its most mature growth stage. The periphery of the discoidal form is mammilated, with rows of spindles gradually enlarging in size and occasionally branching, radiating from a centre (Fig. 11a). Facies interpretation by Sarkar et al. (2008) indicates its exclusive occurrence at the high littoral – low supralittoral zone, where mixing of meteoric water with hyper-saline water is thought to have facilitated early cementation to preserve a microbial mat-ground feature in its intact form. They cannot readily be equated with any specific MRS in the Schieber (2004) classification

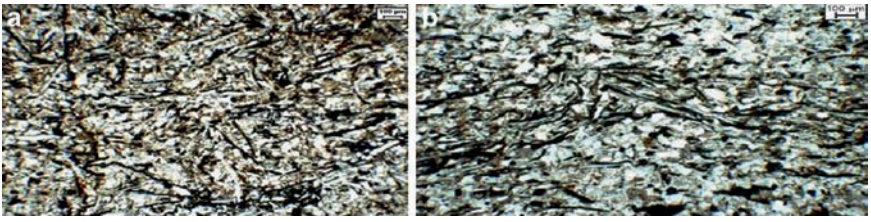

Figure 10. Random grain fabric (**a**) and wavy-crinkly laminae (**b**) within the Sonia Sandstone.

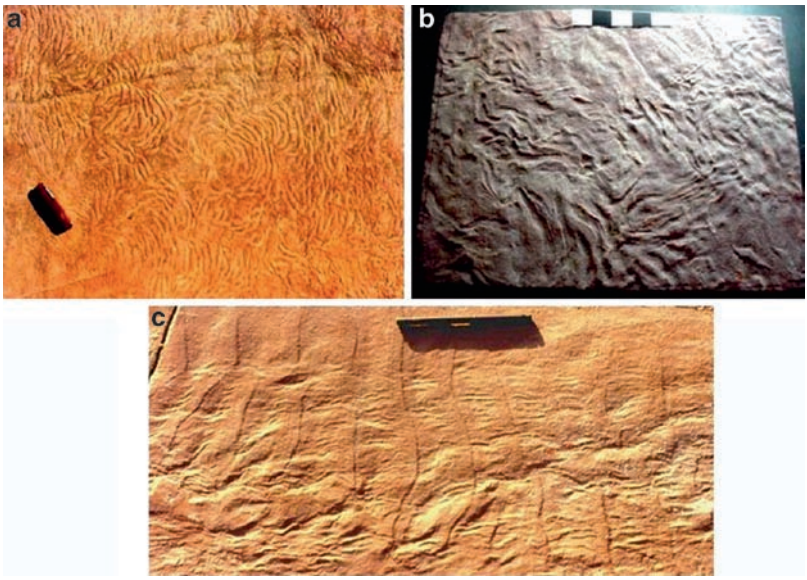

Figure 11. (**a**) Concentrically arranged discoidal forms in inferred mat grounds within the Sonia Sandstone. (**b**) Crumpled, deformed sandstone bed surface. (**c**) Possible soft sediment deformation of a rippled sandstone bed. Both (**b**) and (**c**) may reflect essentially soft sediment deformation processes rather than being direct mat proxies.

scheme, beyond a broad assignment to mat growth features. Another MRS found in the Sonia Sandstone comprises a sub-millimeter-thick sandstone sheet that appears crumpled and shows small horizontal drag folds in diverse orientations (Fig. 11b), resting on an undeformed dark pyritic lamina. The drag folds imply high-flow shear on a mat surface, a condition best satisfied in the low littoral zone, where this structure possibly formed (Fig. 9b). Alternatively, this MRS may merely reflect load structures formed at the base of a sandstone bed and preserved through the presence of an underlying microbial mat. Another possible MRS (Fig. 11c; see also Sarkar et al., 2008) cannot be related to any specific feature in the Schieber (2004) classification and may have formed through intra-stratal deformation of a rippled sandstone bed, possibly through soft sediment processes; this feature seems to be ubiquitous in distribution over the entire shallow subtidal to supralittoral paleoenvironmental range.

'Mat-induced' (mi) structures include polygonal desiccation or sand cracks (surface cracks in Schieber's 2004 classification), ripple-top sand cracks, flattened polygonal ridges and bulges (Fig. 12). None of these structures show preference for any specific sector of the possible paleoenvironmental range in the Sonia Sandstone coastal setting (Fig. 9b). However, they do occur broadly over an essentially littoral – lower supralittoral range of settings, as shown in the latter figure.

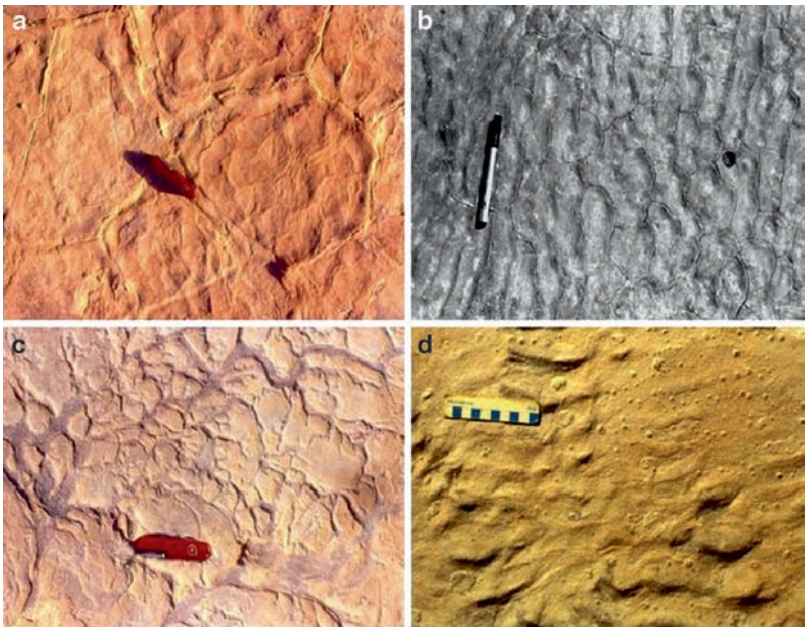

Figure 12. A set of MRS within the Sonia Sandstone, which Sarkar et al. (2008) describe as 'mat-induced' structures: (**a**) polygonal desiccation cracks (sand cracks), (**b**) ripple-top sand cracks, (**c**) flattened polygonal ridges of uncertain origin, and (**d**) bulges, which are probably mat-overgrown relict ripples, with overgrown and stabilized PS-domes at the *top right*.

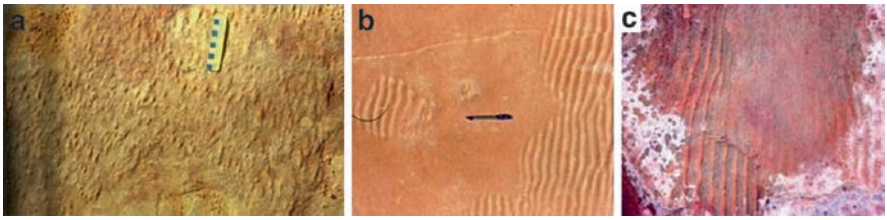

Figure 13. 'Mat protected' MRS within the Sonia Sandstone: (**a**) setulfs; (**b**) ripple patches, and (**c**) palimpsest ripples.

As a third group of MRS identified within the Sonia Sandstone, 'mat-protected' (mp) structures are thought to have been formed without direct influence of a microbial mat, but growth of the latter ensured their preservation. They include setulfs (cf. Friedman and Sanders, 1974; Bottjer and Hagadorn, 2007) (Fig. 13a), ripple patches (Noffke et al., 2001a; Bouougri and Porada, 2002; Sarkar et al., 2004) (Fig. 13b) and mat-protected ripples or palimpsest ripples (Fig. 13c) immediately underlying high-energy products, such as a set of planar laminae or large scale cross-strata. While the setulfs have been found in the littoral–supralittoral

transition, the ripple patches and palimpsest ripples are products of the Sonia Sandstone littoral zone deposits, especially in its upper portion (Fig. 9b). Setulfs in the Sonia Sandstone possess the same orientation as the associated eolian cross-strata and are thus inferred to have formed under action of strong wind on damp sand (Sarkar et al., 2008; see also Bottjer and Hagadorn, 2007); these conditions are most likely best satisfied in the supralittoral zone. On the other hand, the high-littoral zone with intermittent sedimentation favored mat growth that contributed to the preservation of delicate ripples in whole or in part during occasions of high-energy sediment input. All three structures, setulfs, ripple patches and palimpsest ripples, may also be preserved without a protecting mat on top. If such structures have formed through their normal genetic processes, then rapid covering of them through low-energy sedimentation will quite likely ensure their survival in the rock record as easily as a microbial mat cover may have achieved. However, if high-energy sedimentation processes are interpreted from succeeding clastic sediment beds, then the preservation of such structures can be ascribed to a microbial influence with much greater confidence. In all such field examples, it is imperative to ensure that thin mud layers are absent at the contacts between sandy beds bearing such structures and the succeeding arenitic bed; if such muds are present, they could have provided protection for preservation analogous to that derived from mats and an interpretation of these features as MRS is no longer justifiable.

4.3. VINDHYAN SUPERGROUP (~1.7–0.6 GA), BHANDARA CRATON

Sandstone and shale dominate in the 4.5-km-thick Vindhyan Supergroup that is well exposed in central India (Fig. 14a; Bose et al., 2001). This very thick Supergroup consists of only two sequences, separated from each other by an unconformity and its laterally correlatable conformity (Bose et al., 2001). Both sequences developed in an intra-cratonic basin, with a rift interpreted in the case of the lower Vindhyan and a sag basin inferred for the upper Vindhyan (Bose et al., 1997; Sarkar et al., 2002). Except for the basal formation, and another dominantly tuffaceous one (Porcellanite Formation), all the formations constituting the Supergroup and straddling across the unconformity are generally coarsening-upward with a lower shaley and an upper sandy part; carbonates intervene at certain intervals (Fig. 14b).

The oldest rock radiometrically dated in the Vindhyan Supergroup is from the Porcellanite Formation in the lower Vindhyan, giving a U-Pb zircon age a bit older than 1.6 Ga (Rasmussen et al., 2002; Ray et al., 2002) (Fig. 14b). The Rohtas Formation, the youngest of the lower Vindhyan units, yields an age of ca. 1.6 Ga (Fig.14b; Rasmussen et al., 2002; Ray et al., 2003; Sarangi et al., 2004). In the upper Vindhyan, Ray et al. (2003) fixed the age of the Ganurgarh Shale and the Bhander Limestone at 0.75 and 0.6 Ga, respectively. The youngest population of detrital zircon grains in the Upper Bhander Sandstone at the top of the Supergroup yields

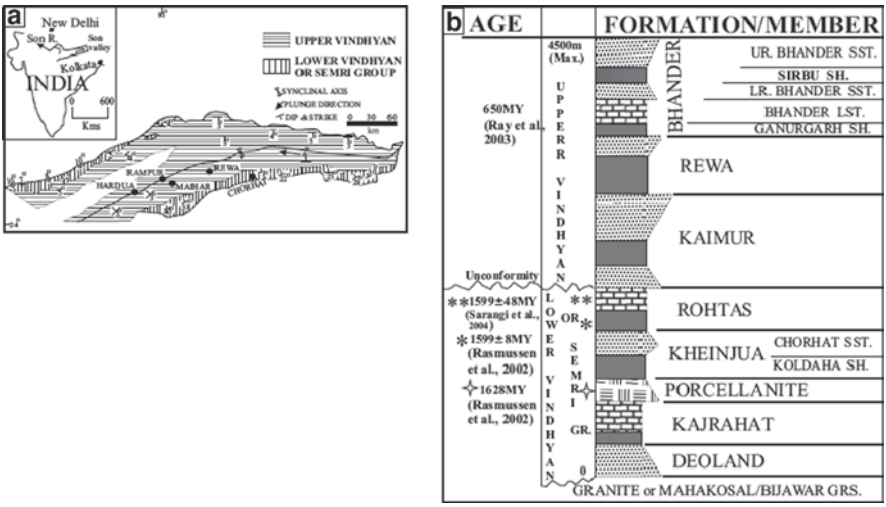

Figure 14. (**a**) Map showing outcrop distribution of the Vindhyan Supergroup in central India, and (**b**) a schematic summary of its stratigraphy. (Modified after Bose et al., 2001.)

an age of ca. 1,020 Ma, fixing the possible maximum age limit of the host sediment (Malone et al., 2008).

The inferred paleoenvironments of deposition of the Vindhyan Supergroup range from marine outer shelf to subaerial, through inner shelf, shoreface and the coastal tract (Singh, 1973; Akhtar, 1996; Bose et al., 2001). Secular swings through this depositional range caused repeated alternations between transgressive systems tracts (TSTs) and highstand system tracts (HSTs), both below and above the medial unconformity. The shale members in their basal parts (Fig. 14b) are assigned to the inner and outer shelf and developed into fining- and deepening-upward TSTs till the maximum flooding surfaces were reached. In contrast, the sandstone members largely constitute the HSTs, their marine influenced parts just straddling a shoreline setting.

MRS have been described from various stratigraphic intervals of the Vindhyan Supergroup, both from the TSTs and HSTs, but so far only from marine deposits (Sarkar et al., 2004, 2005, 2006; Bose et al., 2007; Banerjee and Jeevankumar, 2005; Banerjee et al., 2006). The shales in the TSTs are variously interbedded with storm-deposited sandstones, in the form mostly of lenticular sheets, and becoming reducing in thickness upward. The dominant structure within the shales is planar laminae, and in the condensed zone their sets may alternate with darker massive shale, and organic carbon content may exceed 1%. The interbedded sandstones generally have sharp bases and exhibit various kinds of current features; internally, they may either be massive, planar laminated or ripple laminated, and sandstone-tops are wave-rippled. The MRS reported from the shales of the TSTs, such as from the Rampur Shale and the Bijaigarh Shale include: wavy and crinkly carbonaceous laminae, rolled-up and

folded carbonaceous laminae and pyritic laminae (Fig. 15a, b; Banerjee et al., 2006; Sur et al., 2006). It is thought that a slow rate of sedimentation and a generally low energy milieu favored growth of microbial mats on the sea-floor, while occasional high energy input during storms caused deformation, tearing and transportation of mat fragments. The soles of the interbedded storm sandstones, such as those at the base of the deepest shelf succession of the Sirbu Shale bear various impressions, which appear to be mat fragments (Fig. 16a, b; Bose et al., 2007).

Interbedded sandstones at the bases of the HSTs have largely analogous characteristics, except for thickness, as those of the TSTs. In the HSTs of the Koldaha Shale (Banerjee and Jeevankumar, 2005) and of the Sirbu Shale, the MRS comprise mostly wrinkle structures, Kinneyia and 'elephant skin textures'. These MRS formed originally on bed-tops, but occur either at sandstone bed-tops or at the bases of the beds as bed-sole impressions (Fig. 17a–d). These MRS can be ascribed to flow shear, rapid loading of mats (producing small-scale load structures, preserved at bed-soles through underlying cohesive mats), minute slumps or fluid trapping, with the 'elephant skin textures' interpreted as reflecting reticulate mat growth processes (Porada and Bouougri, 2007b and references therein). For the interpreted fluid trapping, the inferred mat-related bed is underlain locally by a crinkled, dark-colored

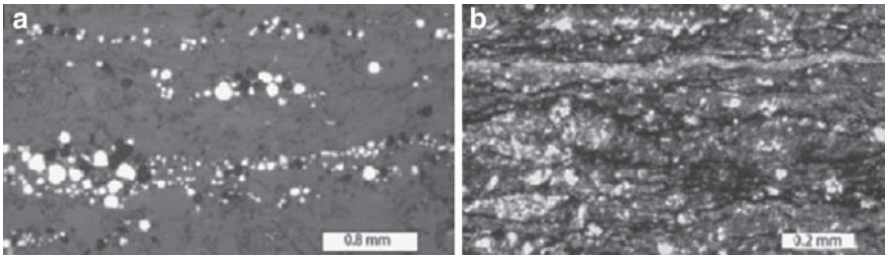

Figure 15. (**a**) Photomicrograph under reflected light showing wavy pyritic laminae within the Bijaigarh Shale. (**b**) Transmitted light photograph showing wavy, crinkly carbonaceous laminae within the Rampur Shale.

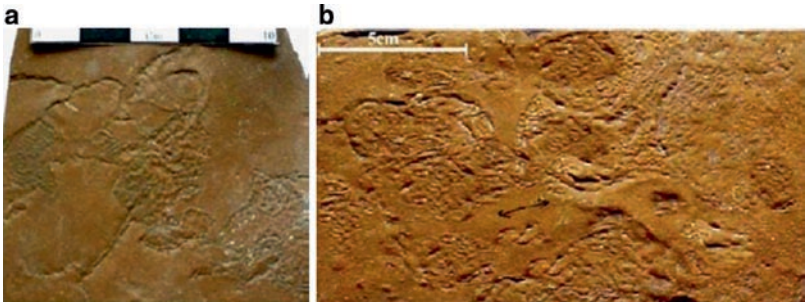

Figure 16. (**a**) Impressions of inferred mat fragments on a sandstone bed-sole. (**b**) Impression of inferred bed-sole mat fragments, with *arrow* indicating bidirectional paleocurrent directions.

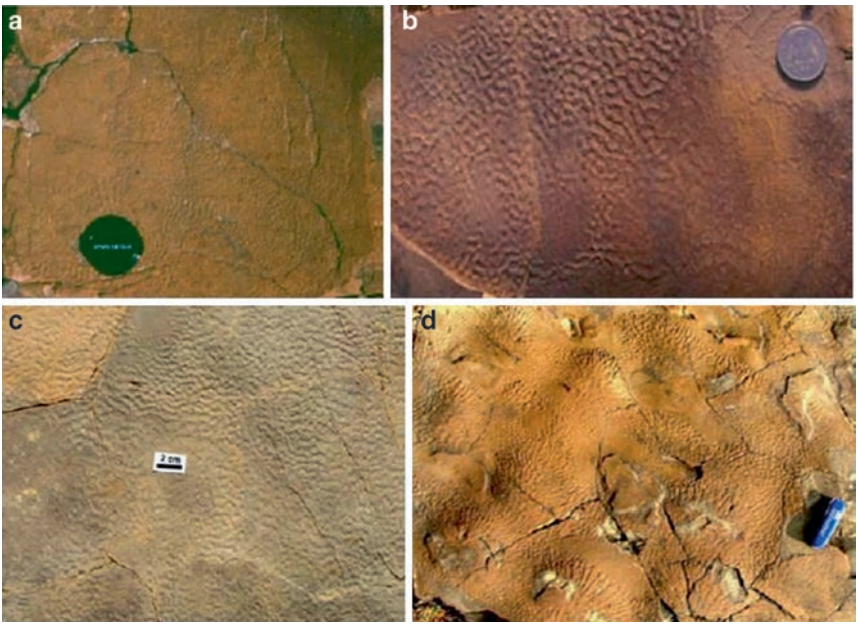

Figure 17. MRS present in the highstand systems tract sandstones (at bed-tops or their soles) of the Koldaha Shale (**a** and **b**) and the Sirbu Shale (**c** and **d**). (**a**) Wrinkle structures, (**b**) and (**c**) Kinneyia, (**d**) 'elephant skin texture'. (Lens cap diameter = 4.1 cm, pen knife = 7.3 cm long, coin diameter = 2.3 cm.)

lamina enriched in pyrite. Tops of sandstone beds in the Sirbu Shale also bear circular or elliptical forms made of minute concentrically arranged bead-like structures, possibly created by ring-like propagation of growth fronts of chemotactic bacteria releasing recurrent pulses of chemical compounds in response to their concentration gradients (Gerdes, 2007).

In the marine parts of the sandstone members of the HSTs, such as the Chorhat Sandstone, the members are made up of well-sorted sandstone beds, generally amalgamated, but separated by mud flasers only in the littoral zone. Large-scale cross-beds, wave ripples, interference ripples, plane beds and occasional massive beds with sole current structures characterize them. The tops of these beds bear a wide variety of MRS (Sarkar et al., 2006): palimpsest ripples (showing replication of older ripples; cf. Seilacher, 1999; Fig. 18a), ripple patches, *Astropolithon* with radiating cracks formed by gas escape having pierced mats (cf. Dawson, 1878; Fig. 18b), mat curls (Fig. 18c; see also Sarkar et al., 2004) and sand chips (cf. Pflüger and Gresse, 1996; Fig. 18d) formed through transportation of torn mat fragments. Close-set load structures, possibly reflecting rapid loading on gelatinous extra polymeric substance of freshly formed microbial mats, are described through their casts at sandstone bed-soles in the Chorhat Sandstone by Sarkar et al. (2004) who infer them to have formed in the neritic zone. *Manchuriophycus* (cf. Häntzschel, 1975; Cloud, 1973; Fig. 18e) and Kinneyia ripples (cf. Martinsson, 1965; Fig. 18f) are also

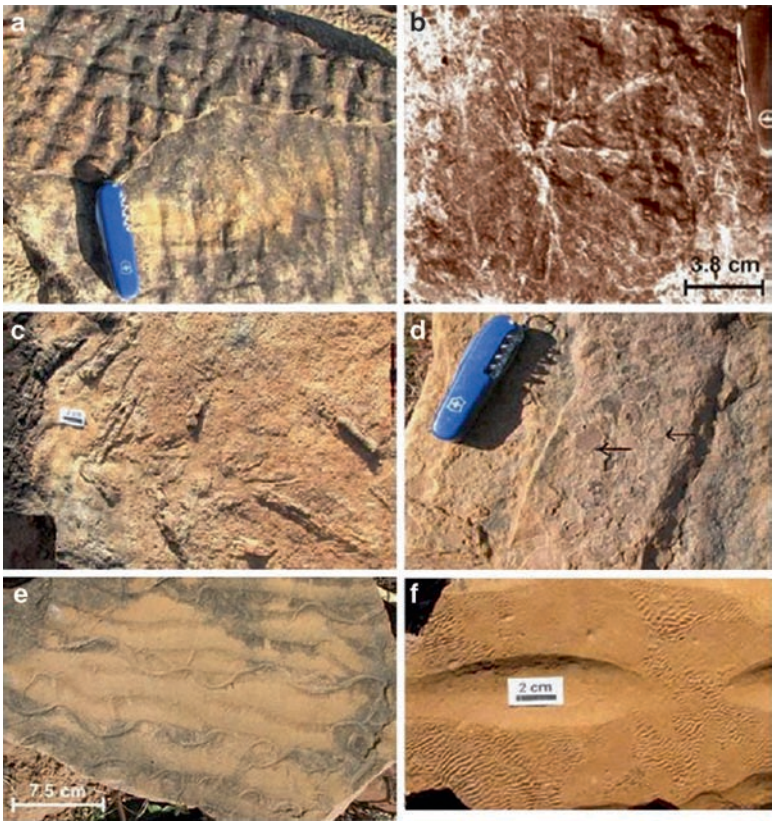

Figure 18. Field photographs of MRS from the highstand systems tract deposits of the Chorhat Sandstone: (**a**) palimpsest ripples, (**b**) *Astropolithon*, (**c**) mat curls or roll-ups, (**d**) microbial sand chips, (**e**) *Manchuriophycus*, (**f**) Kinneyia structures. (Pen knife = 7.3 cm in length.)

thought to have formed in the littoral zone, while varieties of sand cracks and ridges as well as wrinkle marks do not show any particular paleoenvironmental preference within a general shoreline/littoral setting. Sand chips, wrinkle marks and Kinneyia ripples are found also on the beds of the Lower and the Upper Bhander Sandstone, where they formed mostly in a littoral palaeoenvironment.

5. Influence of Mat Growth on Facies Stacking Patterns and Sequence Stratigraphic Architecture

The influence of microbial mats on sedimentation has been investigated in numerous publications, particularly from the point of view of process sedimentology and the mechanisms of formation of microbial activity related sedimentary structures.

At larger scales of observation, however, the stratigraphic context of microbial mats is less understood, and the relationship between the occurrence of microbial mats and the architecture of the stratigraphic record has only begun to be documented (e.g., Sarkar et al., 2005). We examine here the current understanding of the position and role of microbial mats within a sequence stratigraphic framework.

The abundance of microbial mats within depositional environments appears to have changed through geological time, with the highest proliferation inferred during the Palaeo- to Mesoproterozoic and partly Neoproterozoic (e.g., Pflüger and Sarkar, 1996; Eriksson et al., 2000; Schopf, 2004; Altermann, 2004; Sarkar et al., 2005). Recently, strong evidence for microbial mats influencing clastic sedimentation during the Meso- to Neoarchaean period has also been found (e.g., Noffke et al., 2003, 2006a, b). The exceptional preservation of some Precambrian sedimentary-basin-fills, such as in South Africa and India (e.g., work by Eriksson et al., 2000; Noffke et al., 2003, 2006b; Banerjee and Jeevankumar, 2005; Sarkar et al., 2005) has allowed for more insights into the role of microbial mats on sedimentation within a sequence stratigraphic framework. However, the broad siliciclastic shelf environments that accompanied Proterozoic continental and supercontinental environments (e.g., Eriksson et al., 2004) vastly expanded suitable marine settings conducive to flourishing microbial mat communities. The Proterozoic dominance of these microorganisms was followed by a sharp decline at the onset of the Phanerozoic with the rapid growth of grazing metazoan communities (Grotzinger, 1990).

Work on the Palaeoproterozoic and Neoproterozoic successions of central and western India (Banerjee and Jeevankumar, 2005; Sarkar et al., 2005) has documented trends that are in contrast to what is normally observed in the case of Phanerozoic sedimentary basins. Notably, the studied Precambrian sequences lack well-developed transgressive systems tracts, and are dominated by stacked prograding and aggrading 'normal regressive' systems tracts that may be separated only by thin veneers of transgressive deposits, often reduced to transgressive lags (e.g., Sarkar et al., 2005). In contrast, many Phanerozoic sequences include fully developed transgressive systems tracts, which consist of all depositional systems from fluvial, to coastal (particularly estuarine) and fully marine. Therefore, the formation and the degree of preservation of transgressive deposits may represent a key difference between Precambrian and Phanerozoic sequence stratigraphic architectures.

In a most general scenario, a transgressive systems tract consists of transgressive fluvial to coastal facies scoured at the top by transgressive ravinement surfaces, which in turn are onlapped by transgressive shallow-marine strata (Catuneanu, 2006). The absence (or poor development) of the fluvial to coastal section of the Precambrian transgressive systems tracts may be attributed to strong wave scouring in the upper shoreface during transgression, which may have removed much of the underlying section in the processes of shoreline backstepping. This also explains why microbial mats are not documented commonly from coastal to shoreface systems of Precambrian age, even though grazing organisms (common in the shoreface

environment during the Phanerozoic) were not present during that time. The amount of erosion associated with transgressive wave-ravinement surfaces is generally within a range of 20 m for the Phanerozoic (Demarest and Kraft, 1987), with exceptional values of 40 m recorded along the coastline of the present-day Canterbury Plains (Leckie, 1994). The latter magnitude of erosion may have been the norm in the pre-Phanerozoic time, thus explaining the poor preservation of the fluvial to coastal portion of the Precambrian transgressive systems tracts. The transgressive wave-ravinement processes can, however, not be used to explain the poor development of the marine shale portion of the Precambrian transgressive systems tracts (e.g., Catuneanu and Eriksson, 1999; Sarkar et al., 2005), as the transgressive shale accumulates on top of the wave-ravinement surfaces. The issue of the thin or absent transgressive shale of Precambrian sequences has been tackled by Sarkar et al. (2005), who interpreted that low sea-floor gradients, promoting rapid transgressions, coupled with a low sediment supply, may explain the observed lack of significant development of transgressive shale in the studied Proterozoic sections. In these case studies, the transgressive systems tract is typically reduced to a transgressive lag, which is preserved between stacked normal-regressive systems tracts of prograding and aggrading deposits. Aggradation under normal regressive conditions, in spite of the low sediment supply, was attributed to the prolific growth of microbial mats below the fairweather wave-base, within the shelf setting, which prevented deeper-water current reworking of sediments by the organic binding of particles (Sarkar et al., 2005). The preferential preservation of microbial mat-related structures within the deeper (shelf) portions of parasequences, unaffected by fairweather wave reworking, has also been documented by Banerjee and Jeevankumar (2005).

Whether or not these observed trends can be generalized still remains a question that requires further research. It is possible that insufficient Precambrian successions have been studied so far to draw meaningful conclusions.

6. Discussion

The fact that clastic sediment MRS have been studied most commonly from shallow marine intertidal–supratidal and hypersaline lagoonal settings, in modern environments (including also salt works and laboratory simulations) and their inferred ancient analogs (e.g., Gerdes et al., 1985a, b, c; Schneider, 1995; Noffke et al., 2001b; Gerdes and Klenke, 2003; Gerdes, 2007) has encouraged some workers to see this relationship as making the MRS, as a group of sedimentary structures, at least partially diagnostic of such (paleo)environments (e.g., Noffke et al., 2006b; Noffke, 2007). Within these two littoral settings, in ancient and modern examples, specific subenvironments have also been related to different sets of MRS (e.g., Gerdes et al., 2000; Noffke et al., 2001b; Parizot et al., 2005; Bose and Chafetz, 2009). In contrast, there is a widely held view, based partly on commonly observed opportunistic mat development as well as studies of modern and ancient clastic settings, that the MRS, as an entity, are essentially non-facies-specific, within an environmental continuum from

fully subaqueous shallow marine through coastal settings, sabkhas, and into continental environments including lakes, rivers and even deserts (Schieber, 1998; Eriksson et al., 2000; Schieber, 2007a; Schieber et al., 2007c; Gerdes, 2007). However, it should be noted that the abundance and variety of MRS is normally greatest within littoral sandstone settings (Schieber et al., 2007c).

An important facet of MRS in any setting is that the genesis of the microbial mats, at least that part encompassing colonization and establishment of the mats themselves, requires generally low energy levels combined with periods (weeks to months) of non-burial (Gerdes, 2007). However, for the formation of many MRS, high-energy events are necessary, such as for example in the genesis of many mat destruction features such as mat chips, roll-ups and mat fragments. Another caveat is that inferred paleoenvironments for MRS from ancient settings are interpretations rather than the observations implicit in modern settings. Also, detachment and transport of mat fragments may result in their preservation in sites other than those of their original formation (e.g., Fagerstrom, 1967; Schieber, 1999; Gerdes, 2007). The importance of microtopographic changes needs to be taken into account as well; these can be very rapid, and can alter the type and preservation of specific MRS within the littoral continuum, particularly at the subtidal–intertidal interface. The mats themselves and their MRS can also lead to microtopographic effects, as can factors like capillary water movement and evaporative pumping (as discussed in Section 3).

Schieber et al. (2007b) stress that despite metazoan grazing having reduced mat (and therefore also resultant MRS) abundance within many Phanerozoic settings, this has done little to affect the broad environmental adaptations of the mats and the MRS. It can also be added here that the deleterious effects of Phanerozoic metazoans on microbial mat development has perhaps been overstated. The fact that the onset of metazoan grazing activities began approximately at the Neoproterozoic–Phanerozoic boundary should not necessarily mean that there will be significant differences in the environmental relationships of mats and the concomitant MRS preserved within clastic sediments, between Precambrian-aged rocks and those of Phanerozoic affinity. The commonly held assertion that Phanerozoic-modern mats are essentially restricted to stressed environments such as hypersaline lagoons and partly desiccating tidal flats and are absent to rare in permanently subaqueous shallow marine settings may thus be an over-simplification.

Turning to the case studies within this paper, the ‘pre-eminence’ of the shallow marine intertidal–supratidal setting for developing mats and preserving MRS (as a group of features) has to be questioned. For the ca. 2.1 Ga Magaliesberg Formation from Kaapvaal, the dominant MRS is seen in the common succession of thin sandstone beds bearing palimpsest ripples – these beds are exposed over areas of several tens of meters or even a couple of hundred meters in each of two dimensions (e.g., Fig. 6). The near-perfect preservation of these ripples over large surface areas and in many succeeding beds supports a subtidal setting, where lack of exposure prevented any mat destruction and particularly any desiccation features from forming, and thus perfectly preserved large ripple fields due to

biostabilization. Of course, rapid covering of such rippled sandstone surfaces by relatively low energy deposition of further sand would equally preserve the ripples perfectly; however, such sand-depositing events are not that common. The predominant MRS within the Magaliesberg Formation is thus plausibly largely of mat-related origin, and of deeper water affinity than the intertidal-supratidal setting referred to above. The next most common MRS in this case study comprise cracked sandstone layers and polygonal petee ridges; both features occur essentially on earlier rippled sandstones and within exposures of several meters to tens of meters in each of two dimensions. Their origin can be ascribed to mat desiccation for the sand cracks and either decay-related gas or wind/water currents disturbing partially detached mats for the petee ridges (as discussed in Section 4.1); petees may also commonly form from lateral mat growth, through accommodation of lateral mat expansion (e.g., Bouougri et al., 2007). These Magaliesberg MRS can thus be interpreted as reflecting relatively uncommon exposure of biostabilized rippled sandstone bed surfaces, due to water level changes of greater magnitude and lesser frequency than those of tidal origin; in other words, water level changes due to relative sea level changes, most probably of local origin (through tectonics and sedimentation/erosion patterns) rather than eustatic genesis. Other MRS in the Magaliesberg Formation only occur locally and on small preserved bed portions. Taking this case study as a whole, the vast majority of the identified MRS thus reflect a shallow subtidal setting with only subordinate MRS from shallower environments within the intertidal–supratidal continuum.

The second case study, that of the ca. 0.6 Ga Sonia Sandstone, India, encompasses an inferred paleoenvironmental range from shallow subtidal to supralittoral, analogous to that interpreted for the Magaliesberg epeiric paleocoastline. Once again, a wide paleoenvironnmental range is interpreted for the identified MRS, but in this case study, the majority of the MRS appear to have been located within an essentially littoral to supralittoral setting (Section 4.2). Discoidal mat growth features are ascribed to a high littoral–low supralittoral setting, certain wrinkle-like structures are seen as being of low littoral affinity, and a set of MRS whose preservation reflected mat influences are interpreted as littoral–supralittoral features.

The final case study presented here, the ca. 1.7–0.6 Ga Vindhyan Supergroup, India, has an inferred paleoenvironmental range even wider than the two previous examples, from marine outer shelf through inner shelf, shoreface (cf., shallow subtidal) and the coastal (cf., intertidal/littoral and superlittoral) environments right through to subaerial settings (e.g., Bose et al., 2001; Section 4.3). The deeper shelf settings are dominated by MRS in shaly rocks reflecting predominantly low energy mat growth punctuated by uncommon storm-related mat-destruction features; the latter are complemented by mat fragments at the soles of storm-deposited sandstone beds (Bose et al., 2007); however, care should be taken when comparing sandy and muddy sediment-hosted MRS within paleoenvironmental interpretations. Within highstand systems tract deposits of the Vindhyan, MRS that are related to littoral environments are relatively common, and formed through a wide range of

genetic influences including mat growth, desiccation and physical mat destructive influences (Section 4.3). MRS interpreted as load-formed features at the contacts of sandstone soles with underlying mat-bound muds occur in neritic (cf., shelf) zone deposits (Sarkar et al., 2004). Other MRS, notably sand cracks and ridges, show no preferential environmental affinities within a broad littoral–supralittoral setting. Taken together, the Vindhyan case study suggests a range of settings (an inferred deep shelf to littoral continuum predominates) for the MRS, which is considerably broader than intertidal–supratidal niches.

7. Conclusions

It should be emphasized that this paper essentially examines the (paleo)environmental affinity of the MRS as a group of structures rather than the relationship between individual MRS and genetic influences from the settings where they form. All three case studies documented here provide analogous conclusions: (1) although littoral–supralittoral MRS are often considerable in number and variety, they cannot be seen as predominant; (2) MRS ascribed to permanently subaqueous, presumably subtidal settings are essentially just as common as those in (1); (3) in some cases, MRS show no preferential relationship to specific environments or subenvironments. The common epicratonic epeiric marine clastic basins of the Proterozoic period (cf. Eriksson et al., 2004) developed pervasive shallow marine sandy and muddy deposits, ascribed to a continuum of environments ranging from shelf to supralittoral, and within such deposits it is the better preserved littoral sandstones that attract most of the attention, with their range of well-preserved MRS. The thinner deeper shelf sandstones and mudrocks are generally less well studied, this possibly leading to viewpoints supportive of a supposedly strong tidal–supratidal MRS relationship. Growth of microbial mats within these Proterozoic deposits very possibly also influenced sedimentary architecture and sequence stratigraphic trends, as discussed in Section 5. However, other factors such as sediment supply and low sea-floor gradients in intracratonic and epicratonic basins also played a role (e.g., Sarkar et al., 2005; Banerjee and Jeevankumar, 2005; Catuneanu and Eriksson, 2007). More case studies of the interplay of microbial mat growth and sequence stratigraphy are needed to constrain the general trends discussed in Section 5.

There have been relatively few studies attempting to establish the environmental affinities of individual MRS or groups thereof (see, however, Section 3), and this needs to be remedied so that the MRS can take their place amongst the physically (and chemically) formed sedimentary structures familiar to geologists and sedimentologists. Certainly, much work has been done on the genetic processes responsible for specific MRS, and these are relatively well understood (e.g., Schieber et al., 2007b). These smaller-scale studies need to be integrated into a larger-scale environmental framework so that sets of MRS can be established that are characteristic of a specific setting, just as is the case for the better-known

physically formed structures. For the MRS, as is already well known for the physically formed structures, certain features will occur in many different environments, while others have a much more restricted range of settings. As such, the MRS will greatly enhance the subtleties of paleoenvironmental interpretation of ancient preserved facies and deposits, and extend the tools available to earth scientists to do such studies.

8. References

Akhtar, K. (1996) Facies, sedimentation processes and environments in the Proterozoic Vindhyan Basin, India, In: A. Bhattacharyya (ed.) *Recent Advances in Vindhyan Geology*. Memoir 36, Geological Society of India, Bangalore, pp. 127–136.

Altermann, W. (2004) Evolution of life and precambrian bio-geology, In: P.G. Eriksson, W. Altermann, D.R. Nelson, W.U. Mueller and O. Catuneanu (eds.) *The Precambrian Earth: Tempos and Events.* Elsevier, Amsterdam, pp. 587–591.

Altermann, W., Kazmierczak, J., Oren, A. and Wright, D.T. (2006) Cyanobacterial calcification and its rock-building potential during 3.5 billion years of earth history. Geobiology **4/3**: 147–166.

Banerjee, S. and Jeevankumar, S. (2005) Microbially originated wrinkle structures on sandstone and their stratigraphic context: palaeoproterozoic Koldaha Shale, central India. Sediment. Geol. **176**: 211–224.

Banerjee, S., Dutta, S., Paikaray, S. and Mann, U. (2006) Stratigraphy, sedimentology and bulk organic geochemistry of black shales from the Proterozoic Vindhyan Supergroup (central India). J. Earth Syst. Sci. **115/1**: 37–47.

Bosch, P.J.A. and Eriksson, P.G. (2008) A note on two occurrences of inferred microbial mat features preserved in the c. 2.1 Ga Magaliesberg Formation (Pretoria Group, Transvaal Supergroup) sandstones, near Pretoria, South Africa. S. Afr. J. Geol. **111**: 251–262.

Bose, S. and Chafetz, H.S. (2009) Topographic control on distribution of modern microbially induced sedimentary structures (MISS): a case study from Texas coast. Sediment. Geol. **213**: 136–149.

Bose, P.K., Banerjee, S. and Sarkar, S. (1997) Slope-controlled seismic deformation and tectonic framework of deposition: Koldaha Shale, India. Tectonophysics **269**: 151–169.

Bose, P.K., Sarkar, S., Chakraborty, S. and Banerjee, S. (2001) Overview of the Meso- to Neoproterozoic evolution of the Vindhyan basin, central India. Sediment. Geol. **141/142**: 395–419.

Bose, P.K., Sarkar, S., Banerjee, S. and Chakraborty, S. (2007) Mat-related features from sandstone of the Vindhyan Supergroup in central India, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 181–188.

Boshoff, A.J. (1992) 'n Studie van die Magaliesberg Formasie in die gebied suid van Bornkhorstspruit. Unpublished Honours Thesis, University of Pretoria, 32 pp.

Bottjer, D. and Hagadorn, J.W. (2007) Mat growth features, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 53–71.

Bouougri, E. and Porada, H. (2002) Mat-related sedimentary structures in Neoproterozoic peritidal passive margin deposits of the West African craton (Anti-Atlas, Morocco). Sediment. Geol. **153**: 85–106.

Bouougri, E.H. and Porada, H. (2007) Siliciclastic biolaminites indicative of widespread microbial mats in the Neoproterozoic Nama Group of Namibia. J. Afr. Earth Sci. **48**: 38–48.

Bouougri, E.H., Gerdes, G. and Porada, H. (2007) Inherent problems of terminology: definition of terms frequently used in connection with microbial mats, In: J. Schieber, P.J. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features*

Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2. Elsevier, Amsterdam, pp. 145–151.

Buick, I.S., Maas, R. and Gibson, R. (2001) Precise U–Pb titanite age constraints on the emplacement of the Bushveld Complex, South Africa. J. Geol. Soc. London **158**: 3–6.

Bumby, A.J., Eriksson, P.G. and Van der Merwe, R. (1998) Compressive deformation of the floor rocks to the Bushveld Complex (South Africa): evidence from the Rustenburg Fault Zone. J. Afr. Earth Sci. **27**: 307–330.

Catuneanu, O. (2006) *Principles of Sequence Stratigraphy*, 1st ed. Elsevier, Amsterdam, 375 pp.

Catuneanu, O. and Eriksson, P.G. (1999) The sequence stratigraphic concept and the Precambrian rock record: an example from the 2.7–2.1 Ga Transvaal Supergroup, Kaapvaal craton. Precambrian Res. **97**: 215–251.

Catuneanu, O. and Eriksson, P.G. (2007) Sequence stratigraphy of the Precambrian. Gondwana Res. **12/4**: 560–565.

Chauhan, D.S. and Ram, B. (1999) Ripple marks and synthesis of Beach Sequences: a study of Early Palaeozoic Jodhpur Sandstone, Western Rajasthan, In: B.S. Palliwal (ed.) *Geological Evolution of Northwestern India*. Scientific Publishers, India, pp. 129–147.

Chauhan, D.S., Mathur, K.M. and Ram, N. (2001) Geological nature of the Pokaran boulder bed: palaeoenvironmental, palaeoclimatic and stratigraphic implications. J. Geol. Soc. India **58**: 425–433.

Cloud, P. (1973) Pseudofossils: a plea for caution. Geology **1**: 123–127.

Davis, R.A. (1968) Algal stromatolites composed of quartz sandstone. J. Sediment. Petrol. **38**: 953–955.

Dawson, J.W. (1878) *Acadian Geology, the Geological Structure, Organic Remains and Mineral Resources of Nova Scotia*. New Brunswick, and Prince Edward Island. Supplement to 2nd edition. Macmillan, London, 102 pp.

Decho, A.W. (1990) Microbial exopolymer secretions in ocean environments: their roles in food webs and marine processes. Oceanogr. Mar. Biol. Ann. Rev. **28**: 73–153.

Decho, A.W. (2000) Expolymer microdomains as a structuring agent for heterogeneity within microbial Biofilms, In: R. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Berlin, pp. 9–15.

Demarest, J.M. and Kraft, J.C. (1987) Stratigraphic record of quaternary sea levels: implications for more ancient strata, In: D. Nummedal, O.H. Pilkey and J.D. Howard (eds.) *Sea Level Fluctuation and Coastal Evolution*. Special Publication 41, Society for Economic Paleontologists and Mineralogists, Tulsa, OK, pp. 223–239.

Eriksson, P.G., Reczko, B.F.F., Boshoff, A.J., Schreiber, U.M., Van der Neut, M. and Snyman, C.P. (1995) Architectural elements from lower proterozoic braid-delta and high-energy tidal flat deposits in the Magaliesberg Formation, Transvaal Supergroup, South Africa. Sediment. Geol. **97**: 99–117.

Eriksson, P.G., Van der Merwe, R. and Bumby, A.J. (1998) The Palaeoproterozoic Woodlands Formation of eastern Botswana – northwestern South Africa: lithostratigraphy and relationship with Transvaal basin inversion structures. J. Afr. Earth Sci. **27**(3–4): 349–358.

Eriksson, P.G., Simpson, E.L., Eriksson, K.A., Bumby, A.J., Steyn, G.L. and Sarkar, S. (2000) Muddy roll-up structures in siliciclastic interdune beds of the ca. 1.8 Ga Waterberg Group, South Africa. Palaios **15**: 177–183.

Eriksson, P.G., Altermann, W., Catuneanu, O, van der Merwe, R. and Bumby, A.J. (2001) Major influences on the evolution of the 2.67–2.1 Ga Transvaal basin, Kaapvaal craton. Sediment. Geol. **141–142**: 205–231.

Eriksson, P.G., Altermann, W., Eberhardt, L., Ahrend-Heidbrinck, S. and Bumby, A.J. (2002) Palaeoproterozoic epeiric sea palaeoenvironments: the Silverton Formation (Pretoria Group, Transvaal Supergroup), South Africa, In: W. Altermann and P.L. Corcoran (eds.) *Precambrian Sedimentary Environments: A Modern Approach to Ancient Depositional Systems*. Special Publication 33, International Association of Sedimentologists. Blackwell, Oxford, pp. 351–367.

Eriksson, P.G., Altermann, W., Nelson, D.R., Mueller, W.U. and Catuneanu, O. (eds.) (2004) *The Precambrian Earth: Tempos and Events*. Elsevier, Amsterdam, 941 pp.

Eriksson, P.G., Schieber, J., Bouougri, E., Gerdes, G., Porada, H., Banerjee, S., Bose, P.K. and Sarkar, S. (2007a) Classification of structures left by microbial mats in their host sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 39–52.

Eriksson, P.G., Porada, H., Banerjee, S., Bouougri, E., Sarkar, S. and Bumby, A.J. (2007b) Mat destruction features, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 76–105.

Fagerstrom, J.A. (1967) Development, flotation and transportation of mud crusts-neglected factors in sedimentology. J. Sediment. Petrol. **37**: 73–79.

Friedman, G.M. and Sanders, J.E. (1974) Positive-relief bedforms on modern tidal-flat that resemble molds of flutes and grooves: implications for geopetal criteria and for origin and classification of bedforms. J. Sediment. Petrol. **44**: 181–189.

Gavish, E., Krumbein, W.E. and Halevy, J. (1985) Geomorphology, mineralogy and groundwater chemistry as factors of the hydrodynamic system of the Gavish Sabkha, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems: The Gavish Sabkha*. Springer, Berlin, pp. 186–217.

Gehling, J.G. (1999) Microbial mats in terminal Proterozoic siliciclastics: Ediacaran death masks. Palaios **14**: 40–57.

Gehling, J.G. (2000) Environmental interpretation and a sequence stratigraphic framework for the terminal Proterozoic Ediacara Member within the Rawnsley Quartzite, South Australia. Precambrian Res. **100**: 65–95.

Gerdes, G. (2007) Structures left by modern microbial mats in their host sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 5–38.

Gerdes, G. and Klenke, T. (2003) Geologische Bedeutung ökologischer Zeiträume in biogener Schichtung (Mikrobenmatten, potentielle Stromatolithe). Mitt. Ges. Geol. Bergbaustud. Österr **46**: 35–49.

Gerdes, G. and Klenke, T. (2007) States of biogenic bedding as records of the interplay of ecologic time and environment (a case study of modern siliciclastic sediments, Mellum Island, southern North Sea). Senckenbergiana Maritima **37/2**: 129–144.

Gerdes, G. and Krumbein, W.E. (1987) Biolaminated deposits, In: G.M. Bhattacharya, G.M. Friedmann, H.J. Neugebauer and A. Seilacher (eds.) *Lecture Notes in Earth Sciences 9*. Springer, Berlin, pp. 1–183.

Gerdes, G., Krumbein, W.E. and Holtkamp, E.M. (1985a) Salinity and water activity related zonation of microbial communities and potential stromatolites of the Gavish Sabkha, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems: The Gavish Sabkha*. Springer, Berlin, pp. 238–266.

Gerdes, G., Krumbein, W.E. and Reineck, H.E. (1985b) The depositional record of sandy, versicoloured tidal flats (Mellum Island, southern North Sea). J. Sediment. Petrol. **55**: 265–278.

Gerdes, G., Krumbein, W.E. and Reineck, H.E. (1985c) Verbreitung und aktuogeologische Bedeutung mariner mikrobieller Matten im Gezeitenbereich der Nordsee. Facies **12**: 75–96.

Gerdes, G., Claes, M., Dunajtschik-Piewak, K., Riege, H., Krumbein, W.E. and Reineck, H.-E. (1993) Contribution of microbial mats to sedimentary surface structures. Facies **29**: 61–74.

Gerdes, G., Klenke, T. and Noffke, N. (2000) Microbial signatures in peritidal siliciclastic sediments: a catalogue. Sedimentology **47**: 279–308.

Gerdes, G., Porada, H. and Bouougri, E.H. (2008) Bio-sedimentary structures evolving from the interaction of microbial mats, burrowing beetles and the physical environment of Tunisian coastal sabkhas. Senckenbergiana Maritima **38**: 45–58.

Golubic, S. (1976) Organisms that build stromatolites, In: R.W. Walter (ed.) *Stromatolites: Developments in Sedimentology 20*. Elsevier, Amsterdam, pp. 113–126.

Grotzinger, J.P. (1990) Geochemical model for Proterozoic stromatolite decline. Am. J. Sci. **290**: 80–103.

Hagadorn, J.W. and Bottjer, D.J. (1999) Restriction of a Late Neoproterozoic biotope: suspect-microbial structures and trace fossils at the Vendian-Cambrian transition. Palaios **14**: 73–85.

Hagadorn, J.W., Pflüger, F. and Bottjer, D.J. (1999) Unexplored microbial worlds. Palaios **14**: 1–2.
Häntzschel, W. (1975) Trace fossils and problematica, In: C. Teichert (ed.) *Treatise on Invertebrate Paleontology*, Part W, Miscellanea, Supplement 1. Geological Society of America and University of Kansas Press, Boulder, CO and Lawrence, KS, pp. W1-W269.
Hoffman, P. (1976). Stromatolite morphogenesis in Shark Bay, Western Australia, In: R.M. Walter (ed.) *Stromatolites: Developments in Sedimentology 20.* Elsevier, Amsterdam, pp. 261–271.
Horodyski, R.J. (1982) Impressions of algal mats from the Middle Proterozoic Belt Supergroup, northwestern Montana. Sedimentology **29**: 285–289.
Hsü, K. and Siegenthaler, C. (1969) Preliminary experiments on hydrodynamic movement induced by evaporation and their bearing on the dolomite problem. Sedimentology **12**: 11–25.
Kendall, C.G.S.C. and Skipwith, P.A.d'E. (1969) Geomorphology of a recent shallow-water carbonate province: Khor Al Bazam, Trucial Coast, Southwest Persian Gulf. Geol. Soc. Am. Bull. **80**: 865–892.
Kinsman, D.J.J. and Park, R.K. (1976). Algal belt and coastal sabkha evolution, Trucial Coast, Persian Gulf, In: R.M. Walter (ed.) *Stromatolites: Developments in Sedimentology 20*. Elsevier, Amsterdam, pp. 421–433.
Krumbein, W.E. and Cohen, Y. (1977) Primary production, mat formation and lithification: contribution of oxygenic and facultative anoxygenic cyanobacteria, In: E. Flügel (ed.) *Fossil Algae*. Springer, Berlin, pp. 37–56.
Krumbein, W.E. and Werner, D. (1983) The microbial silica cycle, In: W.E. Krumbein (ed.) *Microbial Geochemistry*. Blackwell, Oxford, pp. 125–157.
Krumbein, W.E., Paterson, D.M. and Stal, L. (eds.) (1994) *Biostabilization of Sediments*. Bibliotheks- und Informationssystem der Universität Oldenburg, Oldenburg, 526 pp.
Krumbein, W.E., Brehm, U., Gerdes, G., Gorbushina, A.A., Levit, G. and Palinska, K.A. (2003) Biofilm, biodictyon, biomat, microbialites, oolites, stromatolites, geophysiology, global mechanism, parahistology, In: W.E. Krumbein, G.A. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms*. Kluwer, Dordrecht, pp. 1–27.
Leckie, D.A. (1994) Canterbury Plains, New Zealand – implications for sequence stratigraphic models. Am. Assoc. Petrol. Geol. Bull. **78**: 1240–1256.
Logan, B.W., Hoffman, P. and Gebelein, C.D. (1974). Algal mats, cryptalgal fabrics, and structures, Hamelin Pool, Western Australia. AAPG Mem. **22**: 140–194.
Malone, S.J., Meert, J.G., Banerjee, D.M., Pandit, M.K., Tamrat, E., Kamenov, G.D., Pradhan, V.R. and Sohl, L.E. (2008) Paleomagnetism and detrital zircon geochronology of the Upper Vindhyan Sequence, Son Valley and Rajasthan, India: A ca. 1000 Ma age for the Purana Basins? Precambrian Res. **164**: 137–159.
Martinsson, A. (1965) Aspects of a middle Cambrian thanatotope on Öland. Geologiska Föreningens I Stockholm Förhandlingar **87**: 181–230.
Miall, A.D. (1985) Architectural-element analysis; a new method of facies analysis applied to fluvial deposits. Earth Sci. Rev. **22**: 261–308.
Monty, C.L.V. (1979) The origin and development of cryptalgal fabrics, In: R.M. Walter (ed.) *Stromatolites: Developments in Sedimentology 20*. Elsevier, Amsterdam, pp. 193–259.
Noffke, N. (2007) Microbially-induced sedimentary structures (MISS) of Early and Middle Archaean Ages – Moodies Group, Pongola Supergroup, Witwatersrand Supergroup (South Africa), In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 153–157.
Noffke, N. and Krumbein, W.E. (1999) A quantitative approach to sedimentary surface structures contoured by the interplay of microbial colonization and physical dynamics. Sedimentology **46**: 417–426.
Noffke, N., Gerdes, G., Klenke, T. and Krumbein, W.E. (2001a) Microbially induced sedimentary structures – a new category within the classification of primary sedimentary structures. J. Sediment. Res. **A71**: 649–656.
Noffke, N., Gerdes, G., Klenke, T. and Krumbein, W. (2001b) Microbially induced sedimentary structures indicating climatological, hydrological and depositional conditions within Recent and Pleistocene coastal facies zones (Southern Tunesia). Facies **44**: 23–30.

Noffke, N., Hazen, R. and Nhleko, N. (2003) Earth's earliest microbial mats in a siliciclastic marine environment (2.9 Ga Mozaan Group, South Africa). Geology **31**: 673–676.

Noffke, N., Beukes, N. and Hazen, R. (2006a) Spatial and temporal distribution of microbially induced sedimentary structures: a case study from siliciclastic storm deposits of the 2.9 Ga old Witwatersrand Supergroup, South Africa. Precambrian Res. **146**: 35–44.

Noffke, N., Eriksson, K.A., Hazen, R.M. and Simpson, E.L. (2006b) A new window into Early Archean life: microbial mats in Earth's oldest siliciclastic deposits (3.2 Ga Moodies Group, South Africa). Geology **34**: 253–256.

Pareek, H.S. (1984) Pre-quaternary geology and mineral resources of northwestern Rajasthan. Mem. Geol. Surv. India **115**: 1–95.

Parizot, M., Eriksson, P.G., Aifa, T., Sarkar, S., Banerjee, S., Catuneanu, O., Altermann, W., Bumby, A.J., Bordy, E.M., Louis van Rooy, J. and Boshoff, A.J. (2005) Suspected microbial mat-related crack-like sedimentary structures in the Palaeoproterozoic Magaliesberg Formation sandstones, South Africa. Precambrian Res. **138**: 274–296.

Park, R.K. (1977) The preservation potential of some recent stromatolites. Sedimentology **24**: 485–506.

Pflüger, F. (1999) Matground structures and redox facies. Palaios **14**: 25–39.

Pflüger, F. and Gresse, P.G. (1996) Microbial sand chips – a non-actualistic sedimentary structure. Sediment. Geol. **102**: 263–274.

Pflüger, F. and Sarkar, S. (1996) Precambrian bedding planes – bound to remain. Geol. Soc. Am. Abstr. Program. **28**: 491.

Porada, H. and Bouougri, E. (2007a) 'Wrinkle Structures' – a critical review, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 135–144.

Porada, H. and Bouougri, E. (2007b) Wrinkle structures – a critical review. Earth Sci. Rev. **81**: 199–215.

Porada, H., Bouougri, E.H. and Ghergut, J. (2007) Hydraulic conditions and mat-related structures in Tidal Flats and Coastal Sabkhas, In: J. Schieber, P.J. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 258–265.

Rasmussen, B., Bose, P.K., Sarkar, S., Banerjee, S., Fletcher, I.R. and McNaughton, N.J. (2002) 1.6 Ga U–Pb zircon ages for the Chorhat Sandstone, Lower Vindhyan, India: possible implication for early evolution of animals. Geology **30**: 103–106.

Ray, J.S., Martin, M.W. and Veizer, J. (2002) U–Pb zircon dating and Sr isotope systematics of the Vindhyan Supergroup, India. Geology **30**: 131–134.

Ray, J.S., Veizer, J. and Davis, W.J. (2003) C, O, Sr and Pb isotope systematics of carbonate sequences of the Vindhyan Supergroup, India: age, diagenesis, correlation and implications for global events. Precambrian Res. **121**: 103–140.

Reineck, H.-E. (1979) Rezente und fossile Algenmatten und Wurzelhorizonte. Natur und Museum **109**: 290–296.

Reineck, H.-E., Gerdes, G., Claes, M., Dunajtschik, K., Riege, H. and Krumbein, W.E. (1990) Microbial modification of sedimentary surface structures, In: D. Heling, P. Rothe, U. Förstner and P. Stoffers (eds.) *Sediments and Environmental Geochemistry*. Springer, Berlin, pp. 254–276.

Riding, R.E. and Awramik, S.M. (2000) *Microbial Sediments*. Springer, Berlin, 331 pp.

Sarangi, S., Gopalan, K. and Kumar, S. (2004) Pb–Pb age of earliest megascopic eukaryotic alga bearing Rohtas Formation, Vindhyan Supergroup, India: implications for Precambrian atmospheric oxygen evolution. Precambrian Res. **132**: 107–121.

Sarkar, S., Chakraborty, S., Banerjee, S. and Bose, P.K. (2002) Facies sequence and cryptic imprint of sag tectonics in late Proterozoic Sirbu Shale, central India, In: W. Altermann and P.L. Corcoran (eds.) *Precambrian Sedimentary Environments: A Modern Approach to Ancient Depositional Systems.* Blackwell, Oxford, pp. 369–382.

Sarkar, S., Banerjee, S. and Eriksson, P.G. (2004) Microbial mat features in sandstones illustrated, In: P.G. Eriksson, W. Altermann, D.R. Nelson, W.U. Mueller and O. Catuneanu (eds.) *The Precambrian Earth: Tempos and Events*. Special Publication of the International Association of Sedimentologists 33. Elsevier, Amsterdam, pp. 673–675.

Sarkar, S., Banerjee, S., Eriksson, P.G. and Catuneanu, O. (2005) Microbial mat control on siliciclastic Precambrian sequence stratigraphic architecture: examples from India. Sediment. Geol. **176**: 195–209.

Sarkar, S., Banerjee, S., Samanta, P. and Jeevankumar, S. (2006) Microbial mat-induced sedimentary structures in siliciclastic sediments: examples from the 1.6 Ga Chorhat Sandstone, Vindhyan Supergroup, MP, India. J. Earth Syst. Sci. **115/1**: 49–60.

Sarkar, S., Bose, P.K., Samanta, P., Sengupta, P. and Eriksson, P.G. (2008) Microbial mat-mediated structures in the Ediacaran Sonia sandstone, Rajasthan, India and their implications for Proterozoic sedimentation. Precambrian Res. **162**: 248–263.

Schieber, J. (1986) The possible role of benthic microbial mats during the formation of carbonaceous shales in shallow Mid-Proterozoic basins. Sedimentology **33**: 521–536.

Schieber, J. (1998) Possible indicators of microbial mat deposits in shales and sandstones: examples from the Mid-Proterozoic Belt Supergroup, Montana, USA. Sediment. Geol. **120**: 105–124.

Schieber, J. (1999) Microbial mats in terrigenous clastics: the challenge of identification in the rock record. Palaios **14**: 3–12.

Schieber, J. (2004) Microbial mats in the Siliciclastic Rock Record: a summary of the diagnostic features, In: P.G. Eriksson, W. Altermann, D.R. Nelson, W.U. Mueller and O. Catuneanu (eds.) *The Precambrian Earth: Tempos and Events. Developments in Precambrian Geology 12*. Elsevier, Amsterdam, pp. 663–673.

Schieber, J. (2007a) Microbial mats on muddy substrates – examples of possible sedimentary features and underlying processes, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 117–133.

Schieber, J. (2007b) Flume experiments on the durability of sandy microbial mat fragments during transport, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 248–257.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (2007a) Prologue: an introduction to microbial mats, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 1–3.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (eds.) (2007b) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, 311 pp.

Schieber, J., Bose, P.K., Eriksson, P.G. and Sarkar, S. (2007c) Palaeogeography of microbial mats in terrigenous clastics – environmental distribution of associated sedimentary features and the role of geologic time, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 267–275.

Schneider, J. (1995) Eindunstung von Meerwasser und Salzbildung in Salinen. Kali Steinsalz **11**: 325–330.

Schopf, J.W. (2004) Earth's earliest biosphere: status of the Hunt, In: P.G. Eriksson, W. Altermann, D.R. Nelson, W.U. Mueller and O. Catuneanu (eds.) *The Precambrian Earth: Tempos and Events.* Elsevier, Amsterdam, pp. 516–539.

Seilacher, A. (1964) Sedimentological classification and nomenclature of trace fossils. Sedimentology **3**: 253–256.

Seilacher, A. (1999) Biomat-related lifestyles in the Precambrian. Palaios **14**: 86–93.

Singh, I.B. (1973) Depositional environment of the Vindhyan Sediments in the Son Valley Area, In: V.K. Verma (ed.) *Recent Researches in Geology*, Vol. 1. Hindusthan Publishing, New Delhi, pp. 140–152.

Sur, S., Schieber, J. and Banerjee, S. (2006) Petrographic observations suggesting of microbial mats from Rampur shale and Bijoygarh Shale, Vindhyan basin, India. J. Earth Syst. Sci. **115**: 61–66.

Tanner, W.F. (1967) Ripple mark indices and their uses. Sedimentology **9**: 89–104.

Tanner, W.F. (1971) Numerical estimates of ancient waves, water depths and fetch. Sedimentology **16**: 71–88.

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MICROBIALLY RELATED STRUCTURES IN SILICICLASTIC SEDIMENT RESEMBLING EDIACARAN FOSSILS: EXAMPLES FROM INDIA, ANCIENT AND MODERN

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1. Introduction

Ediacaran fossils represent a distinct group of large and structurally complex, enigmatic, soft-bodied organisms dominating the end-Precambrian (Ediacaran) oceans, with an age range from 630 to 542 Ma (Martin et al., 2000; Knoll et al., 2004, 2006). Microbes constituted the Precambrian biosphere almost entirely, and formed mats on wet sediment surfaces in the absence of grazers and burrowers. Evidence for microbial mats in the rock record dates back to 3.5 Ga (Altermann et al., 2006; Altermann, 2008) and mats continue to exist today, although largely confined to stressful environments due to metazoan activities. The Precambrian sedimentation system was significantly influenced by the microbes which, among other influences, impart unusual cohesiveness to sands, generating a wide range of microbial mat related structures (MRS) (e.g., Gerdes et al., 2000; Eriksson et al., 2000; Noffke et al., 2001, 2002, 2003; Sarkar et al., 2004, 2005, 2006, 2008; Schieber, 2004; Banerjee and Jeevankumar, 2005; Parizot et al., 2005; Schieber et al., 2007 and references therein). Microbial colonies often occur as discoidal mounds, spheres, or have honeycomb geometries, and are characterized by division of the colonies into concentric zones and wedge-shaped radially oriented sectors (Gerdes et al., 1994; Shapiro and Dworkin, 1997; Brehm et al., 2003; Thar and Kühl, 2005); they thereby bear an uncanny resemblance to some purported fossils of Ediacaran age (Grazhdankin and Gerdes, 2007). Many examples of Ediacaran fossils have been re-interpreted as pseudofossils since their initial descriptions, thus reducing the spectrum of Ediacaran fossil diversity (Pickerill and Harris, 1979; Bland, 1984; Sun, 1986a; Farmer et al., 1992; Pflüger, 1995; Buatois and Mangáno, 2003; Seilacher et al., 2005; Seilacher, 2007). The most commonly mistaken fossils include discoidal structures with central depression ("medusoids")

that predate the first appearance of the diverse Ediacaran fossil assemblage and which are related to microbial colonies (Grazhdankin and Gerdes, 2007). In general, microbially originated sinuous cracks (*Manchuriophycus*) on sandstone beds are often confused with metazoan burrows belonging to *Cochlichnus* (Kulkarni and Borkar, 1996). MRS such as wrinkle structures and related "elephant skin" structures have been confused with *Protopaleodictyon* (Durand and Aceñolaza, 1990) and *Squamodictyon* (Durand et al., 1994). Gas bubbles may produce millimeter- to centimeter-scale depressions on mud-free sandstone bed surfaces made cohesive by in situ mat growth, and are often misconstrued as inclined burrows (Seilacher, 2007). Analogously, microbially originated petee ridges on sandstone beds are frequently confused with horizontal burrows (Singh and Sinha, 2001). Microbial mat decay produces rounded saucer-like shallow depressions with radial cracks (*Astropolithon*), and these are quite likely to be confused with jellyfish impressions (Glaessner and Wade, 1966).

This paper concentrates on some microbial mat related structures in Indian Proterozoic successions that have been or are likely to be confused with Ediacaran fossils. A few of them belonging to the Chorhat sandstone of roughly 1.6 Ga age, long predate the Ediacaran fossil assemblage. Others belong to the possibly Neoproterozoic Sirbu shale and Sonia sandstone of India. The rest of the examples are cited from the modern hypersaline coastal plains of the Gulf of Cambay in India. The intention of this chapter is to make general readers aware of the pitfalls in identifying fossils, especially those of the Ediacaran assemblage in the Proterozoic, and their alternative as well as more readily acceptable explanations.

2. Geological Background of the Ancient Formations

The aforementioned Chorhat sandstone belongs to the lower part and the Sirbu Shale belongs to the upper part of the 4.5 km-thick unmetamorphosed and little deformed Vindhyan Supergroup. Both are best studied in the area around Uchaira and Chorhat in the Son valley in central India (Fig. 1a, b; Bose et al., 2001). Whereas the Chorhat yields a radiometric age just at the transition between the Meso and the Paleoproterozoic (~1.6 Ga, Rasmussen et al., 2002a; Ray et al., 2002; Sarangi et al., 2004; Ray, 2006), the Sirbu Shale is considered to be late Neoproterozoic (0.6 Ga) on the basis of Sr-isotope ratios of the underlying Bhander Limestone (Ray et al., 2003; Ray, 2006). Both formations were studied in their prograding siliciclastic wave-dominated open shelf facies successions (Tables 1 and 2; Sarkar et al., 2002, 2006), where excellent microbial mat structures have already been reported (Seilacher et al., 1998; Sarkar et al., 2004, 2005, 2006; Sarkar and Banerjee, 2007; Bose et al., 2007).

The Sonia sandstone, dominantly arenitic, is the basal formation of the 2 km-thick Marwar Supergroup, which like the Vindhyan Supergroup, consists of largely undeformed and unmetamorphosed sedimentary rocks and is well

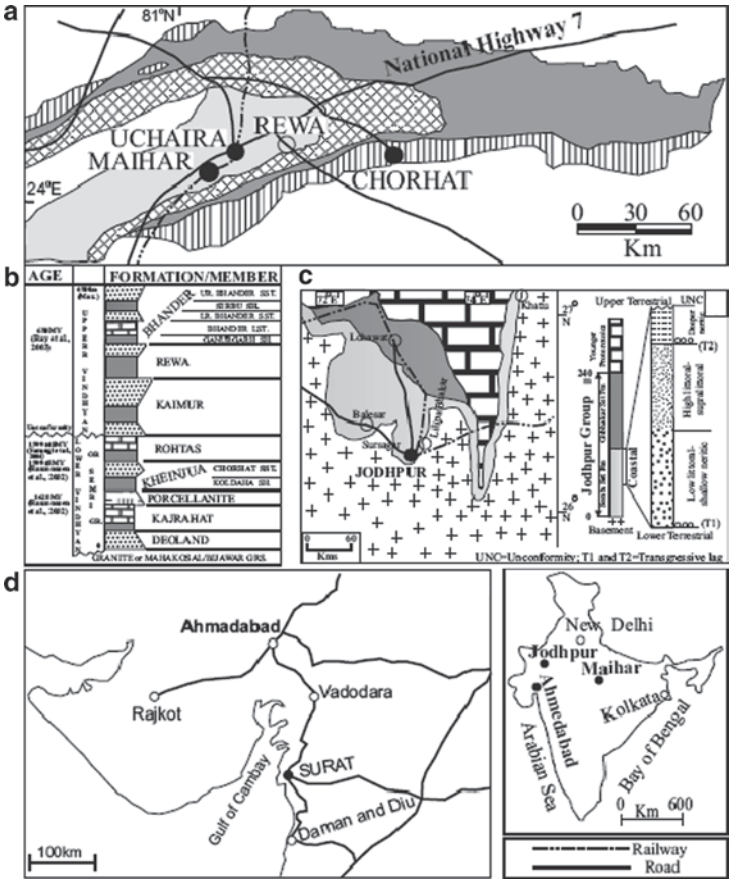

Figure 1. (**a**) Geological map showing outcrops of the Vindhyan exposures in the Son valley, study areas marked in solid circles. (**b**) Necessary stratigraphic details of the Vindhyan Supergroup. (**c**) Geological map showing outcrop distribution of the Jodhpur Group, study area marked in solid circle (*left*) and relevant stratigraphic details (*right*). (**d**) Geographic map showing location of Gulf of Cambay and study area (solid circle) (map of India within inset).

exposed around Jodhpur City, Rajasthan, India (Fig. 1c; Pareek, 1984; Roy and Jakhar, 2002). Resting on Malani volcanics, the Sonia sandstone has a lower fluvial interval and an upper 60-m thick coastal marine interval (Sarkar et al., 2008; Eriksson et al., this volume). The latter interval comprises generally of well-sorted sandstone and presents a rich assemblage of microbial mat structures (Sarkar and Banerjee, 2007; Sarkar et al., 2008). The age of the Sonia sandstone is generally considered to be close to 600 Ma (Rathore et al., 1996, 1998). However, recent Pb-isotopic study reveals an 800 Ma age for the youngest population of detrital

Table 1. Description and interpretation of marine facies in Chorhat sandstone (After Sarkar et al., 2006).

Facies	Description	Interpretation
C	Medium-grained sandstone with adhesion laminae, translatent strata, and isolated cross-sets (<32 cm thick) of grainflow-grainfall origin. Maximum thickness 8 m	Aeolian sandsheet of erg-margin wet system (supratidal-erg margin)
B	Fine- to medium-grained sandstone beds generally thicker than 10 cm, often amalgamated, massive or quasiplanar, and wavy laminated. Wave ripples, ripples migrating along troughs of larger ripples, parting lineation, rill marks present on sandstone bed surfaces. Thinner muddy siltstone of cm scale locally present. Wart marks and adhesion ripples occur locally. Maximum thickness is 45 m	Shallow shelf, within fair-weather wave base with intermittent exposure (shallow subtidal to intertidal)
A	Fine-grained sandstone-siltstone interbedding. Sandstone beds are tabular in shape, often less than a centimeter thick; relatively thicker ones overall graded, have sharp erosional bases riddled with gutters and prods followed up by quasiplanar laminae, hummocky cross-stratification and finally wave ripples on bed-tops. Amalgamation of sandstone beds maximum thickness 15 m	Siltstone beds are autochthonous, sandstone beds formed during storms events (deeper subtidal)

Table 2. Description and interpretation of the shelf facies of Sirbu Shale (After Sarkar et al., 2005).

Facies	Description	Interpretation
E	Sandstone – sandy siltstone interbedded facies up to 90 cm thick. Laterally extensive sandstone beds generally have flat bases with comparatively larger gutters, and tool marks, often amalgamate	Proximal shelf above fair-weather wave base
D	Sandstone – shale interbedded facies up to 73 cm thick. The sandstone beds are sheet like and 10.5–15 cm thick. Gutters, still larger in dimension as well as tool marks are present. Mud clasts are concentrated at the base of sand beds. Sandstone beds locally amalgamate with top wave-reworked	Offshore between fair-weather and storm wave bases
C	Alternating shale and siltstone beds of comparable thickness, and fair degree of lateral persistence. Facies thickness ranges up to 56 cm. Siltstone beds greater than 9 cm thick, have sharp bases but with tops grading into mudstone, gutters (larger in dimension), and tool marks are common	Offshore setting more proximal with respect to setting for facies B
B	Gray shale, interbedded with siltstone lenses ranging from 5 to 8.5 cm in thickness impersistent lateral extent, facies thickness up to 45 cm. Siltstone beds massive, graded, or planar laminated with numerous sole features and wave rippled at top have gradational upper contacts with shales	Distal offshore, relatively proximal with respect to facies A site
A	Dark green shale <22 cm-thick incorporating submillimeter-thick planar silt interlaminae. Lenticular massive or planar laminated siltstone beds <3 cm thick with sharp and planar bases with sole features locally present	Distal offshore; bottom touched only by exceptionally strong storms

Table 3. Description and interpretation of marine facies in Sonia Sandstone (After Sarkar et al., 2008).

Facies	Description	Interpretation
C	Relatively less sorted and still coarser grained (Mdϕ ~0.8) sandstone, >25 m thick, with large-scale, normally graded cross-strata, consists two subfacies. Subfacies C_1 is widespread, characterized by 40–50 cm-thick cosets of troughs separated by 2–3 cm thick wave rippled sheets. Subfacies C_2 characterized by chevron-like criss-cross arrangement of tabular cross-strata in 70–90 cm thick co-sets	Deeper neritic (C1) and wave-dominated shoal (C2)
B	Well-sorted sandstone, 28 m thick, relatively coarser than facies A (Mdϕ ~1.5) with adhesion laminae, inversely graded translatent strata, very low amplitude impact ripples, regular alternations of large scale (20 cm thick) grain-flow and grain-fall strata and wave ripples	High littoral to supralittoral with coastal dunefield
A	Coarse to medium grained (Mdϕ ~1.7), 30 m thick, well-sorted sandstone consists of alternating sets of tabular cross-strata and planar laminae with wave ripples migrating along troughs of larger ripples, local occurrence of mud drapes, and the herringbone cross-bedding azimuth warrants distinction of a subfacies A2	Low littoral to shallow neritic (A1) and upward transition from shallow subtidal to low intertidal (A2)

zircons in this unit (Malone et al., 2008). Facies identified within the coastal interval indicates paleogeography ranging from shallow neritic to supralittoral (Table 3, Fig. 1).

3. Sedimentologic Frame of the Modern Setting

The extensive intertidal−supratidal flats in the Gulf of Cambay on the western coast of India are hypersaline in character (Fig. 1d; Ghosh et al., 2009). The N-S oriented Gulf is macrotidal, the tidal range exceeding 10 m. The western flank of the Gulf is dominated by carbonate sedimentation, while its eastern flank is characterized by siliciclastic sedimentation in several tide-dominated estuaries. Microbiota colonize extensive areas along the coast, and especially on the northern flank of Narmada Estuary, north of Surat, where hypersalinity inhibits invasion of other organisms and a wide variety of microbial mat related structures occur (Saha, 2009). The funnel-shaped Narmada Estuary is a tide-dominated estuary with well-developed sand flat, mud flat, and salt flat facies zones on both sides of the estuarine channel (Saha et al., 2007; Saha, 2009). The microbial mat growth is common along the sand flats and mud flats on the northern sides of the estuarine channel.

4. Microbial Mat-Related Structures Resembling Ediacaran Fossils

4.1. FEATURES IN THE CHORHAT SANDSTONE

The subtidal- to intertidally-deposited Chorhat sandstone exhibits a spectacular variation of microbially originated structures (Sarkar et al., 2004, 2006; Sarkar and Banerjee, 2007). The following discoidal forms, which occur in association with abundant and unambiguous evidence of prolific microbial mat growth in the Paleoproterozoic Chorhat sandstone, are readily explicable in terms of growth or decay of mats; however, they closely resemble features recorded as Ediacaran fossils in different corners of the world. All these features are essentially found in the middle shallow subtidal to intertidal portion of the Chorhat sandstone (facies B in Table 1).

1. Low-relief domes, circular in plan and with a thin rim, occur locally on beds of mud-free sandstone of subtidal origin (Fig. 2a). The domes have an average relief of about 0.5 cm and diameter of about 1 cm. Some of the domes have small ruptures up to half a centimeter in diameter, in the form of craters on their summits (Fig. 2b, c). These features essentially resemble discoidal Ediacaran fossils or "medusoids" described by some authors (e.g., Pickerill and Harris, 1979; Sun, 1986a, b; Fedonkin, 1990; Cruse, et al., 1993; Cruse and Harris, 1994; De, 2003, 2006). Figure 2c appears very similar to Ichnogenus *Bergaueria*, which is

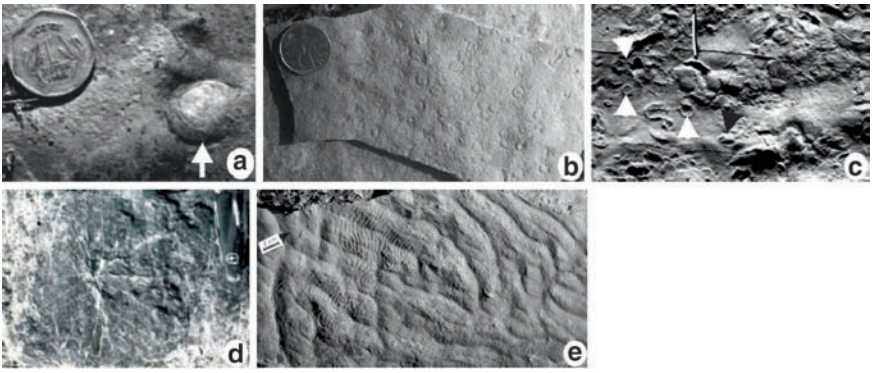

Figure 2. (**a**) Field photograph showing sand bulge with rim around (*arrow*) on Chorhat sandstone bed surfaces (coin diameter = 2.5 cm). (**b**) Numerous domes with craters on the bedding surface resembling Ediacaran fossil *Bergaueria* (coin diameter = 1.7 cm). (**c**) Domes with craters (white arrows), a dome without crater is marked by a black arrow (match stick length = 4.1 cm). (**d**) Domal features with radial cracks; note clear lobate projections on the bottom left of the feature. (**e**) Near-concentric band on a wave rippled sandstone surface (Note the fading of ripple relief immediately below the concentric feature) (Swiss knife = 7.5 cm).

considered as a resting trace or dwelling burrow of a shallow water coelenterate (compare with Fig. 4 in Cruse and Harris, 1994; see also Cruse et al., 1993). The examples cited from the Chorhat sandstone, nonetheless, belong to an age far older than Ediacaran (Knoll et al., 2006). These sand bulges are interpreted to have formed because of expulsion of pressurized gases through a cohesive microbial mat surface. The gas would have been produced by the decay of buried microbial mat (cf., Pflüger, 1999; Dornbos et al., 2007; Schieber et al., 2007). The presence of craters on top of some of them further corroborates this contention, presumably reflecting the zones through which fluid and sediment actually flowed (cf., Dornbos et al., 2007).

2. Some saucer-like structures on bed surfaces having a larger diameter (averaging about 12.5 cm) are characterized by radiating cracks, a central crater-like depression of average diameter about 2.3 cm, and lobate margins, similar to "*Astropolithon*" (Fig. 2d; Dawson, 1878). Gas expulsion through a cohesive microbial mat cover is the most likely mechanism for the origin of these structures; the radial cracks are strongly supportive of this idea, intimating cohesiveness of mud-free surficial sands (Seilacher et al., 2005; Seilacher, 2007; Dornbos et al., 2007; Schieber et al., 2007). The feature could be related to "discoidal microbial colony," representing localized concentrations of microbial mass of discoidal morphology with wedge-shaped, radially oriented sectors and lobate projections (Grazhdankin and Gerdes, 2007). Essentially similar structures of Ediacaran affinity have been recorded as basal holdfasts of frondose organisms comparable with *Inaria* (Gehling, 1988). However, those cited here from the Chorhat sandstone formed about 1,000 million years earlier.
3. Low-relief, incomplete circular features, exhibiting internal concentric bands marked by alternate dark and light appearance, often occur on sandstone bed surfaces showing two sets of ripples (Fig. 2e). The concentric bands are more pronounced in the troughs of the ripples, and exhibit slight offset of the concentric bands passing from crests to the adjoining troughs. The width of the bands is fairly consistent within the concentric structure and is 0.3 cm on average. Diameter of the overall circular feature is approximately 20.5 cm. Similar features on rippled sandstones from Ediacaran age have been related to *Cyclomedusa* (Sprigg, 1949; see also McCall, 2006 for more reports) or *Kullingia concentrica* (Kulling, 1964, 1972; Foyn and Glaessner, 1979). The feature appears to be related to microbial mat, as it is best developed when the primary ground relief caused by rippling is reduced owing to thicker microbial mat growth within ripple troughs (cf., "leveling"; Noffke et al., 2002). The features resemble peculiar concentric ring-shaped structures, known as "fairy rings," which developed on microbial mat covered modern hypersaline environments (Gerdes et al., 1993, 1994; Grazhdankin and Gerdes, 2007). Gerdes (2007) considered that the concentric microwaves initiated by gas bubbling through small exit points within the mat surfaces propagate nutrient fronts, which may trigger chemotactic responses.

4.2. FEATURES IN THE SIRBU SHALE

Microbial mat features at the soles of shelf storm sandstone beds (positive hyporelief) have already been reported from the Sirbu shale (Sarkar and Banerjee, 2007). However, similar features occur on bedding surfaces (positive epirelief) locally. The majority of these features are present within the offshore-originated facies B and C of the Sirbu shale (Table 2). Some of these features that have exact equivalents in the Ediacaran fossil assemblage are discussed below:

1. On the upper surface of the rippled sandstone beds, millimeter-scale radial grooves alternating with millimeter-scale ridges (height up to 1.5 mm) occur preferably on the crests of the ripples, forming peculiar circular to elliptical patterns in places (Fig. 3a). Similar-looking features were inferred initially to be *Arumberia*, interpreted as cup-shaped animal traces of coelenterate grade preserved as positive epireliefs (Glaessner and Walter, 1975). *Arumberia* was subsequently related to growth-related features of microbial mats (Bland, 1984; McIlroy and Walter, 1997; Noffke, 2007). It is believed that the miniscule ridges are possibly related to the action of currents on soft, gelatinous microbial mat cover (McIlroy and Walter, 1997; McIlroy et al., 2005).
2. Roughly discrete circular, wrinkled masses are occasionally preserved as positive hyporelief features on the soles of fine-grained storm sandstone beds bearing distinct prod marks, some of them overprinting the wrinkled masses (Fig. 3b).

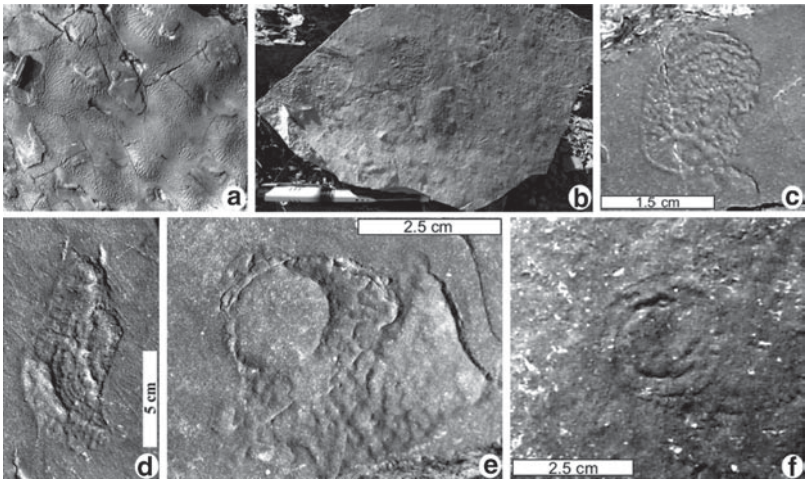

Figure 3. (**a**) Field photograph showing alternate ridges and grooves (*Arumberia*) on a rippled sandstone surface (Swiss knife = 7.5 cm). (**b**) Wrinkled masses with circular outlines on the sole of a sandstone bed bearing abundant tool marks (pen length = 14 cm). (**c**) Subrounded impression showing wrinkled mass. (**d**) Lenticular mat fragment impression showing internal concentric and radial feature on left. Note the torn boundary of the feature at the right. (**e**) Nearly circular feature delimited by a thin, raised rim and wrinkled frill toward bottom. (**f**) Circular impression with concentric bands at the sole of a sandstone bed.

The nearly circular masses have diameters ranging from 2.8 to 3.6 cm. Internally, these bodies bear discontinuous and millimeter-high wrinkles. The feature resembles the Ediacaran fossil *Nimbia* (Fedonkin, 1980), which is reported from the soles of some sandstone turbidite beds (see also De, 2006; McCall, 2006). In the absence of any unequivocal characteristics of Ediacaran fossils, the feature most likely represents impressions of torn pieces of microbial mats derived from the shallower parts of the sea by storm actions (Sarkar and Banerjee, 2007). Alternatively, the wrinkled mass could represent impressions of "discoidal microbial colonies," which were carried by the storm currents and subsequently deposited on the deeper shelf.

3. Positive epirelief features exhibiting an ovate outline defined by sharp but partly eroded peripheral ridges are often found on the bedding surfaces of storm sandstone beds (Fig. 3c). The long dimension of the subrounded features is on average 1.8 cm. Internally, the features exhibit a wrinkled appearance consisting of minute, discontinuous ridges often occurring in concentric fashion. The features resemble the Ediacaran fossil *Kaisalia* as reported in the literature (Fedonkin, 1984; see also De, 2006). However, the ovate, wrinkled mass is also comparable with "discoidal microbial colonies" (cf., Grazhdankin and Gerdes, 2007).

Figure 3d is a positive hyporelief feature of a discrete lenticular fragment with a smooth left margin, which is curved without any marked undulation. In contrast, the straight margin is beveled, angular, and a bit irregular. It bears prominent ridges (positive on the bed sole) that conform to the seemingly intact left margin, and another set of finer straight and radiating ridges cut across them. The ridges radiating from an unknown center and maintaining an orthogonal relationship with the left margin are almost certainly biogenic in nature. The feature appears to be a torn piece of a microbial mat (Sarkar and Banerjee, 2007). The semicircular outline and the minute ridges within it suggest that the original feature is comparable to partially preserved "discoidal microbial colonies" showing both radial and concentric features (Grazhdankin and Gerdes, 2007).

Nearly circular, positive hyporelief features of 2.0 cm diameter, delimited by a thin raised rim (now seen as a hair-thin furrow) occur at the soles of the sandstone beds within the Sirbu shale (Fig. 3e). An irregular wrinkled frill area of variable thickness occurs immediately outside the circular feature on one side. The frill bears two sets of wrinkles. The rimmed, circular, structureless part resembles some kind of substrate attachment. The circular feature appears similar to *Cyclomedusa radiata* (Sprigg, 1949; see also De, 2006). But its origin can also be explained as mat fragment impressions (Sarkar and Banerjee, 2007). The circular impression of an object with a diameter of 2.6 cm and with concentric bands with an average width of 4.5 mm separated by thin ridges is now represented in reverse (sole markings) as furrows (Fig. 3f). The feature resembles medusoid (*Cyclomedusa davidi*) forms of Ediacaran fossils (Sprigg, 1947; Wade, 1968; Ford, 1968; Fritz et al., 1983; Sun, 1986a; Cruse and Harris, 1994). However, similar concentric bands can also be found in both modern and ancient "discoidal microbial colonies" (Gerdes et al., 1994; Grazhdankin and Gerdes, 2007).

4.3. FEATURES IN THE SONIA SANDSTONE

Microbial mat related structures occur within the coastal marine interval of the Sonia sandstone exhibiting abundant emergence features (facies A and B in Table 3). Sarkar et al. (2008) recently reported microbial mat related sedimentary structures within the overall sedimentation background of the Sonia sandstone. The Ediacaran-like features from the Sonia sandstone are described below.

1. The rippled sandstone bed surfaces in the Sonia sandstone exhibit small bulges similar to those of the Chorhat sandstone (Fig. 4a). Sizes of the bulges vary from 2.8 to 3.7 cm. Miniature craters, having an average diameter of 0.9 cm, may occur in places inside the small bulges (Fig. 4b). The structures lack the radial ridges described in Fig. 2d.

   The small bulges are essentially similar to the gas domes formed by gas entrapment underneath a mat, and are ascribed to a similar origin to those found within the Chorhat sandstone (Fig. 2a–c). Gas domes form when there is enough pressure generated by the continued decay of microbial mat at its base, so that ascending gas can rupture through the mat cover (Dornbos et al., 2007).
2. Discoidal, positive epirelief features with circular outer rim and central flat-lying and featureless portions occur on some bedding surfaces (Fig. 4c). Average diameter of the discs is 32 cm. The feature is morphologically as well as dimensionally similar to the *Ediacaria* sp. (Sprigg, 1947;

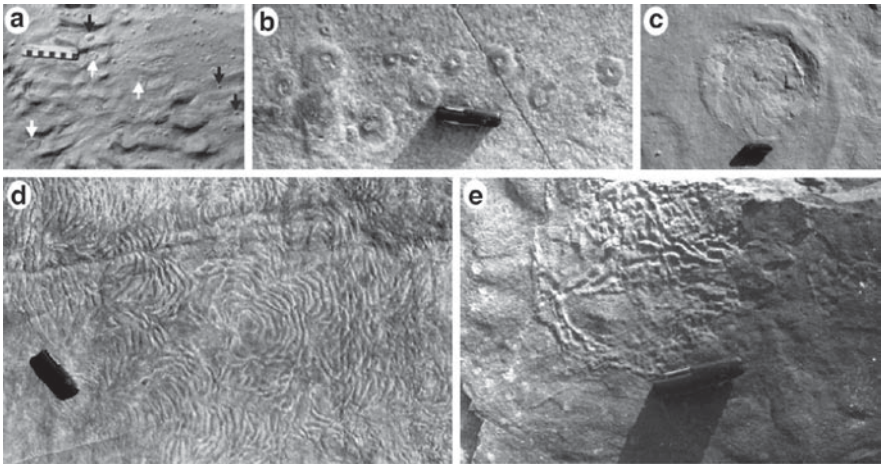

Figure 4. (**a**) Small bulges (*black arrows*) on a sandstone bed surface. Note association with minute gas domes with craters at their centers (white arrows). (**b**) Bulges with craters at their centers in the same sandstone. (**c**) Discoidal impression with circular outer rim. (**d**) Concentric structures resembling *Paleopascichnus* on a sandstone bed (Swiss knife = 7.5 cm, pen length = 14.1 cm) (**e**) Partially preserved near-circular wrinkled masses resembling Edicaran fossil *Kaisalia*.

see McCall, 2006 for more records). In the absence of any definite Ediacaran fossils within the Sonia sandstone, we are inclined to believe that the feature represents microbial mat decay-related gas domes.

3. The rippled sandstone bed surfaces in a few places exhibit complex segments consisting of semicircular to curved bands arranged in parallel (Fig. 4d). The relief of the bands is very small, varying from 3 to 6 mm. The width of the individual structure may vary from 3.2 to 16.5 cm. The width of the semicircular to curved bands within the structures is fairly consistent and is 1.1 cm on average. The structures become discontinuous against the underlying ripples in places. Essentially similar features in rocks of Ediacaran age have been identified as *Paleopascichnus*, described as meandering feeding burrows (Glaessner, 1969; Fedonkin, 1977; Jenkins, 1995). However, Haines (1990) and Seilacher et al. (2005) considered a microbial origin for these features. Haines (2000) noted that the features bear resemblance to the growth pattern of modern, shallow marine brown algae *Padina* (Lobban and Wynne, 1981).
4. Partially preserved, near-circular wrinkled masses with sharp boundaries are occasionally found on the bedding surfaces (Fig. 4e). A peripherally raised rim is well preserved on one side of the feature and is partly eroded. Diameter of the feature is 18.5 cm and the miniature ridges within the wrinkled mass are up to 4 mm high. The features resemble the Ediacaran fossil *Kaisalia* (Fedonkin, 1984; De, 2006). However, similar features can be produced by "discoidal microbial colonies" (Grazhdankin and Gerdes, 2007).

4.4. MAT FEATURES FROM THE MODERN GULF OF CAMBAY

Microbial mats are well developed on modern intertidal and supratidal flats of the Gulf of Cambay and growth, destruction, and decay of microbial mats generates a wide range of features (Saha, 2009). Only those resembling discoidal Ediacaran fossils are described here. Roughly circular patches showing internal wrinkle features often occur on partly dried-up microbial mat covered sands, which otherwise exhibit irregular wrinkle features oriented randomly (Fig. 5a, b). The circular features gradationally pass to wrinkle features at places (Fig. 5b).

It appears that the wrinkles develop when soft, gelatinous mat starts shrinking during the neap tide in the upper intertidal to lower supratidal zones. The concentration of microbial mass creates the peculiar creasing effect within the circular patches because of differential shrinking in response to gradual dehydration. The circular features are essentially the impressions of "discoidal microbial colonies." Exactly similar features are observed in the Late Ediacaran rocks of the White Sea area, northwestern Russia (D. Grazhdankin, 2008, personal communication). The circular features provide modern analogs to the features resembling Ediacaran fossils observed in the Sirbu shale and Sonia sandstone (Figs. 3b–d and 4e).

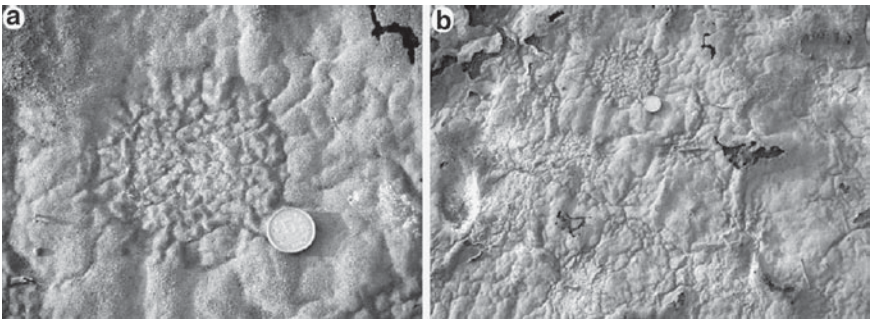

Figure 5. (**a**) Field photograph showing partly dried up "discoidal microbial colony" with circular outline and wrinkled appearance on modern intertidal environment. (**b**) Field photograph showing several "discoidal microbial colonies" (Note gradational transition of the microbial colonies to the wrinkled microbial mass) (coin diameter = 2.2 cm).

5. Discussion

Ediacaran fossils represent a distinct group of enigmatic, large, and structurally complex soft-bodied organisms dominating the end-Precambrian (Ediacaran) oceans (Narbonne, 1998, 2005). Ediacaran fossils are either considered as the oldest metazoans (Narbonne, 2005) or as bizarre, mattress-like forms, known as Vendobionts (Seilacher, 2007). The Ediacaran fossils are dominated by discoid, stalked-frondose, and segmented morphological forms (Mapstone and McIlroy, 2006). The most important element for the Ediacaran fossils includes morphologically complex, leaf-shaped frond leaves or petaloids exhibiting fractal growth patterns (Laflamme and Narbonne, 2008).

Although the leaf-shaped frondose forms unequivocally represent Ediacaran fossils, the discoidal forms (medusoids) are found from rocks considerably older than the Ediacaran (Walter, 1972; Cloud, 1973; Sun, 1986a, b; Cruse et al., 1993; Cruse and Harris, 1994; Rasmussen et al., 2002b; Bengtson et al., 2007; Grazhdankin and Gerdes, 2007). The discoidal Ediacaran fossils from the Stirling Range Formation of Western Australia have been reinterpreted later as pseudofossils, when the absolute age of the rocks was found to be close to 1.8 Ga (Rasmussen et al., 2002b; Bengtson et al., 2007). Discoidal fossils similar to those occurring in the Vendian Twitya Formation can be found in rocks older than Neoproterozoic (Hofmann, 1985; Rasmussen et al., 2002b; Terleev et al., 2006). All the discoidal features can reasonably be related genetically to the microbially originated gas domes or "discoidal microbial colonies." A number of discoidal structures have even been re-interpreted as scratch circles produced by objects rooted to soft sediment surfaces (Grazhdankin, 2000, 2003; Jensen et al., 2002; Mapstone and McIlroy, 2006). The most important macroscopic feature of the discoidal microbial morphs is the partitioning of the colonies into concentric and radial zones. Concentric ring patterns in recent "discoidal microbial colonies" provide excellent

analogs to the concentric zonation of the medusoid fossils (Grazhdankin and Gerdes, 2007). However, some of the discoidal features consisting of lobes radiating out from a central circle (*Mawsonites*) are still being debated, as to whether they are true Ediacaran fossils (Sun, 1986b; van Loon, 2008) or microbial mat decay related gas domes (Seilacher et al., 2005; Seilacher, 2007). Other examples of pseudo-Ediacaran fossils described in this paper include the purported Ediacaran body fossils *Arumberia*, which are now considered to be a microbial mat related structure (Mapstone and McIlroy, 2006; Noffke, 2007). The peculiar curved and meandering features of *Paleopascichnus*, considered earlier as Ediacaran trace fossils, have been reinterpreted as microbially related structures (Haines, 2000; Seilacher et al., 2005; Seilacher, 2007).

The microbial origin of the above features therefore reduces the range of Ediacaran fossils, which can be linked to metazoan phylogenies. Microbial mats and "discoidal microbial colonies" occurring in hypersaline depositional conditions provide excellent analogs to some of the features resembling Ediacaran fossils. Fairy-ring features developing in modern hypersaline environments provide a reasonable explanation for similar features found in pre-Ediacaran rocks (e.g. Fig. 1e). Circular impressions with internal wrinkles observed on modern hypersaline intertidal flats of the Gulf of Cambay are comparable to similar features from the Precambrian sandstones, which resemble Ediacaran fossils (Figs. 3b–d, and 4e). Microbial mat decay related gas domes observed in modern hypersaline siliciclastic depositional environments (Gerdes, 2007) are comparable with those observed in ancient rock records, often interpreted misleadingly as Ediacaran fossils.

Many of the examples cited are found in rocks older than the Ediacaran age. Microbial mat related structures and "discoidal microbial colonies" offer alternative and readily acceptable mechanisms for the features observed within the Chorhat sandstone. Recent radiometric age dating by Malone et al. (2008) and "medusoid" fossil reports from the Sirbu shale (De, 2003, 2006) are contradictory. It is important to note that other evidence of an end-Precambrian age for the Sirbu shale is derived from indirect means, like carbon isotope stratigraphy of a limited number of samples (Friedman et al., 1996), purported fossil records (Venkatachala et al., 1996) and Sr isotopes of limestones (Ray et al., 2003). All these methods have serious limitations (see Malone et al., 2008). Microbial mat related structures and "discoidal microbial colonies" offer alternative and more readily acceptable explanations for the features resembling Ediacaran fossils within the Sirbu shale. The same is the case for the Sonia sandstone. In the absence of any confirmed radiometric dates, the latter formation was believed earlier to be ca. 600 Ma, based on the presence of purported Ediacaran fossils (Raghav et al., 2005) and radiometric dating of the underlying volcanics (Rathore et al., 1996, 1998). However, the recent date by Malone et al. (2008) suggests an age of at least 800 Ma for this stratigraphic unit. Microbial mat related structures and "discoidal microbial colonies" provide alternative explanations for the features resembling Ediacaran fossils.

6. Conclusions

1. Microbial mats and discoidal microbial colonies produced wide ranging nonactualistic features in the Precambrian Vindhyan Supergroup and Jodhpur Group, resembling Ediacaran fossils.
2. Discoidal features showing a central depression as well as radial and concentric patterns are found in the 1.6 Ga Chorhat sandstone, and which appear similar to Ediacaran fossils described in the literature. The features can be related to microbial mat decay originated gas domes or "discoidal microbial colonies." Similar features therefore should be carefully avoided for the correlation of end-Precambrian stratigraphic successions.
3. If the recent age data are true, then all the reported Ediacaran fossils by De (2003, 2006) from the Sirbu shale actually belong to microbial mat related structures.
4. Modern clastic sediments deposited in hypersaline conditions often colonized by "discoidal microbial colonies" provide useful analogs to the features resembling Ediacaran fossils, but found in considerably older rocks.

7. References

Altermann, W. (2008) Accretion, trapping and binding of sediment in Archean stromatolites – morphological expression of the antiquity of life. Space Sci. Rev. **135**: 55–79.

Altermann, W., Kazmierczak, J., Oren, A. and Wright, D.T. (2006) Cyanobacterial calcification and its rock-building potential during 3.5 billion years of earth history. Geobiology **4**: 147–166.

Banerjee, S. and Jeevankumar, S. (2005) Microbial mat response to physical process generating varied forms of wrinkle marks in sand: evidences from Mesoproterozoic Koldaha Shale, central India. Sediment. Geol. **176**: 211–224.

Bengtson, S., Rasmussen, B. and Krapež, B. (2007) The Paleoproterozoic megascopic Stirling biota. Paleobiology **33**: 351–381.

Bland, B.H. (1984) *Arumberia* Glaessner & Walter, a review of its potential for correlation in the region of the Precambrian–Cambrian boundary. Geol. Mag. **121**: 625–633.

Bose, P.K., Sarkar, S., Chakraborty, S. and Banerjee, S. (2001) Overview of the Meso- to Neoproterozoic evolution of the Vindhyan basin, central India. Sediment. Geol. **141/142**: 395–419.

Bose, P.K., Sarkar, S., Banerjee, S. and Chakraborty, S. (2007) Mat-related features from the Vindhyan Supergroup in central India, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, O. Catuneanu and W. Altermann (eds.) *An Atlas of Microbial Mat Features Preserved Within the Clastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 181–188.

Brehm, U., Krumbein, W.E. and Palinska, K.A. (2003) Microbial spheres from microbial mats, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms: A Natural History of Life on Earth.* Kluwer, Dordrecht, pp. 151–160.

Buatois, L.A. and Mangáno, M.G. (2003) La ichnofauna de la Formación Puncoviscana en el noroeste argentine: implicancias en la colonizacion de fondos oceánicos y reconstrucción de paleoambientes y paleoecosistemas de la transición precámbrica-cámbrica. Ameghiniana **40**: 103–117.

Cloud, P. (1973) Pseudofossils: a plea for caution. Geology **1**: 123–127.

Cruse, T. and Harris, L.B. (1994) Ediacaran fossils from the Stirling Range Formation, Western Australia. Precambrian Res. **67**: 1–10.

Cruse, T., Harris, L.B. and Rasmussen, B. (1993) The discovery of Ediacaran trace and body fossils in the Stirling Range Formation, Western Australia: implications for sedimentation and deformation during the 'Pan-African' orogenic cycle. Aust. J. Earth Sci. **40**: 293–296.

Dawson, J.W. (1878) *Supplement to the Second Edition of Acadian Geology, Acadian Geology, the Geological Structure, Organic Remains and Mineral Resources of Nova Scotia, New Brunswick, and Prince Edward Island*, 3rd ed. Macmillan, London, 102 pp.

De, C. (2003) Possible organisms similar to Ediacaran forms from the Bhander Group, Vindhyan Supergroup, Late Neoproterozoic of India. J. Asian Earth Sci. **21**: 387–395.

De, C. (2006) Ediacara fossil assemblage in the upper Vindhyans of central India and its significance. J. Asian Earth Sci. **27**: 660–683.

Dornbos, S.Q., Noffke, N. and Hagadorn, J.W. (2007). Mat decay features, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, O. Catuneanu and W. Altermann (eds.) *An Atlas of Microbial Mat Features Preserved Within the Clastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 106–110.

Durand, F.R. and Aceñolaza, F.G. (1990) Carateres biofaunísticos, paeocológicos y paleogeográficos de la la Formación Puncoviscana (Precámbrico Superior-Cámbrico Inferior) del Noroeste Argentino, In: F.G. Aceñolaza, H. Miller and A.J. Toselli (eds.) *El Ciclo Pampeano en el Noreste Argentino, Serie Correlación Geológica*, Vol. 4, pp. 71–112.

Durand, F.R., Lech, R.R. and Tortello, M.F. (1994) Nuevas evidencias paleotológicas en el basamento precámbrico-cámbrico del noroeste aregentino. Acta Geol. Leopoldensia **39**: 691–701.

Eriksson, P.G., Simpson, E.L., Eriksson, K.A., Bumby, A.J., Steyn, G.L. and Sarkar, S. (2000) Muddy roll-up structures in siliciclastic interdume beds of the c. 1.8 Ga Waterberg Group, South Africa. Palaios **15**: 177–183.

Farmer, J., Vidal, G., Moczydlowska, M., Strauss, H., Ahlberg, P. and Siedlecka, A. (1992) Ediacaran fossils from the Innerelv Member (late Proterozoic) of the Tanafjorden area, northwestern Finnmark. Geol. Mag. **129**: 181–195.

Fedonkin, M.A. (1977) Precambrian–Cambrian ichnocoenoses of the east European platform, In: T.P. Crimes and J.C. Harper (eds.) *Trace Fossils 2*. Geol. J. **9**: 183–193.

Fedonkin, M.A. (1980) New representatives of Precambrian coelenterates on the north of the Russian Platform. Paleontologičeskij Žurnal **2**: 7–15.

Fedonkin, M.A. (1984) *Promorfologia vendckikh Radiala (Promorphology of the Vendian Radiala). Stratigrafiya I Paleontologiya Drevnevshego Fanerozova.* Nauka, Moscow, pp. 30–58 (in Russian).

Fedonkin, M.A. (1990) Systematic description of Vendian metazoan, In: B.S. Sokolov and A.B. Iwanoski (eds.) *The Vendian System: Paleontology 1*. Springer, Berlin, pp. 71–120.

Ford, T.D. (1968) The Precambrian palaeontology of Chamwood Forest, In: A.M. Evans, T.D. Ford and J.R.L. Allen (eds.) *Precambrian Rocks: The Geology of the East Midlands.* Leicester University Press, Leicester, pp. 12–19.

Foyn, S. and Glaessner, M.F. (1979) Platysolenites, other animal fossils, and the Precambrian–Cambrian transition in Norway. Nordisk Geologiska Tidskrift **59**: 25–46.

Friedman, G.M., Chakraborty, C. and Kolkas, M.M. (1996) $\delta^{13}C$ excursion in the End-Proterozoic strata of the Vindhyan basin (central India): its chronostratigraphic significance. Carb. Evap. **11**: 206–212.

Fritz, W., Narbonne, G. and Gordey, S. (1983) Strata and trace fossils near the Precambrian–Cambrian boundary, Mackenzie, Selwyn and Wernecke Mountains, Yukon and Northwest Territories. Curr. Res. B Geological Surv. Can. Pap. **83-1** B: 365–375.

Gehling, J.G. (1988) A cnidarian of actinian-grade from the Ediacaran Pound Subgroup, South Australia. Alcheringa **12**: 299–314.

Gerdes, G. (2007) Structures left by modern microbial mats in their host sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, O. Catuneanu and W. Altermann (eds.) *An Atlas of Microbial Mat Features Preserved Within the Clastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 5–38.

Gerdes, G., Claes, M., Dunajtschik-Piewak, K., Riege, H., Krumbein, W.E. and Reineck, H.-E. (1993) Contribution of microbial mats to sedimentary surface structures. Facies **29**: 61–74.

Gerdes, G., Krumbein, W.E. and Reineck, H.-E. (1994) Microbial mats as architects of sedimentary surface structures, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments.* Bibliotheks und Informationssytem der Carl von Ossietzky Universität, Oldenburg, Germany, pp. 165–182.

Gerdes, G., Klenke, T. and Noffke, N. (2000) Microbial signatures in peritidal siliciclastic sediments: a catalogue. Sedimentology **47**: 279–308.

Ghosh, A., Saha, S., Saraswati, P.K., Banerjee, S. and Burley, S. (2009) Intertidal foraminifera in the macrotidal Estuaries of the Gulf of Cambay: implications for interpreting sea level change in palaeo-estuaries. Mar. Petrol. Geol. **26**: 1592–1599.

Glaessner, M.F. (1969) Trace fossils from the Precambrian and basal Cambrian. Lethaia **2**: 369–373.

Glaessner, M.F. and Wade, M. (1966) The late Precambrian fossils from the Ediacara, South Australia. Palaeontology **9**: 599–628.

Glaessner, M.F. and Walter, M.R. (1975) New Precambrian fossils from the Arumbera Sandstone, Northern territory, Australia. Alcheringa **1**: 11–28.

Grazhdankin, D. (2000) The Ediacaran genus *Inaria*: a taphonomic/morphodynamic analysis. N. Jb. Geol. Palaont. Abh. **216**: 1–34.

Grazhdankin, D. (2003) Structure and depositional environment of the Vendian complex in the Southeast White Sea area. Stratigr. Geol. Correlation **11**: 313–331.

Grazhdankin, D. and Gerdes, G. (2007) Ediacaran microbial colonies. Lethaia **40**: 201–210.

Haines, P.W. (1990) A late Proterozoic storm-dominated carbonate shelf sequence: the Wonoka Formation in the central and southern Flinders Ranges, South Australia. Geol. Soc. Aust. Spec. Publ. **16**: 177–198.

Haines, P.W. (2000) Problematic fossils in the late Neoproterozoic Wonoka Formation, South Australia. Percambrian Res. **100**: 97–108.

Hofmann, H.J. (1985) The mid-Proterozoic Little Dal macrobiota, Mackenzie Mountains, north-west Canada. Palaeontology **28**: 35–39.

Jenkins, R.J.F. (1995) The problems and potential of using animal and trace fossils in terminal Proterozoic biostratigraphy. Precambrian Res. **73**: 51–69.

Jensen, S., Gehling, J.G., Droser, M.L. and Grant, S.W.F. (2002) A scratch circle origin for the medusoids fossil Kullingia. Lethaia **35**: 291–299.

Knoll, A.H., Walter, M.R., Narbonne, G. and Christie-Blick, N. (2004) A new period for the geologic time-scale. Science **305**: 621–622.

Knoll, A.H., Walter, M.R., Narbonne, G.M. and Christie-Blick, N. (2006) The Ediacaran Period: a new addition to the geologic time scale. Lethaia **39**: 13–30.

Kulkarni, K.G. and Borkar, V.D. (1996) Occurrence of *Cochlichnus hitchcock* in the Vindhyan Supergroup (Proterozoic) of Madhya Pradesh. J. Geol. Soc. India **47**: 725–729.

Kulling, O. (1964) Oversiktover Noverbitensfjallens Kaledonberggrund. Sveriges Geologiska Undersokning. Ser. Ba **19**: 1–166.

Kulling, O. (1972) The Swedish caledonites, In: T. Strand and O. Kulling, O. (eds.) *Scandenavian Caledonites.* Wiley, London, pp. 147–302.

Laflamme, M. and Narbonne, G.M. (2008) Ediacaran fornds. Palaeogeogr. Palaeoclimatol. Palaeoecol. **258**: 162–179.

Lobban, C.S. and Wynne, M.J. (1981) *The Biology of Seaweeds.* Botanical Monographs, Vol. 17. University of California Press, Berkeley, CA.

Malone, S.J., Meert, J.G., Banerjee, D.M., Pandit, M.K., Tamrat, E., Kamenov, G.D., Pradhan, V.R. and Sohl, L.E. (2008) Paleomagnetism and detrital zircon geochronology of the upper Vindhyan sequence, Son Valley and Rajasthan, India: a ca. 1000 Ma closure age for the Purana basins? Precambrian Res. **164**: 137–159.

Mapstone, N.B. and McIlroy, D. (2006) Ediacaran fossil preservation: taphonomy and diagenesis of a discoid biota from the Amadeus basin, central Australia. Precambrian Res. **149**: 126–148.

Martin, M.W., Grazhdankin, D.V., Bowring, S.A., Evans, D.A.D., Fedonkin, M.A. and Kirschvink, J.L. (2000) Age of Neoproetrozoic bilateral trace and body fossils, White Sea, Russia: implications for metazoan evolution. Science **288**: 841–845.

McCall, G.J.H. (2006) The Vendian (Ediacaran) in the geological record: enigmas in geology's prelude to Cambrian explosion. Earth Sci. Rev. **77**: 1–229.

McIlroy, D. and Walter, M.R. (1997) A reconsideration of the biogenicity of *Arumberia banksi* Glaessner and Walter. Alcheringa **21**: 79–80.

McIlroy, D., Crimes, T.P. and Pauley, J.C. (2005) Fossils and matgrounds from the Neoproterozoic Longmyndian Supergroup, Shropshire, UK. Geol. Mag. **142**: 441–455.

Narbonne, G.M. (1998) The Ediacara biota: a terminal Neoproterozoic experiment in the evolution of life. GSA Today **8**: 1–6.

Narbonne, G.M. (2005) The Ediacara biota: Neoproterozoic origin of animals and their ecosystems. Ann. Rev. Earth Planet. Sci. **33**: 421–442.

Noffke, N. (2007) Microbially induced sedimentary structures in Archean sandstones: a new window into early life. Gondwana Res. **11**: 336–342.

Noffke, N., Gerdes, G., Klenke, T. and Krumbein, W.E. (2001) Microbially induced sedimentary structures – a new category within the classification of primary sedimentary structures. J. Sediment. Res. **71**: 649–656.

Noffke, N., Knoll, A.H. and Gritzinger, J.P. (2002) Sedimentary controls on the formation and preservation of microbial mat in siliciclastic deposits: a case study from the Upper Proterozoic Nama Group, Namibia. Palaios **17**: 533–544.

Noffke, N., Hazen, R. and Nhieko, N. (2003) Earth's earliest microbial mats in a siliciclastic marine environment (2.9 Ga Mozaan Group, South Africa). Geology **31**: 673–676.

Pareek, H.S. (1984) Pre-quaternary geology and mineral resources of northwestern Rajasthan. Mem. Geol. Surv. India **115**: 1–95.

Parizot, M., Eriksson, P.G., Aifa, T., Sarkar, S., Banerjee, S., Catuneanu, O., Altermann, W., Bumby, A.J., Bordy, E.M., Louis van Rooy, J. and Boshoff, A.J. (2005) Microbial mat-related crack-like sedimentary structures in the c. 2.1 Ga Magaliesberg Formation sandstones, South Africa. Precambrian Res. **138**: 274–296.

Pflüger, F. (1995) Morphodynamik, aktualismus und sedimentstrukturen. Neues Jahrbuch für Geol. und Palaontol. Abh. **195**: 75–83.

Pflüger, F. (1999) Matground structures and redox facies. Palaios **14**: 25–39.

Pickerill, R.K. and Harris, I.M. (1979) A reinterpretation of *Astroplotihon hindii* Dawson 1878. J. Sediment. Petrol. **49**: 1029–1036.

Raghav, K.S., De, C. and Jain, R.L. (2005) The first record of Vendian Medusoids and trace fossil-bearing algal matgrounds from the basal part of the Marwar Supergroup of Rajasthan, India. Indian Miner. **59**: 23–30.

Rasmussen, B., Bose, P.K., Sarkar, S., Banerjee, S., Fletcher, I.R. and McNaughton, N.J. (2002a) 1.6 Ga U–Pb zircon ages for the Chorhat Sandstone, Lower Vindhyan, India: possible implication for early evolution of animals. Geology **30**: 103–106.

Rasmussen, B., Bengtson, S., Fletcher, I.R. and McNaughton, N.J. (2002b) Discoidal impressions and trace-like fossils more than 1200 million years old. Science **296**: 1112–1115.

Rathore, S.S., Venkatesan, T.R. and Srivastava, R.K. (1996) Rb–Sr and Ar–Ar systematics of Malani volcanic rocks of southwest Rajasthan: evidence for a younger post-crystallization thermal event. Proc. Indian Acad. Sci. **105**: 131–141.

Rathore, S.S., Venkatesan, T.R. and Srivastava, R.K. (1998) Rb–Sr isotope dating of Neoproterozoic (Malani Group) magmatism from southwest Rajasthan, India: evidence of younger pan-African thermal event by ^{40}Ar–^{39}Ar studies. Gond. Res. **2**: 271–281.

Ray, J.S. (2006) Age of the Vindhyan Supergroup: a review of the recent findings. J. Earth Sys. Sci. **115**: 149–160.

Ray, J.S., Martin, M.W. and Veizer, J. (2002) U–Pb zircon dating and Sr isotope systematics pf the Vindhyan Supergroup, India. Geology **30**: 131–134.

Ray, J.S., Veizer, J. and Davis, W.J. (2003) C, O, Sr and Pb isotope systematics of carbonate sequences of the Vindhyan Supergroup, India: age, diagenesis, correlations and implications for global events. Precambrian Res. **121**: 103–140.

Roy, A.B. and Jakhar, S.R. (2002) *Geology of Rajasthan (Northwest India) Precambrian to Recent.* Scientific Publishers, Jodhpur, India, 421 pp.

Saha, S. (2009) Modern and Pleistocene coastal sediments of the Gulf of Khambhat – an analogue for understanding Oligo-Miocene hydrocarbon reservoirs of the Surat depression. Unpublished Ph.D. thesis, Indian Institute of Technology Bombay, India, 314 pp.

Saha, S., Ghosh, A., Burley, S., Banerjee, S. and Saraswati, P.K. (2007) Tidal bars in a macrotidal estuarine embayment: the Gulf of Khambhat, western India. Indian J. Petrol. Geol. **15**: 69–78.

Sarangi, S., Gopalan, K. and Kumar, S. (2004) Pb–Pb age of earliest megascopic, eukaryotic alga bearing Rohtas Formation, Vindhyan Supergroup, India: implications for Precambrian atmospheric oxygen evolution. Precambrian Res. **132**: 107–121.

Sarkar, S. and Banerjee, S. (2007) Some unusual and/or problematic features, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, O. Catuneanu and W. Altermann (eds.) *An Atlas of Microbial Mat Features Preserved Within the Clastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 145–147.

Sarkar, S., Chakraborty, S., Banerjee, S. and Bose, P.K. (2002) Facies sequence and cryptic imprint of sag tectonics in late Proterozoic Sirbu Shale, central India, In: W. Altermann and P. Corcoran (eds.) *Precambrian Sedimentary Environments: A Modern Approach to Ancient Depositional Systems*, Spec. Publ. Int. Ass. Sedimentologists 33. Blackwell Science, Oxford, pp. 369–382.

Sarkar, S., Banerjee, S. and Eriksson, P.G. (2004) Microbial mat features in sandstones illustrated, In: P.G. Eriksson, W. Altermann, D.R. Nelson, W.U. Mueller and O. Catuneanu (eds.) *The Precambrian Earth: Tempos and Events*. *Developments in Precambrian Geology 12*. Elsevier, Amsterdam, pp. 673–675.

Sarkar, S., Banerjee, S., Eriksson, P.G. and Catuneanu, O. (2005) Microbial mat control on siliciclastic Precambrian sequence stratigraphic architecture: examples from India. Sediment. Geol. **176**: 195–209.

Sarkar, S., Banerjee, S., Samanta, P. and Jeevankumar, S. (2006) Microbial mat-induced sedimentary structures and their implications: examples from Chorhat Sandstone, M.P., India. J. Earth Sys. Sci. **115**: 49–60.

Sarkar, S., Bose, P.K., Samanta, P., Sengupta, P. and Eriksson, P.G. (2008) Microbial mat-mediated structures in the Ediacaran Sonia sandstone, Rajasthan, India and their implications for Proterozoic sedimentation. Precambrian Res. **162**: 248–263.

Schieber, J. (2004) Microbial mats in the siliciclastic rock record: a summary of diagnostic features, In: P.G. Eriksson, W. Altermann, D.R. Nelson, W.U. Mueller and O. Catuneanu (eds.) *The Precambrian Earth: Tempos and Events*, *Developments in Precambrian Geology 12*. Elsevier, Amsterdam, pp. 663–673.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (eds.) (2007) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geology 2*. Elsevier, Amsterdam, 311 pp.

Seilacher, A. (2007) *Trace Fossil Analysis.* Springer, Berlin, Heidelberg, 226 p.

Seilacher, A., Bose P.K. and Pflüger, F. (1998) Triploblastic animals more than 1 billion years ago: trace fossil evidence from India. Science **282**: 80–83.

Seilacher, A., Buatois, L.A. and Mangáno, M.G. (2005) Trace fossils in the Ediacaran-Cambrian transition: behavioral diversification, ecological turnover and environmental shift. Palaeogeogr. Palaeoclimatol. Palaeoecol. **227**: 323–356.

Shapiro, J.A. and Dworkin, M. (eds.) (1997) *Bacteria as Multicellular Organsims.* Oxford University Press, Oxford, 466 pp.

Singh, S.P. and Sinha, P.K. (2001) Vindhyan Supergroup of Bihar – an overview, In: S.P. Singh (ed.) *Precambrian Crustal Evolution and Mineralisation in India*. South Asian Association of Economic Geologists, Patna, pp. 107–125.

Sprigg, R.C. (1947) Early Cambrian (?) jellyfishes from the Flinders ranges, South Australia. Trans. Royal Soc. South Aust. **71**: 212–224.

Sprigg, R.C. (1949) Early Cambrian 'jellyfishes' of Ediacara, South Australia and Mount John, Kimberley District, Western Australia. Trans. Royal Soc. S. Aust. **73**: 72–99.

Sun, W. (1986a) Precambrian medusoids: the *Cyclomedusa* plexus and *Cyclomedusa*-like pseudofossils. Precambrian Res. **31**: 325–360.

Sun W. (1986b) Late Precambrian scyphozoan medusa *Mawsonites randellensis* sp. nov. and its significance in the Ediacaran metazoan assemblage, South Australia. Alcheringa **10**: 169–181.

Terleev, A.A., Postnikov, A.A., Kochnev, B.B., Nagovitsin, K.E., Grazhdankin, D.V. and Stanevisch, A.M. (2006) Ranneproterozoiskaia biota iz udokanskoi serii zapadnoi chasti aldanskogoshchita (Rossiia) (Early Proterozoic biota from the Udokan Group of the western Aladan Shield, Russia), In: S.V. Rozhnov (ed.) *Evolyutsiia Biosfery I Bioraznoobraziia*. KMK, Moscow, 271–281 (in Russian).

Thar, R. and Kühl, M. (2005) Complex pattern formation of marine gradient bacteria explained by a simple computer model. FEMS Microbiol. Lett. **246**: 75–79.

van Loon, A.J. (2008) The nature of Mawsonites (Ediacara fauna). Gond. Res. **14**: 175–182.

Venkatachala, B.S., Sharma, M. and Shukla, M. (1996) Age and life of the Vindhyans – facts and conjectures. Mem. Geol. Soc. India **36**: 137–165.

Wade, M. (1968) Preservation of soft-bodied animals at Ediacara, South Australia. Lethaia **1**: 238–267.

Walter, M.R. (1972) Tectonically deformed sand volcanoes in a Precambrian greywacke, Northern Territory of Australia. J. Geol. Soc. Australia **18**: 395–399.

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DOI 10.1007/978-90-481-3799-2_7,

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OSMOTROPHIC BIOFILMS: FROM MODERN TO ANCIENT

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1. Introduction

Biofilms and microbial mats can be regarded as among the most vigorous and ancient ecosystems found on Earth (Noffke et al., 2006). In contrast with planktonic microbes, the extracellular polymeric substance in which they are usually embedded renders the microbes in biofilms relatively resilient to adverse environmental changes (Costerton and Stoodley, 2003). Biofilms are usually multilayered and involve consortia of microbes, in which all the materials needed to sustain life can be recycled (Stolz, 2003). A viable microbial mat is therefore quite likely to contain a mixture of autotrophic fixers of carbon dioxide, heterotrophic consumers that feed by ingestion of organic particles, and others that have adapted to recycle the preformed organic matter by means of enzymatic digestion and absorption through the cell wall – called osmotrophy.

It might seem self-evident that such osmotrophy must have existed since the very earliest times. But chemotrophic, heterotrophic, and osmotrophic components have received little attention in the geological literature, so that it is widely assumed that most microbial mats have accumulated from the effects of photoautotrophic organisms such as cyanobacteria, as illustrated, for example, by much of the literature on stromatolites (Schopf et al., 1984; Schopf and Klein, 1992; Grotzinger and Knoll, 1999; Allwood et al., 2006), and microbially induced sedimentary structures (e.g., Noffke et al., 2001; Schieber et al., 2007).

In this chapter, we attempt to redress this imbalance, by drawing attention to evidence of possible and probable osmotrophs from modern to Proterozoic deep marine to subaerial environments. Modern osmotrophs can be the predominant component of mats in a wide range of environments, including caves and karstic fissures beyond the reach of sunlight, forests and woodland soils, toxic and acid waste sites, anoxic lakes and ponds, and even the seafloor below the reach of sunlight (Fig. 1) (Varnam and Evans, 2000; Krumbein et al., 2003; Ross, 2006). In spite of the great diversity of osmotrophs thriving in these settings, most may be referred either to prokaryotic actinobacteria or to the various eukaryotic fungal groups (see Margulis and Schwartz, 1988; Lecointre and Guyader, 2006). In modern environments, both actinobacteria and fungi can play

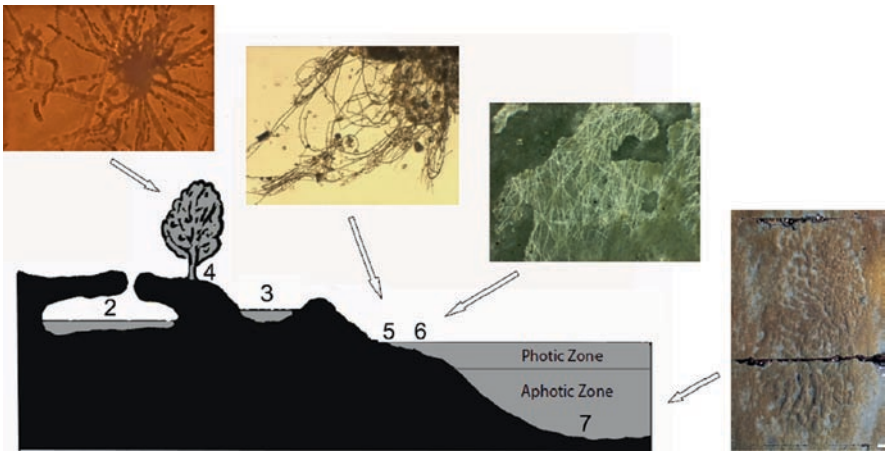

Figure 1. Summary diagram of the settings for candidate osmotrophic biofilms in the fossil record, numbered according to the sections under which they are outlined in the text, with representative fossil or modern material from each environment. For explanation of inset images, see later figure captions.

an important role in the recycling of organic components ranging from animal and vegetable detritus to organic rich fluids. Both groups also tend to form dense networks of filaments, called hyphae, in which organic breakdown is aided by the formation of numerous branches or bridges between nutrient-rich areas. Such osmotrophs help induce organic breakdown by the secretion of enzymes, which may help dissolve and digest algal materials (agarolytic), chitinous materials (chitinolytic), or woody materials (cellulolytic) (e.g., Varnam and Evans, 2000). The evolution of these metabolic pathways is most likely to have had a major impact on the history of biogeochemical cycling on the planet, and even on the very nature of the fossilization itself.

Eukaryotic fungi and prokaryotic actinobacteria (formerly called actinomycetes) are ancient types of osmotrophic organism (e.g., Margulis and Schwartz, 1988), and although both might be expected to have a fossil record that stretches far back into the Precambrian (Heckman et al., 2001), their remains have received little attention so far. Under each heading, we begin by exploring some modern examples where osmotrophs play key roles in microbial communities, before examining possible analogs within the fossil record.

2. Osmotrophic Biofilms in Subterranean Caves and Karsts

The subterranean cave environment, with its aphotic, low-nutrient conditions, provides unique challenges for would-be colonizers. Yet even the deepest cave environments have been found to harbor life adapted to these extreme conditions (see Northup and Lavoie, 2001; Engel, 2007). There has been relatively little work

on fungi in subterranean environments (Reitner et al., 2006), and fossil fungi from caves and karst are rarely reported. The preservation of fossil fungal filaments in karst environments in the vadose zone described by Kretzschmar (1982) and Duane (2003) form rare exceptions. Yet dense mats of actinobacterial filaments and ascomycete fungi found in caves such as Altamira, Spain, and Lascaux, France (Cañaveras et al., 2001; Dupont et al., 2007) indicate that actinobacteria and fungi can penetrate hypogean caverns readily.

Of particular interest here are the remarkable ecosystems that have been discovered in isolated, aphotic, oxygen-limited cave systems associated with sulfide-rich waters, which, with limited or no input of allochthonous organic carbon and with photoautotrophy impossible, rely on chemoautotrophy. Such sulfidic karst systems, fed by springs and streams rich in H_2S, include the Frasassi Gorge systems, central Italy (Vlasceanu et al., 2000); Lower Kane Cave, Wyoming (Engel et al., 2004b); Cueva de Villa Luz, Mexico (Hose et al., 2000); Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico (Cunningham et al., 1995). These caves contain widespread microbial mats with fungi and heterotrophs feeding on the organic matter generated by sulfide-oxidizing proteobacteria, mostly Epsilonproteobacteria (Mattison et al., 1998; Engel et al., 2004a; Campbell et al., 2006).

The most astonishing of these isolated ecosystems is perhaps Movile Cave, Romania, which appears to be driven entirely by chemolithoautotrophy (Lascu et al., 1993; Sarbu et al., 1994). Sarbu et al. (1994) found floating and submerged anaerobic mats of tangled mycelium and bacteria in airbells in the cave, identifying *Penicillium* sp., *Trichoderma* sp., and *Plasmopora* sp., as well as oomycetes. These chemoautotrophs support diverse ecosystems of ciliates, rotifers, copepods, amphipods, and abundant *Collembola* hexapods, forming terrestrial equivalents to those of deep-sea hydrothermal vents (Karl et al., 1980; Jannasch, 1985; Sarbu et al., 1994), and deep-sea cold seeps (Levin, 2005), though cave chemoautotrophic systems appear to lack endosymbiotic elements (Kinkle and Kane, 2000) and have slower rates of primary productivity (Poulson and Lavoie, 2000; Porter et al., 2009).

Karstification by sulfuric acid speleogenesis resulting from oxidation of H_2S to H_2SO_4 in caves with springs rich in dissolved sulfides (Egemeier, 1981; Jagnow et al., 2000) may involve Bacteria and Archaea (Hose et al., 2000; Engel et al., 2004b). Microbial communities of autotrophs, heterotrophs, and osmotrophs, albeit of minimal diversity (Macaladay et al., 2007), have been found in the highly acidic microenvironments of active sulfidic caves such as Cueva de Villa Luz (Hose and Pisarowicz, 1999; Hose et al., 2000) and those at Frasassi Gorge (Vlasceanu et al., 2000).

Fungi and other microbes also appear to play a role in the formation of various speleothems, which may be considered examples of erosional and depositional biokarst (Viles, 1984). Their roles may be passive, in providing nucleation centers for precipitation (Went, 1969), or active, through mechanical disturbance or chemical activity by the production of acids or enzyme products, and can involve fungi in conjunction with bacteria (Verrecchia et al., 2003). The role of fungi and bacteria in the formation of Moonmilk or Mondmilch

(Boston et al., 2001), a white, cottage-cheese-like paste of microcrystalline gypsum often found in caves, remains uncertain, although studies of fiber calcite crystals in Moonmilk from Altamira Cave by Cañaveras et al. (2006) show strong evidence supporting actinobacterial activity (unlike the fungal origin proposed by Verrecchia and Verrecchia (1994)).

Complex, ancient cave systems such as Lechuguilla Cave contain a remarkable range of microbially mediated speleothems, or "biothems," including pool fingers, and widespread ferromanganese deposits of possible microbial origin (Cunningham et al., 1995; Davis, 2000; Barton and Northup, 2007). Cunningham et al. (1995) reported 92 species of fungi in the cave and its pools, including *Aspergillus* spp. associated with Fe–Mn encrustations on calcitic folia alongside bacteria, and calcified fossil fungal filaments morphologically similar to *Aspergillus* in areas next to present-day microbial mats.

An understanding of microbial communities in deep, sulfidic caves, especially those within comparable chemoautotrophic or aphotic settings (e.g. Rasmussen, 2000), may provide fresh insights into the lifestyles of early microbial communities on the Precambrian Earth.

3. Osmotrophs in Other Early Terrestrial Settings

Today, modern fungi and actinobacteria participate actively in a range of other terrestrial processes involving calcium carbonate, including the formation of soil calcretes (Preat et al., 2003), endolithic microborings and karstification (Golubic and Schneider, 2003; Verrecchia et al., 2003) and in fluvial and terrestrial systems (e.g., Neu et al., 2003; Preat et al., 2003).

In the fossil record, fungal hyphae have been reported from the early Devonian Rhynie chert biota of Scotland, where some appear to have entered into symbiotic associations with the earliest land plants (see authors in Trewin and Rice, 2004). Further evidence for terrestrial fungal remains has come from microfossils preserved in calcrete paleosols of Devonian, Carboniferous, and Cretaceous age (Preat et al., 2003). Records of putative fungal fossils from ancient terrestrial deposits remain rare. The earliest terrestrial calcretes familiar to us are from the 1,200 Ma Stoer Group of Northwest Scotland, and are currently under investigation for evidence of their microbial components.

4. Osmotrophic Biodictyons in Ancient Tree Resins and Soils

Osmotrophic actinobacteria can be found living abundantly today in terrestrial soils, where they play an important role in breaking down organic matter. Actinobacterial colonies can be distinguished morphologically from the hyphae of basidiomycete and ascomycete fungi by their small cell diameter (typically ≤ 1 μm across), and by rounded to bacillate cell shapes. Like fungi, they typically show aggregations

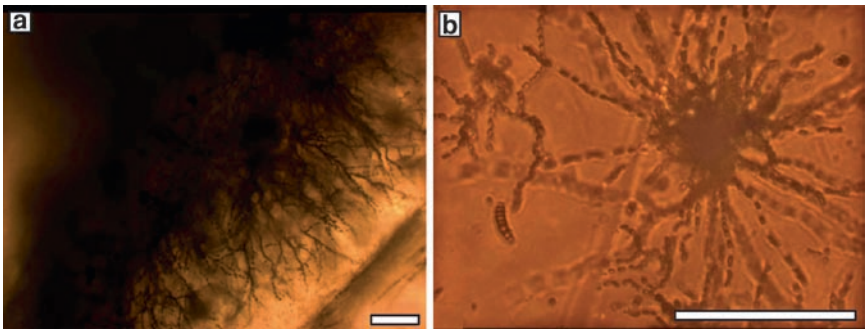

Figure 2. (**a**) Light micrograph of the margins of modern cherry tree resin (reddish color) showing a marginal zone (at *left*), darkened by the activities of actinobacterial hyphae, which can be seen penetrating into the resin itself (at *right*). (**b**) Light micrograph of a radiating actinobacterial colony within the modern resin. Transmitted light micrograph images taken with Automontage software. Both scale bars = 100 μm.

into networks and stellate cell colonies, but with the additional feature of associations with numerous tiny cells, called conidia (see Margulis and Schwartz, 1988, p. 71). Such actinobacterial communities today are typically dominated by the species of *Streptomyces* (Varnam and Evans, 2000).

In searching for active fossilization of actinobacterial colonies, we have succeeded in finding them living as pioneer colonists, both on and within the soft resins of modern cherry trees (Fig. 2a), where over a period of several years they contribute actively to the breakdown of these resins into soil components. They arrive on the surface of the resins from cells transmitted through the air, and are then seen to grow and feed by osmotrophy on the still soft surfaces of the tree resin. That this process continues as they become embalmed within the resins is shown by their continued growth and radiation into large star-like colonies within the resin itself (Fig. 2b). Over a period of months to years, we find that these resins become progressively more colonized and degraded, appearing darker and less resinous, until they are permeated by cracks, which emanate from the outer surface inward, and eventually force the resin to crumble away and fall from the bark surface.

Comparable structures and processes have now been observed in amber, a fossilized resin, obtained by us from as early as the lowest Cretaceous of Hastings (Brasier et al., 2009) and Bexhill (unpublished). Petrographic thin sections of these 130–140 Ma ambers can reveal a great abundance of tiny filaments arranged in radiating networks (Fig. 3a). These filaments can occur in several intergrading forms. The first consists of long and rather straight filaments of near constant (~1 μm) diameter, without any evident cellular constrictions. The second type shows "Y"-shaped and "T"-shaped branchings (Fig. 3b), again in filaments of about 1 μm in diameter. These can be subdivided into cell-like constrictions, often passing into filaments that can be loosely coiled (Fig. 3b). Lastly, we observe isolated cells of rounded or rod-shaped outline, also of a comparable diameter. These isolated cells

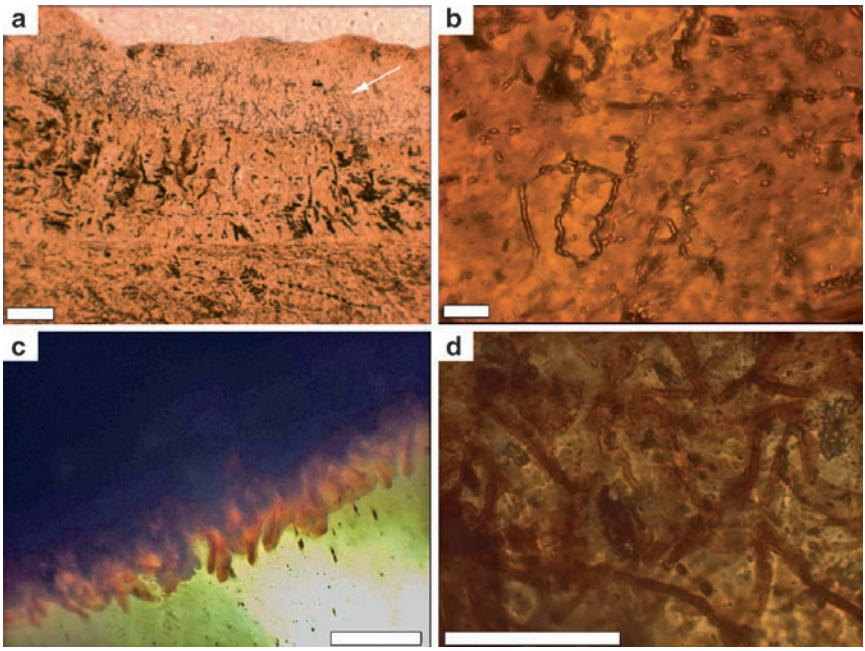

Figure 3. (**a**) Light micrograph of a Cretaceous Hastings amber slice showing three successive resin flows with dark inclusions. Each flow has been colonized and partly broken down by dark bundles of actinobacterial filaments, well seen in the upper resin flow (*white arrow*). (**b**) Petrographic thin section of a slice of Hastings amber formed between portions of fractured and charred tree bark, here preserving arrays of tiny actinobacterial filaments about 1 μm in diameter. Note the presence of filaments with "Y"- shaped branches and of subdivisions into small, cell-like constrictions. Portions of these filaments can also be loosely coiled. Isolated rod-shaped cells are also present. (**c**) Light micrograph of Cretaceous Hastings amber showing a darkened marginal zone (*above*) containing dense populations of cylindrical microtubes, which radiate toward the center of the nodule (*below*). (**d**) Detail of a similar marginal zone in Hastings amber showing filaments with "T" and "Y"-shaped branching. These were putatively made by fungal hyphae before the amber hardened. Transmitted light micrograph images taken with Automontage software. Scale bar for (**a**), (**c**), and (**d**) = 100 μm and for (**b**) = 10 μm.

can be scattered through the amber matrix, especially between and around the filaments (Fig. 3b). Together, these structures closely resemble the colonies and reproductive spores of the living actinobacteria in cherry tree resins outlined earlier. Structures of comparable small size and appearance, also referred to the actinobacteria, have been found in younger amber deposits, notably from the Barremian of the Republic of Lebanon (e.g., Poinar and Milki, 2001), from the Albian-Cenomanian of France (Néraudeau et al., 2008), and from around the margins of Eocene amber from Corbieres in France (Breton, 2007). It needs to be stressed, however, that it is not always easy to draw a distinction between the remains of prokaryotic actinobacteria and those of eukaryotic fungi within amber (Martín-González et al., 2009).

Actinobacteria are not alone in forming biodictyons (network-like transformations of mineral and organic materials), in ancient and modern resins. Zygomycete fungi, basidiomycetes, and ascomycetes also play a major role, as they do in the colonization of rotting wood and other organic substrates in modern forest soils. It follows that fungal hyphae can quite commonly be preserved in amber resins (e.g., *Aspergillus* and *Matonia* from Eocene Baltic amber; Dorfelt and Schmidt, 2005; Schmidt and Dorfelt, 2007), and they may especially be found preserved within the outer marginal zones, where it seems that they have digested tubular cavities in its surface, forming structures sometimes called "feltings." Our early Cretaceous amber nodules from Hastings and Bexhill can also show such darkened marginal zones and biodictyons (Fig. 3c, d). These networks do not arise from modern weathering, since they can be embedded within resin flows. Microscopic examination shows these zones to be made from dense populations of cylindrical microtubes ~10 μm in diameter. Many such microtubes can be seen to penetrate from the outer surface toward the center of the amber nodules (Fig. 3c). Although these microtubes can be very dense and difficult to resolve by optical means, they do not commonly appear to branch, and have been suggested to be of fungal origin (Brasier et al., 2009). Fungal hyphae and marginal microtubules have also been reported from within and around the margins of uppermost Albian to Cenomanian amber nodules in France (e.g., Ascaso et al., 2005) and Germany (Schmidt and Schäfer, 2005). Fungal remains, including the ascomycote *Ramularia*, have also been reported from Triassic ambers (Schmidt et al., 2006).

5. Osmotrophic Mats in Modern Marine Settings

Moving from the land toward the sea, we encounter marginal environments such as deltas, marshes, and mangrove swamps. The fossilization potential of microbes in these habitats would seem to be high, but records of fossilized osmotrophs and their biofilms are scant.

Stands of mangrove cultivated in our tropical marine tanks have allowed us to study the development of extensive fungal mats and networks around dead and decaying subaqueous "aerial" root systems. The exclusion of metazoan grazers by us encouraged the growth of thick, clotted, and fluffy masses of fungal mycelia (Fig. 4a, b) up to a thickness of 3 cm around the margins of decaying mangrove roots. Here, they act as decomposers of recalcitrant lignin and cellulose materials over periods of weeks to years. Transmitted light microscopy reveals these clumps to be composed of dense accumulations of thin (ca. 20 μm diameters), randomly oriented fungal filaments (Fig. 4b–d). These filaments are composed of numerous long and rod-like cells separated by infrequent septa (Fig. 4d), together with "T" and "H" shaped branchings, and sharp bends resulting from the rigidity of the chitinous fungal cell walls (Fig. 4d), all of which appear to be characteristic for basidiomycete fungal hyphae (cf. Margulis and Schwartz, 1988). It is possible to observe "H"-shaped branches forming ladder-like cross-partitions between adjacent filaments (Fig. 4d).

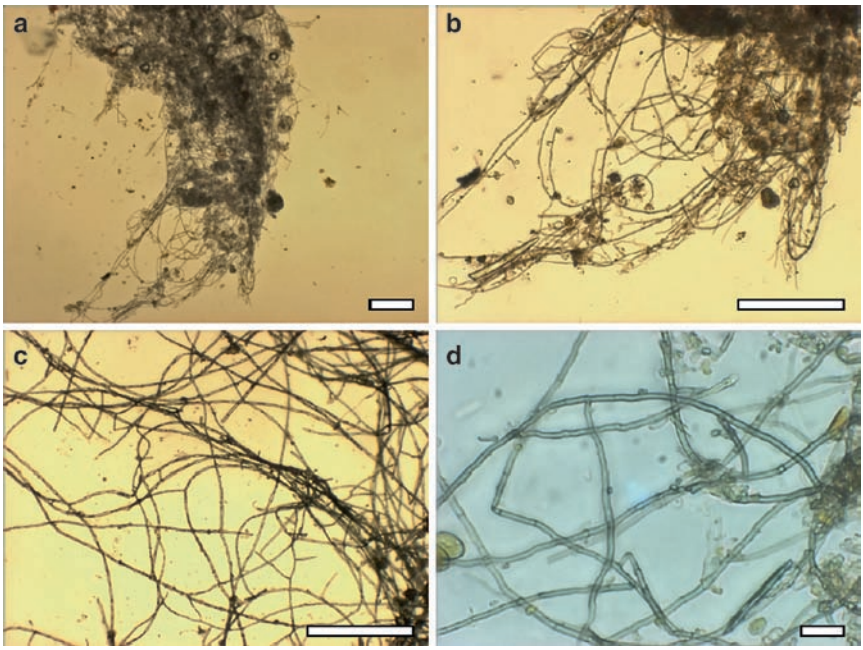

Figure 4. Fungal biofilms growing on a decomposing mangrove root in our marine laboratory in the Department of Earth Sciences, Oxford, UK. (**a, b**) Dense interlocking mesh of fluffy fungal mycelia, often associated with disseminated clotted organic materials and protoctist cells, including diatoms. (**c, d**) Higher magnification of the fungal biofilm in (**a**) and (**b**), showing typical appearance of elongate fungal cells with sparse dividing septa, sharp bends resulting from the rigidity of the chitinous fungal cell walls, and "T" and "H" type branchings. Scale bar (**a**–**c**) = 100 μm, (**d**) = 10 μm.

This distinctive feature is known as hyphal fusion and is a typical synapomorphic characteristic of fungal filaments (Butterfield, 2005). Fossil examples of such fungal biodictyons should be looked for in ancient swamp and mangrove environments, such as those of the Carboniferous coal swamps.

6. Osmotrophic Mats in Ediacaran Marginal Seas

Osmotrophs currently play an important role in many lagoonal and marginal marine settings, as demonstrated by the studies of the Laguna Figueroa of Baja California. Examination of the biofilms and mats from that region has revealed 17 species of cyanobacteria, 14 species of fungi, 75 species of heterotrophic bacteria, nine species of anoxygenic phototrophic bacteria, five species of green algae, 70 species of diatoms, and 19 species of protists (Stolz, 2003). Despite this impressive contribution from osmotrophs, the fossil record of marine microbial mats in

the Phanerozoic remains sparse, perhaps in part owing to disruption of the evidence through bioturbation and grazing. Interestingly, therefore, it is in the Precambrian where the record of microbes and their mats is most striking. A period of particular interest to our research group is the Ediacaran, in which both microbes themselves and microbially induced sedimentary structures (MISS *sensu* Noffke et al., 2001) are preserved not only in shallow water sediments (e.g., the White Sea of Russia and the Flinders Ranges of South Australia; Gehling, 1999; Dzik, 2003), but also in deeper marine environments, such as those of the Avalon region in England, Wales, and Newfoundland (Narbonne et al., 2005).

A variety of macroscopic markings are well known from the latest Ediacaran sediments of the Longmyndian Supergroup, UK, and have been used to infer widespread ancient microbial mats (McIlroy et al., 2005). It is only recently, however, that a range of exceptionally preserved microbes has been described from these sediments. Both filamentous (Fig. 5) and spheromorph microbes can be found preserved in a number of different taphonomic modes (Peat, 1984; Callow and Brasier, 2009). In many cases, these filaments can be present in high

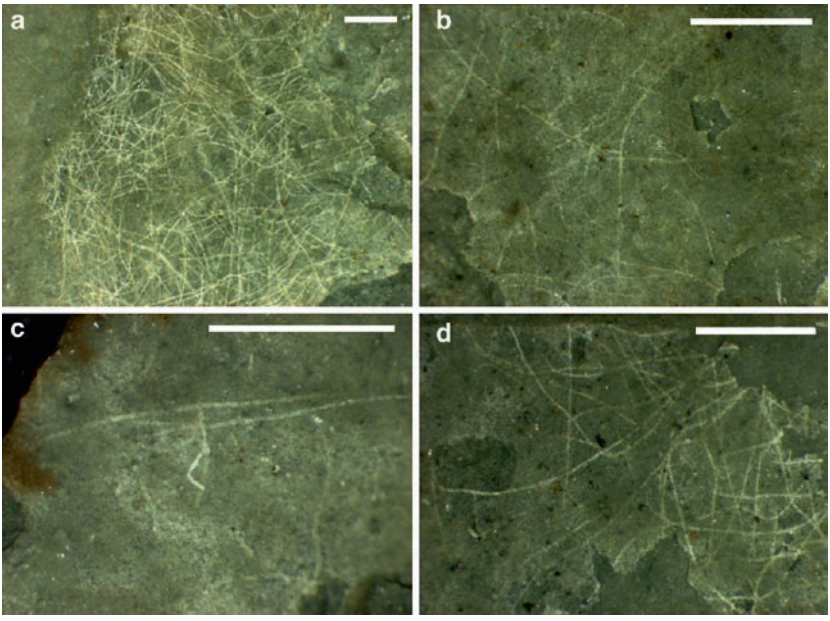

Figure 5. Filamentous microfossils preserved by replacement by aluminosilicate minerals from the late Ediacaran Burway Formation, Longmyndian Supergroup, Shropshire, UK. (**a**) Dense populations of filaments within thin siltstone laminae, showing randomly oriented filaments. These are thought to represent the remains of dense fungal mats on the seafloor. (**b, c**) Low densities of filaments, showing clear "T"-shaped branching structures. (**d**) Filaments showing clear "T"-shaped branching and possible "H"-type cross partitions between filaments, which are used as an indicator of hyphal fusion. All scale bars = 1 mm.

densities, preserved within and on top of the thin sediment laminae (Fig. 5a), indicating the presence of ancient biofilms at the sediment–water interface. While some simple, nondiagnostic, unbranched, and aseptate filaments could represent heterotrophic and osmotrophic bacteria, cyanobacteria, or even eukaryotic algae, others share clear diagnostic morphological features with fungal filaments (Fig. 5b–d). In particular, we draw attention to filamentous microbes preserved in white-colored aluminosilicate minerals within green siltstones of marine turbidite to brackish prodelta fan facies of the Burway Formation, Longmyndian Supergroup. Sometimes these filaments are present in low densities (Fig. 5b–d), and show complex overlapping, branching, and cross-cutting relationships. In other cases, the filaments are present in high densities and can either be randomly oriented or broadly parallel in orientation, possibly reflecting paleocurrents (Fig. 5a).

Thin (ca. 20 μm), long (>1 mm), straight, and sinuous filaments are present, showing "Y" and "T"-shaped branching as well as "H"-shape cross-partitions, which are interpreted as possible indicators of hyphal fusion (Fig. 5d) (Butterfield, 2005). Unfortunately, the coarse preservation within these filaments does not allow us to identify other diagnostic features, such as septa or subcellular structures. We therefore tentatively interpret these communities of microbes from the Longmyndian Supergroup as the remains of dense fungal mats. One of the problems with this, however, is that undifferentiated tubular filaments with "Y" and "T"-shaped junctions that lack clear cell walls could also be the remains of siphonalean green algae. The discovery of better preserved phosphatized or silicified specimens (cf. the lichen-like fossils from the ~580 Ma Doushantuo phosphorites of China; see Yuan et al., 2005), or the recognition of fungal biomarker molecules within these sediments, may help to test this preliminary interpretation.

7. Osmotrophic Mats in Deeper Ediacaran Seas

Most interestingly, microbial mat-type fabrics have been reported from the Ediacaran Conception Group, laid down in waters deeper than 1 km (Wood et al., 2003) and therefore below the reach of sunlight. These include "old elephant skin" and simple wrinkles over large areas of bedding planes (Fig. 6a) and discrete colonies growing on the tops of decaying Ediacaran macroorganisms such as *Bradgatia* and *Charniodiscus* (Fig. 6b). Indeed, some of these decayed organisms have previously been described as distinct taxa (such as *Ivesheadia* and *Shepshedia* of Boynton and Ford, 1995). Our field and laboratory observations suggest that colonization and slow decomposition of the carcasses of these Ediacaran organisms by bacteria and fungi have smoothed out and transformed their profiles (Liu et al., in press). These ecosystems were later smothered by ashes and turbidites, thereby preserving the specimens in a state of partial decay.

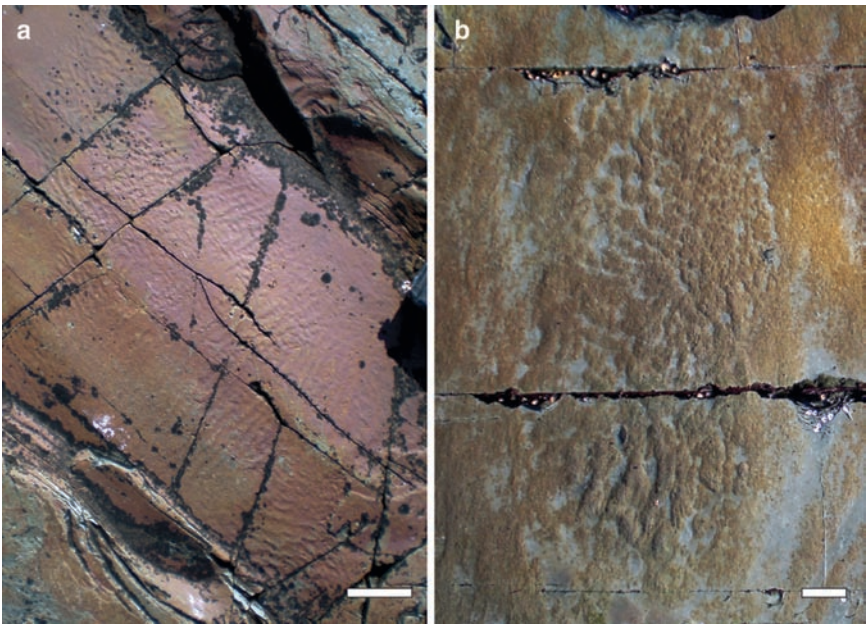

Figure 6. (**a**) Microbially induced wrinkle markings on bedding plane top surfaces from the Ediacaran of Western Head, Newfoundland. Such structures are considered to represent microbial mats on the deep sea floor, and are common in Ediacaran and early Cambrian settings. (**b**) An *Ivesheadia* specimen from the Mistaken Point Formation, Bonavista Peninsula, Newfoundland. Such impressions may record stages in a preservational spectrum documenting the slow decay of Ediacaran macroorganisms such as *Bradgatia* or *Charniodiscus* by microbial activity (see Liu et al., in press). Scale bars both = 5 cm.

Our laboratory experiments attempting to replicate this style of preservation show that when the bodies of invertebrate organisms are left to decay in seawater in the absence of bioturbators and scavengers, their tissues are invariably colonized and broken down by microbial communities, which include both bacteria and fungi, with the process lasting for a period of several weeks. The role of osmotrophs in such environments is likely to be very important, and our studies in this area are ongoing.

8. Osmotrophs and the Cambrian Explosion

Osmotrophic microbes are fundamental to a wide range of biosedimentary processes, ranging from the digestion of relatively refractory organic materials including chitin and cellulose to the provision of suitable substrates for deposit feeders and scavengers on the seafloor. It follows that we might expect to find clear evidence for osmotrophic activity on the seafloor in the distant past, and most especially so at the beginning of the Cambrian Explosion, when extensive

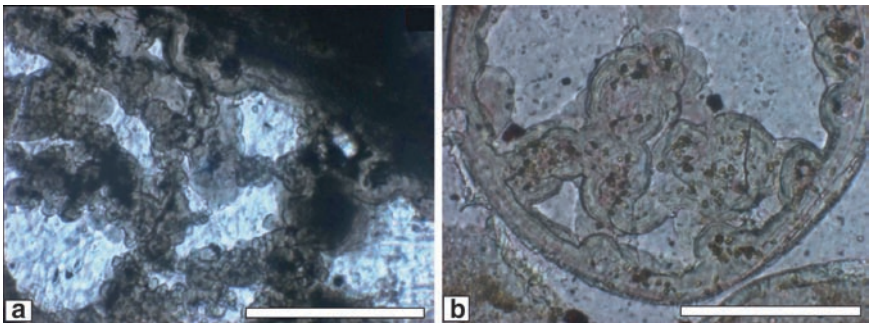

Figure 7. (**a**) Light micrograph of a petrographic thin section from a small cavity within the basal Cambrian Tal phosphorite of India. Note the phosphate-encrusted filaments of possible fungal origin crossing an internal cavity formed within the semi-lithified sediment. Dark cylindrical threads are here coated with botryoidal phosphate to produce a pseudococcoidal appearance. (**b**) A range of similar thread-like features seen within an embryo-like *Olivooides* vesicle, from the basal Cambrian Meishucun phosphorite of Yunnan, China, also of possible fungal origin. Transmitted light micrograph images taken with Automontage software. Both scale bars = 100 μm.

burrowing and scavenging appears to have begun on the seafloor. This bioturbation transformed it from a rather uniform interface to a complexly zoned mixed layer, like that of a modern soil (Brasier, 2009).

Although a major role for fungi in the Cambrian Explosion has long been mooted (e.g., B. Sokolov, 1983, personal communication), the evidence still remains circumstantial or indirect. Narrow threads coated with phosphate are, for example, a familiar feature of the internal cavities of remarkably preserved fossils from the earliest Cambrian (Fig. 7) (see also Brasier and Callow, 2007). Many of these filaments could be the remains of microcoelobionts that lived within the dark, oxygen-depleted but protected cavities of decaying bodies (Fig. 7a). In a few cases, these cavities were wholly enclosed spaces, as with the internal cavities of the "embryos" called *Olivooides* (Fig. 7b). It seems reasonable to speculate, but more difficult to prove, that these microfossils resulted from colonization of decaying animal tissues by osmotrophic fungi and bacteria, and that their hyphae became coated with calcium phosphate during early diagenesis on the seafloor. These secondary coatings of phosphate are much thicker than the original hyphae, and often form botryoidal shapes on the outer surface (Fig. 7a, b).

9. Conclusion

As shown by the examples illustrated earlier, osmotrophs can play a significant role in the formation of modern and ancient biofilms and soils, and may have a much better potential to enter the fossil record than has hitherto been realized.

They deserve to be searched out more vigorously in the fossil record, especially in the following settings where they may be expected to flourish: Precambrian substrates that accumulated before the evolution of metazoan grazers; substrates in which metazoan grazing was inhibited by extreme physical conditions, such as high temperatures or low oxygen; substrates in which the organic matter was relatively refractory, such as algal tissue, chitin, and woody tissue; substrates that were deposited beyond the reach of sunlight. Osmotrophic biofilms may have played an important role in the breakdown of organic matter and in the formation of both marine and terrestrial "soils" during the early history of the Earth, helping to make the Earth a more habitable planet.

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11. References

Allwood, A.C., Walter, M.R., Kamber, B.S., Marshall, C.P. and Burch, I.W. (2006) Stromatolite reef from the Early Archaean era of Australia. Nature **441**: 714–718.

Ascaso, C., Wierzchos, J., Speranza, M., Gutiérrez, J.C., Martín-González, A., De Los Rios, A. and Alonso, J. (2005) Fossil protists and fungi in amber and rock substrates. Micropaleontology **51**: 59–72.

Barton, H.A. and Northup, D.E. (2007) Geomicrobiology in cave environments: past, current and future perspectives. J. Cave Karst Stud. **69**: 163–178.

Boston, P.J., Spilde, M.N., Northup, D.E., Melim, L.A., Soroka, D.S., Kleina, L.G., Lavoie, K.H., Hose, L.D., Mallory, L.M., Dahm, C.N., Crossey, L.J. and Schelble, R.T. (2001) Cave biosignature suites: microbes, minerals, and Mars. Astrobiology **1**: 25–55.

Boynton, H.E. and Ford, T.D. (1995). Ediacaran fossils from the Precambrian (Charnian supergroup) of Charnwood Forest, Leicestershire, England. Mercian Geologist **13**: 165–182.

Brasier, M.D. (2009) *Darwin's Lost World. The Hidden History of Animal Life.* Oxford University Press, Oxford.

Brasier, M.D. and Callow, R.H.T. (2007) Changes in the patterns of phosphatic preservation across the Proterozoic–Cambrian transition. Mem. Assoc. Austral. Palaeontol. **34**: 377–389.

Brasier, M.D., Cotton, L. and Yenney, I. (2010). First report of amber with spider web and microbial inclusions from the earliest Cretaceous (~140 Ma) of Hastings, Sussex. J. Geol. Soc. London. (in press)

Breton, G. (2007). La bioaccumulation de microorganismes dans l'ambre: analyse comparée d'un ambre cénomanien et d'un ambre sparnacien, et de leurs tapis algaires et bactériens. C. R. Palevol. **6**: 125–133.

Butterfield, N.J. (2005). Probable Proterozoic fungi. Paleobiology **31**: 165–182.

Callow, R.H.T. and Brasier, M.D. (2009) A solution to Darwin's dilemma of 1859: exceptional preservation in Salter's material from the late Ediacaran Longmyndian Supergroup, England. J. Geol. Soc. London **166**: 1–4.

Campbell, B.J., Engel, A.S., Porter, M.L. and Takai, K. (2006) The versatile ε-proteobacteria: key players in sulphidic habitats. Nature **4**: 458–468.

Cañaveras, J.C., Sanchez-Moral, S., Soler, V. and Saiz-Jimenez, C. (2001) Microorganisms and microbially induced fabrics in cave walls. Geomicrobiol. J. **18**: 223–240.

Cañaveras, J.C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J.M. and Saiz-Jimenez, C. (2006) On the origin of fiber calcite crystals in moonmilk deposits. Naturwissenschaften **93**: 27–32.

Costerton, J.W. and Stoodley, P. (2003) Microbial biofilms: protective niches in ancient and modern geomicrobiology, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. xv–xxi.

Cunningham, K.L., Northup, D.E., Pollastro, R.M., Wright, W.G. and Larock, E.J. (1995) Bacteria, fungi and biokarst in Luchuguilla Cave, Carlsbad Caverns National Park, New Mexico. Environ. Geol. **25**: 2–8

Davis, D.G. (2000) Extraordinary features of Lechuguilla Cave, Guadalupe Mountains, New Mexico. J. Cave Karst Stud. **62**: 147–157.

Dorfelt, H. and Schmidt, A.R. (2005) A fossil *Aspergillus* from Baltic amber. Mycol. Res. **109**: 956–960.

Duane, M.J. (2003) Unusual preservation of crustaceans and microbial colonies in a vadose zone, northwest Morocco. Lethaia **36**: 21–32.

Dupont, J., Jacquet, C., Dennetiérre, B., Lacoste, S., Bousta, F., Orial, G., Cruard, C., Couloux, A. and Roquebert, M. (2007) Invasion of the French Paleolithic painted cave of Lascaux by members of the *Fusarium solani* species complex. Mycologia **99**: 526–533.

Dzik, J. (2003) Anatomical information content in the Ediacaran fossils and their possible zoological affinities. Integr. Comp. Biol. **43**: 114–126.

Egemeier, S.J. (1981) Cavern development by thermal waters. Nat. Speleol. Soc. Bull. **43**: 31–51.

Engel, A.S. (2007) Observations on the biodiversity of sulfidic karst habitats. J. Cave Karst Stud. **69**: 187–206.

Engel, A.S., Porter, M.L., Stern, L.A., Quinlan, S. and Bennett, P.C. (2004a) Bacterial diversity and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic "*Epsilonproteobacteria*". FEMS Microbiol. Ecol. **51**: 31–53.

Engel, A.S., Stern, L.A. and Bennett, P.C. (2004b) Microbial contributions to cave formation: new insights into sulfuric acid speleogenesis. Geology **32**: 369–372.

Gehling, J.G. (1999) Microbial mats in terminal Proterozoic siliciclastics: Ediacaran death masks. Palaios **14**: 40–57.

Golubic, S. and Schneider, J. (2003) Microbial endoliths as internal biofilms, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 249–264.

Grotzinger, J.P. and Knoll, A.H. (1999) Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? Annu. Rev. Earth Planet. Sci. **27**: 313–358.

Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L. and Hedges, S.B. (2001) Molecular evidence for the early colonization of land by fungi and plants. Science **293**: 1129–1133.

Hose, L.D. and Pisarowicz, J.A. (1999) Cueva de Villa Luz, Tabasco, Mexico: reconnaissance study of an active sulfur spring cave and ecosystem. J Cave Karst Stud. **61**: 13–21.

Hose, L.D., Palmer, A.N., Palmer, M.V., Northup, D.E., Boston, P.J. and Duchene, H.R. (2000) Microbiology and geochemistry in a hydrogen-sulphide-rich karst environment. Chem. Geol. **169**: 399–423.

Jagnow, D.H., Hill, C.A., Davis, D.G., Duchene, H.R., Cunningham, K.I., Northup, D.E. and Queen, J.M. (2000) History of the sulfuric acid theory of speleogenesis in the Guadalupe Mountains, New Mexico. J. Cave Karst Stud. **62**: 54–59.

Jannasch, H.W. (1985) The chemosynthetic support of life and the microbial diversity at deep-sea hydrothermal vents. Proc. Roy. Soc. London B **225**: 277–297.

Karl, D.M., Wirsen, C.O. and Jannasch, H.W. (1980) Deep-sea primary production at the Galapagos hydrothermal vents. Science **207**: 1345–1347.

Kinkle, B.K. and Kane, T.C. (2000) Chemolithoautotrophic microorganisms and their potential role in subsurface environments, In: H. Wilkens, D. Culver and W.F. Humphreys (eds.) *Ecosystems of the World: Subterranean Ecosystems.* Elsevier, Amsterdam, pp. 309–318.

Kretzschmar, M. (1982) Fossile Pilze in Eisen-Stromatolithen von Warstein (Rheinisches Schiefergebirge). Facies **7**: 237–260.

Hose, L.D. and Pisarowicz, J.A. (1999) Cueva de Villa Luz, Tabasco, Mexico: reconnaissance study of an active sulfur spring cave and ecosystem. J. Cave Karst Stud. **61**: 13–21.

Krumbein, W.E., Paterson, D.M. and Zavarzin, G.A. (eds.) (2003) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, 482 pp.

Lascu, C., Popa, R., Sarbu, S.M., Vlasceanu, L. and Prodan, S. (1993) La grotte de Movile: une faune hors du temps. La Réchérche **258**: 1092–1098.

Lecointre, G. and Guyader, H.L. (2006) *The Tree of Life.* Belknap Press, Harvard, 560 pp.

Levin, L.A. (2005) Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanogr. Mar. Biol. Annu. Rev. **43**: 1–46.

Liu, A.G., McIlroy, D., Antcliffe, J.B. and Brasier, M.D. (2010) Post-mortem decay of the Avalonian Ediacara biota and its implications for the early fossil record (in press).

Macaladay, J.L., Jones, D.S. and Lyon, E.H. (2007) Extremely acidic, pendulous cave wall biofilms from the Frasassi cave system, Italy. Environ. Microbiol. **9**: 1402–1414.

Margulis, L. and Schwartz, K.V. (1988) *The Five Kingdoms.* W.H. Freeman, New York.

Martín-González, A., Wierzchos, J., Gutiérrez, J.C., Alonso, J. and Ascaso, C. (2009) Microbial Cretaceous park: biodiversity of microbial fossils entrapped in amber. Naturwissenschaften **96**: 551–564.

Mattison, R.G., Abbiati, M., Dando, P.R., Fitzsimons, M.F., Pratt, S.M., Southward, A.J. and Southward, E.C. (1998) Chemoautotrophic microbial mats in submarine caves with hydrothermal sulphidic springs at Cape Palinuro, Italy. Microb. Ecol. **35**: 58–71.

McIlroy, D., Crimes, T.P. and Pauley, J.C. (2005) Fossils and matgrounds from the Neoproterozoic Longmyndian Supergroup, Shropshire, UK. Geol. Mag. **142**: 441–455.

Narbonne, G.M., Dalrymple, R.W., LaFlamme, M., Gehling, J.G. and Boyce, W.D. (2005) *Life After Snowball: Mistaken Point Biota and the Cambrian of the Avalon.* North American Paleontological Convention Field Trip Guidebook, Halifax, Nova Scotia.

Néraudeau, D., Perrichot, V., Colin, J.-P., Girard, V., Gomez, B., Guillocheau, F., Masure, E., Peyrot, D., Tostain, F., Videt, B. and Vullo, R. (2008) A new amber deposit from the Cretaceous (uppermost Albian-lowermost Cenomanian) of southwestern France. Cretaceous Res. **29**: 925–929.

Neu, T.R., Eitner, A. and Paje, M.L. (2003) Development and architecture of complex environmental biofilms – lotic biofilm systems, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 29–45.

Noffke, N., Gerdes, G., Klenke, T. and Krumbein, W.E. (2001) Microbially induced sedimentary structures – a new category within the classification of primary sedimentary structures. J. Sed. Res. **7**: 649–656.

Noffke, N., Eriksson, K.A., Hazen, R.M. and Simpson, E.L. (2006) A new window into Early Archean life: Microbial mats in Earth's oldest siliciclastic tidal deposits (3.2 Ga Moodies Group, South Africa). Geology **34**: 253–256.

Northup, D.E. and Lavoie, K.H. (2001) Geomicrobiology of caves: a review. Geomicrobiol. J. **18**: 199–222.

Peat, C. (1984) Precambrian microfossils from the Longmyndian of Shropshire. Proc. Geol. Assoc. **5**: 17–22.

Poinar, G.O. and Milki, R. (2001) *Lebanese Amber: The Oldest Insect Ecosystem in Fossilized Resin*. Oregon State University Press, Corvallis, OR.

Porter, M.L., Engel, A.S., Kane, T.C. and Kinkle, B.K. (2009) Productivity–diversity relationships from chemolithoautotrophically based sulfidic karst systems. Int. J. Speleol. **38**: 27–40.

Poulson, T.L. and Lavoie, K.H. (2000) The trophic basis of subsurface ecosystems, In: H. Wilkens, D.C. Culver and W.F. Humphreys (eds.) *Ecosystems of the World: Subterranean Ecosystems.* Elsevier, Amsterdam, pp. 231–249.

Preat, A., Kolo, K., Mamet, B., Gorbushina, A.A. and Gillian, D.C. (2003) Fossil and subrecent fungal communities in three calcrete series from the Devonian Canadian Rocky Mountains, Carboniferous of northern France and Cretaceous of central Italy, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 291–306.

Rasmussen, B. (2000) Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. Nature **405**: 676–679.

Reitner, J., Schumann, G. and Pedersen, K. (2006) Fungi in biogeochemical cycles, In: G.M. Gadd (ed.) *Fungi in Biogeochemical Cycles.* Cambridge University Press, Cambridge, pp. 377–403.

Ross, I.K. (2006) Fungal lives, In: J. Seckbach (ed.) *Life as We Know It*. Springer, Dordrecht, pp. 55–73.

Sarbu, S.M., Vlasceanu, L., Popa, R., Sheridan, P., Kinkle, B.K. and Kane, T.C. (1994) Microbial mats in a thermomineral sulfurous cave, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development, and Environmental Significance.* Springer, Berlin, pp. 45–50.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (eds.) (2007) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record*. Elsevier, Amsterdam, 311 pp.

Schmidt, A.R. and Dorfelt, H. (2007) Evidence of Cenozoic Matoniaceae from Baltic and Bitterfeld amber. Rev. Palaeobot. Palynol. **144**: 145–156.

Schmidt, A.R. and Schäfer, U. (2005) *Leptotrichites resinatus* new genus and species, a fossil sheathed bacterium in alpine Cretaceous amber. J. Paleontol. **79**: 175–184.

Schmidt, A.R., Ragazzi, E., Coppellotti, O. and Roghi, G. (2006) A microworld in Triassic amber. Nature **444**: 835.

Schopf, J.W. and Klein, C. (1992) *The Proterozoic Biosphere.* Cambridge University Press, Cambridge, UK.

Schopf, J.W., Zhu, W.-Q., Xu, Z.-L. and Hse, J. (1984) Proterozoic stromatolitic mmicrobiotas of the 1400–1500 Ma-old Gaoyuzhuang formation near Jixian, northern China. Precambrian Res. **24**: 335–349.

Stolz, J.F. (2003) Structure of marine biofilms – flat laminated mats and modern marine stromatolites, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 65–76.

Trewin, N.H. and Rice, C.M. (eds.) (2004) The Rhynie hot-spring system: geology, biota and mineralization. Trans. R. Soc. Edinb. Earth Sci. **94**, 239 pp.

Varnam, A.H. and Evans, M.G. (2000) *Environmental Microbiology.* Manson Publishing, London, 160 pp.

Verrecchia, E.P. and Verrecchia, K.E. (1994) Needle-fiber calcite: a critical review and proposed classification. J. Sed. Res. **A64**: 650–664.

Verrecchia, E.P., Loisy, C., Braissant, O. and Gorbushina, A.A. (2003) The role of fungal biofilm and networks in the terrestrial calcium carbonate cycle, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms.* Kluwer, Dordrecht, pp. 363–369.

Viles, H.A. (1984) Biokarst. Review and prospect. Prog. Phys. Geogr. **8**: 532–542.

Vlasceanu, L., Sarbu, S.M., Engel, A.S. and Kinkle, B.K. (2000) Acidic cave-wall biofilms located in the Frasassi Gorge, Italy. Geomicrobiol. J. **17**: 125–139.

Went, F.W. (1969) Fungi associated with stalactite growth. Science **166**: 385–386.

Wood, D.A., Dalrymple, R.W., Narbonne, G.M., Gehling, J.G. and Clapham, M.E. (2003) Paleoenvironmental analysis of the late Neoproterozoic Mistaken Point and Trepassey formations, southeastern Newfoundland. Can. J. Earth Sci. **40**: 1375–1391.

Yuan, X., Xiao, S. and Taylor, T.N. (2005) Lichen-like symbiosis 600 million years ago. Science **308**: 1017–1020.

Biodata of **Elizabeth Chacón Baca**, author of ***"Microbial Mats as a Source of Biosignatures"***

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MICROBIAL MATS AS A SOURCE OF BIOSIGNATURES

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1. What Is a Microbial Mat?

Microbial mats are layered organosedimentary structures formed by benthic communities of microorganisms distributed according to their metabolic capacities relative to geochemical gradients (van Gemerden, 1993; Stal et al., 1985), created by the interplay between diverse physical and chemical factors that promote the proliferation of certain species (Stolz, 2000). Microbial mats develop on solid surfaces of abiogenic and biogenic substrates in aquatic or semiaquatic habitats and under a wide variety of environmental conditions such as hydrothermal settings, extremely cold settings, or hypersaline or acidic pools; they are vertical and geochemically stratified carpets where nutrients and metabolites diffuse. Among the main factors for mat development is a founding population that starts to colonize a substrate, a source of energy, and a continuous supply of organic and inorganic metabolites. Spectacular microbial mats have been reported not only from extreme environments of diverse compositional range (Stal, 2000, 2007; Zavarzin, 2001) such as those found in Yellowstone Park (Ward et al., 2006), New Zealand (Jones and Renaut, 2006), or Abu Dhabi (Abed et al., 2008) but also from quite normal environments such as the continental mats developing on deserts, in lacustrine and fluvial systems around the world, or in transitional environments (Stal, 2001) like the tidal flats from the Northern Atlantic (Kremer et al., 2008) and the water blooms from the Baltic Sea (Stal et al., 2003). Microbial mats exhibit a layered structure ranging from a few micrometers up to several centimeters thick displaying a vivid combination of colors and textures (Fig. 1).

Fenchel et al. (1998) documented that some of the first descriptions of microbial mats can be found in the works of Ørsted (1842) and Hofman (1826), who described the laminated and colored sandy sediments of the kilometer-sized tidal flats ecosystem found at the Wadden Sea (Germany). Black (1933) observed species of filamentous cyanobacteria such as *Schizothrix* and *Phormidium* binding a new layer of sediments in the modern carbonates from the Bahamas, a basic feature that he interpreted as annual changes in the sedimentation and tide rates. Microbial mats can be composed of cyanobacteria, iron bacteria, or purple sulfur bacteria supported by allochthonous organic carbon (Fenchel et al., 1998). Among the huge

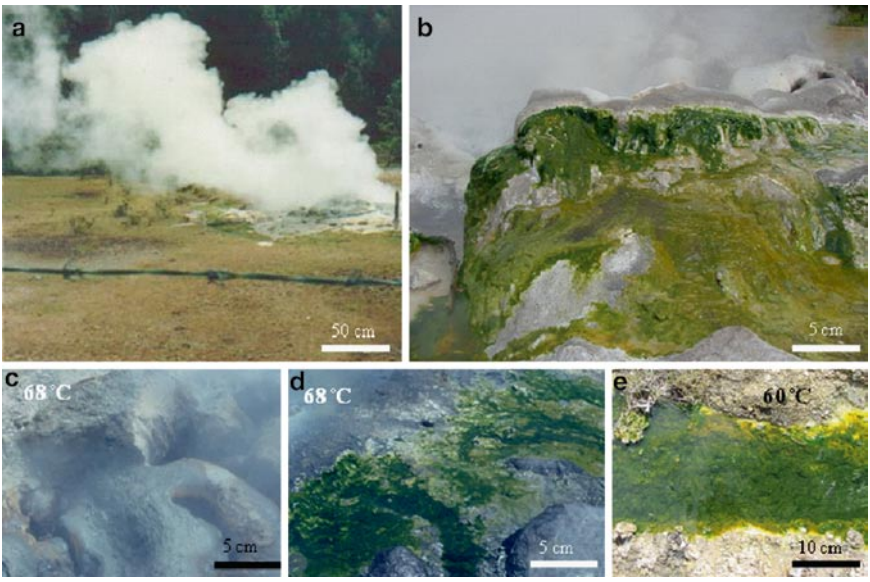

Figure 1. Microbial mats in the sulfurous springs from Los Azufres, Michoacan, Mexico. (**a, b**) A typical spot where microbial mats profusely develop along a temperature gradient; (**c**) silica in situ precipitation; (**d**) close-up of the thermal sulfurous springs where dark and light green mats grow; (**e**) cyanobacterial laminated mats at 60°C.

microbial diversity in nature, cyanobacterial communities stand out as major microbial mat builders, which form by far the most conspicuous mats since the early evolution of life (Awramik, 1977; Stoodley et al., 2002). Cyanobacteria are phototrophic prokaryotes that include unicellular and colonial species organized in different types of filaments or coccoidal pluricellular aggregates. Cyanobacteria form microbial mats on diverse sedimentary surfaces, releasing O_2 as by-product and accumulating reduced carbon and sulfur. Their ability to perform oxygenic photosynthesis based on the activity of photosystem II and I make cyanobacteria the leading evolutionary architects of our modern biosphere.

The release of oxygen as a photosynthetic by-product caused the oxygenation of the entire planet, not only changing the face of the Earth but also determining the course of biological evolution. In addition to being significant primary producers, cyanobacteria also represent the living descents of the ancestral endosymbionts that evolved into chloroplasts (Tomitani et al., 1999; Douglas and Raven, 2003). Although the appearance of oxygenic photosynthesis has been coupled to the evolution of cyanobacteria (Buick, 1992; Kasting and Siefert, 2002), the emergence of cyanobacteria and the origin of photosynthesis are among the most thrilling geobiological events that still wait for elucidation. Some unresolved issues on the evolution of early ecosystems are the timing of the atmospheric transition (Liang et al., 2006; Xiong et al., 2000) or the role of gene transfer and the evolution of oxygen-mediating enzymes (Falkowski, 2006).

Microbial mats display special physical characteristics to behave as structural units: mechanical integrity, stability, and cohesiveness. They are embedded within extracellular polymeric substances or EPS (Weckesser and Drews, 1979; Costerton et al., 1995) composed of highly organized polymers such as cellulose and small molecules such as uronic acid and amino acids among others (Decho et al., 2008). This EPS accumulates outside the cells forming an organic matrix to which microbes attach.

Mineral precipitation occurs within the amorphous exopolymer matrix of EPS, rather than onto the surface of cyanobacterial sheaths (Reid et al., 2000). But at the same time, cyanobacterial mats are complex geochemical factories where geological processes converge with the intrinsic biology of microorganisms. They function as biosystems with an active recycling of O, C, and S derived from photosynthesis, aerobic respiration, fermentation, sulfide oxidation, sulfate reduction, and methano genesis (Fenchel et al., 1998). Modern studies have stressed the importance of light in the generation of microbialites for the stabilization of a mat, emphasizing the role of photosynthesis (Andres and Reid, 2006; Paterson et al., 2008).

Frequently, there is no sharp distinction between the term *biofilm* and *microbial mat*, since they share a common genesis. Biofilms form when microbial communities associate to any surface in which the position and spatial relations of each component cell are dominated by a single microbial population. The reader is referred to Gerdes (2008), Levit (2008), Krumbein (2008), and references therein. In microbial mats, there are several coexisting populations showing a coordinated response − called *quorum sensing* − an emergent mechanism mediated by signal molecules and some type of positioning mechanism (Stoodley et al., 2002). Figure 2 shows some examples of cyanobacteria associated to a modern microbial mat isolated from moderate thermal springs in Linares, Nuevo Leon in northeastern Mexico. The springs of Baño San Ignacio (BSI) represent a mesothermal sulfurous environment with pH fluctuations between 7 and 7.4 and temperatures ranging from 23°C in winter up to 37°C in summer time.

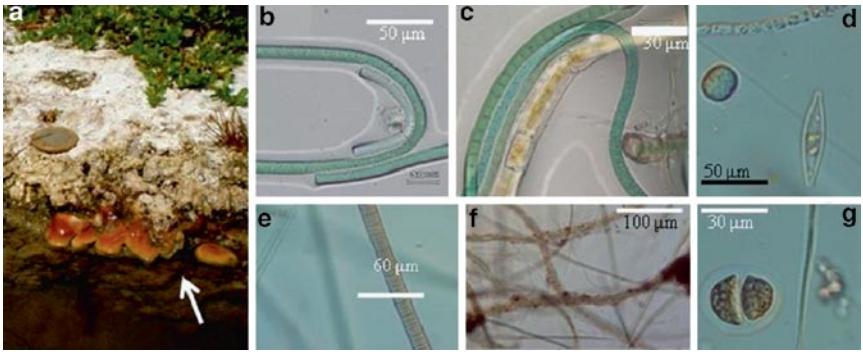

Figure 2. Microbial mats of Baño San Ignacio. (**a**) Orange-colored cyanobacterial mats developing on travertines, (**b**–**g**) the most representative filamentous cyanobacteria in the mat community correspond to species of *Phormidium*, *Oscillatoria*, *Pseudanabaena*, *Schizothrix*, and to several species of *Chroococcus* co-occurring with pennate diatoms.

1.1. MICROBIAL MATS AS PRECURSORS OF STROMATOLITES

The generation of stromatolites has been investigated through the study of modern microbial mat communities from diverse environments, in which the working hypothesis presumes that microbial mats are the precursors of stromatolites (Krumbein, 1983; Krumbein et al., 2003). Indeed, microbial mats are complex biogeochemical systems, in which multiple interactions between microorganisms and mineral interfaces continuously occur. Although this research area has been quite fertile, there is an increasing awareness about the complexity of these biosystems at the spatial microscale parallel to diagenetic changes over time.

Stromatolites are the oldest and most important biosedimentary record of the early Earth, where the first microbial communities developed complex ecological interactions with sedimentation since the Archean (Fig. 3). Though stromatolites are not fossils sensu stricto, they may be considered as macroscopic trace fossils or ichnofossils since they are macroscopic sedimentary relics of former (and lithified) microbial mats. Stromatolite-building microorganisms like cyanobacteria and other heterotrophic bacteria dominated most of the early microbial ecosystems during the Proterozoic, playing a decisive role in the evolution of our biosphere (Grotzinger and Knoll, 1999; Altermann et al., 2006; Kazmierczak et al., 2009; Knoll, 2008), but whether the first Archean stromatolites were built by cyanobacteria or by other anoxygenic photosynthetic organisms is unknown. The oldest record of stromatolites has been firmly established by 3.5 Ga (Allwood et al., 2006; van Kranendonk, 2008 and references therein); in addition to their paleoenvironmental reconstruction, the observed sediment behavior during laminae accretion, precipitation, trapping, and binding in some Archean stromatolites argue in favor of a biogenic origin (Altermann, 2008).

These laminated biodeposits are built by the physical trapping and binding of sedimentary debris and by precipitation on microbial mats as the communities

Figure 3. (**a**) Archean stromatolites (3.5×10^9 years) from the Pilbarra Craton, Australia. (**b**) Archean MISS (microbially induced sedimentary structures) showing fossil multidirected ripple marks from the locality in Pongola Group, South Africa. Picture (**a**) was graciously provided by Professor Andy Knoll. Picture (**b**) was graciously provided by Nora Noffke.

grow (Awramik, 1977). However, other rocks with a stromatolitic texture (laminated) may result from pure chemical precipitation (see Hofmann, 2000). Although most of them occur always associated to shallow waters from supratidal and intertidal settings, stromatolites from deep water environments from the Archean and Proterozoic have also been reported (Hoffman, 1974; Bertrand-Sarfati and Moussine-Pouchkine, 1985; Narbonne and James, 1996; Altermann, 2008; Kah et al., 2009). Today, laminated microbial mats are common in a wide range of environments and probably they were more diverse, abundant, and impressive in Precambrian scenarios. Figure 4 shows a laminated mat slide from Baño San Ignacio (BSI) with alternating layers or light sediments and organics.

Although the most conspicuous feature of stromatolites is their alternated lamination, stromatolites also show a characteristic macroscopic morphology

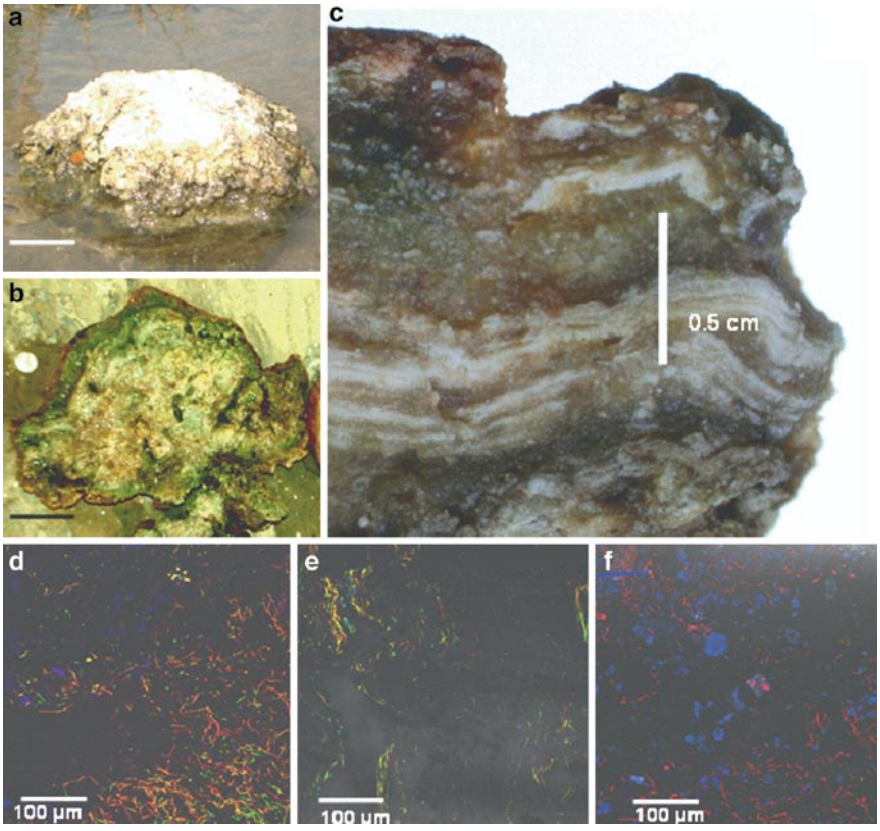

Figure 4. (**a**) Microbial mats associated to travertines in Baño San Ignacio; (**b**) cross section of the fungal-like texture of the layered mat; (**c**) mat slice showing a clear defined lamination; (**d**–**f**) natural fluorescence of a fresh natural slide of the mat showing diverse microbiota with in situ carbonate precipitates.

(macrostructure) and a distinctive microscopic fabric (microstructure). Among the macroscopic attributes of stromatolites are their accretion habit, surface morphology, dimensions, and composition, whereas their microscopic characteristics include configuration, linkage, spacing, and relief of lamination (Hofmann, 2000). Cyanobacterial mat communities have influenced carbonate precipitation and dissolution as long-term geomorphological agents, recycling carbon and carbonates at global scales (Schneider and LeCampion-Alsumard, 1999; Kazmierczak and Altermann, 2002; Kazmierczak et al., 2009).

1.2. ACCRETION OF A MICROBIAL MAT

One preliminary requirement for the colonization of a given substrate and further development of a mat is the establishment of the founder community. Once a surface is colonized, the whole community continues the stabilization process (Reid et al., 2000). Mats are rich in mucilaginous material derived from cyanobacteria; precisely this sticky material allows the continuing trapping of sedimentary debris (Riding, 2000). In some well-studied examples in which stromatolites mostly trap and bind sediments, cohesion plays a prominent role in the construction of the organic framework (Verrecchia et al., 1995). In other stromatolites, however, precipitation is the dominant process for accretion (Altermann, 2008 and references therein). Verrecchia et al. (1995) analyzed the role of sediment cohesion in the construction of an organic framework and the importance of dead organic matter during postmortem mineralization.

Several other observations have emphasized the role of heterotrophs in $CaCO_3$ precipitation, promoted by sulfate reduction during stromatolite lithification (Dupraz et al., 2004; Visscher et al., 1998; Andres and Reid, 2006; Dittrich and Sibler, 2005; Obst et al., 2005; Vasconcelos et al., 2006; Tourney and Ngwenya, 2009) and during micritic laminae production (Visscher et al., 2000). Actually, there is experimental evidence regarding the templating role of EPS in $CaCO_3$ precipitation (Dupraz and Visscher, 2005; Obst et al., 2005; Bontognali et al., 2008). In addition, Stephens et al. (2008) have confirmed the role of sulfate-reducing bacteria (SRB) in $CaCO_3$ precipitation on several lithifying mats, reporting EPS-degrading spirochetes that produce low molecular organic compounds. The role of heterotrophic respiration has also been acknowledged in the recent microbialites of Cuatro Cienegas, Mexico (Breitbart et al., 2008).

Is the stratification in microbial mats equivalent to stromatolitic lamination? The microscopic characteristic of lamination may be related to species interaction and diurnal changes in some key factors (nutrients, light, oxygen, and ionic composition of water, and spatial organization within the microbial mat); however, the temporal scales between the lamination in a mat and that of stromatolites may reflect quite different processes. According to their sources and metabolic needs, microbial communities distribute vertically, resulting in layered mats (Costerton et al., 1995; Reid et al., 2003). Microbial mats may trap and concentrate

carbonate minerals by the microscale impacts of sedimentary particles that adhere to the mucilage. The cohesive nature of the mucopolysaccharides of cyanobacterial sheaths is an important factor for the accretion of some stromatolites, since it acts as sedimentary glue that enhances further sediment trapping (Stoodley et al., 2002). Of course, under streaming water conditions and high energy, the glue effect may be null. Whereas in binding stromatolites the overgrowth activity and the roughness of the mat surface play critical roles, morphology does in trapping stromatolites. Microbial baffling and trapping may concentrate sedimentary particles, stabilize them against erosion, and increase their preservation potential (Noffke and Paterson, 2008). Grotzinger and Knoll (1999) identified three laminae-forming processes: mat growth, sedimentary deposition, and mineral precipitation, among which mineral precipitation played a significant role in the accretion of Precambrian stromatolites. Quantitative data from modern microbialites have shown the additional importance of early lithification to these processes (Dupraz and Visscher, 2005). Early lithification is thought to be driven by a metabolically induced increase of $CaCO_3$ saturation state in modern mats (Aloisi, 2008). This calcifying ability in mats may have well evolved since Neoarchean times (Kazmierczak et al., 2009).

1.3. CALCIFICATION AS AN EXAMPLE OF A UBIQUITOUS MICROBIAL–MINERAL INTERACTION

Calcification refers to the precipitation of common carbonate minerals such as calcite, aragonite, and dolomite on the surface of the microbial mat. Microorganisms promote calcification using ionic proton pumps or secreting specific enzymes as some eukaryotes do. To what extent cyanobacteria influence precipitation has been difficult to determine. During the last 2 decades, numerous works have analyzed and compiled far-reaching observations concerning calcification on mats. It has been normally associated to the photosynthetic uptake of CO_2, followed by a rise in alkalinity (Merz-Preiß and Riding, 1999; Ludwig et al., 2005), but not in all cases (Kremer et al., 2008).

As mentioned earlier, diverse studies have showed the role of cyanobacterial EPS in promoting carbonate nucleation (Aloisi et al., 2006; Dittrich et al., 2003; Dittrich and Sibler, 2006; Benzerara et al., 2006). More recent observations are detailing how EPS influences carbonate nucleation (Kremer et al., 2008; Bissett et al., 2008; Sanchez-Roman et al., 2008; Tourney and Ngwenya, 2009; Altermann et al., 2006; Kazmierczak et al., 2009).

In hypersaline mats, Dupraz et al. (2004) demonstrated that $CaCO_3$ is not associated with cyanobacterial sheaths, but rather with the EPS degradation that raises the alkalinity, promoting a massive precipitation. Calcification occurs naturally when there is a local saturation of calcium ions and carbonate ions, in which $CaCO_3$ supersaturation, pH, temperature, and chemical activity are the determining factors (Arp et al., 1999). Cyanobacteria and other bacteria degrade organic

matter generating microchemical gradients concentrated at the sediment–mineral interface, provoking lithification in decaying mats (Stolz et al., 2001; Andres and Reid, 2006). Kempe and Kazmierczak (1990) suggested a periodic calcification in vivo on superficial cyanobacterial layers by low Mg calcite and an early post-mortem calcification of cyanobacterial aggregates below the mats surface by aragonite. Later, Arp et al. (2003) emphasized the importance of diffusion rates and microgradients for the binding of divalent ions. They proposed that $CaCO_3$ nucleation starts in the EPS matrix with reduced diffusion rates and microgradients, exposing acid groups to which divalent cations can bind; in such a case, eventually EPS may also prevent calcification (Arp et al., 2003).

The many faces of the interaction between microbes and mineral surfaces have been constant and persistent since early evolution, and the sedimentary record reveals this long and continued relationship between carbonates and cyanobacteria (Seong-Joo et al., 2000). Many algae and bacteria species induce the precipitation of minerals as a consequence of their metabolic activities, but how to distinguish between minerals precipitated spontaneously from those biologically induced; do they co-occur in the matrix of microbial mats?

The answer to this question would provide important clues to address biogenicity, a capital issue in astrobiology. Minerals produced biologically may display a distinctive spatial and temporal arrangement along a former organic matrix, around cells, oriented in the extracell matrix or aligned in stratified mats. This three-dimensional (3D)-spatial arrangement could indicate biogenicity. Lowenstam and Weiner (1989) differentiated biologically initiated calcification from biologically controlled ones; however, this distinction fades as geochemical processes take place on the micrometer-scale, for instance, in the matrix of a microbial mat. Within this organic matrix, the interactions of mineral surfaces, organics, and passing ions diffusing over geochemical gradients at the micrometer scale are multiple. Figure 5 shows a selective calcification on the external margins of a laminated mat where carbonate crystals precipitate randomly around filaments.

2. Microbial Mats as a Source of Diverse Biosignatures

Since microbial mats form by the manifold interactions between sediments and microbes, the gamma of microbial biosignatures produced within the framework of a microbial mat is potentially high. A biosignature can be defined as any macroscopic or microscopic physical or chemical evidence of life (fossil or extant) preserved in terrestrial or extraterrestrial rocks. The early fossil record of life reveals well-preserved biosignatures in the form of stromatolites, microfossils, microbial ichnofossils, and chemical fossils such as biomarkers and isotopic biosignatures (Fig. 6). The potential preservation of biosignatures within a microbial mat may include several types of cell remains, sediments, and minerals ranging from several meters to a few micrometers. Since the early 1980s, the search for biosignatures to document the oldest record of life has been conducted through

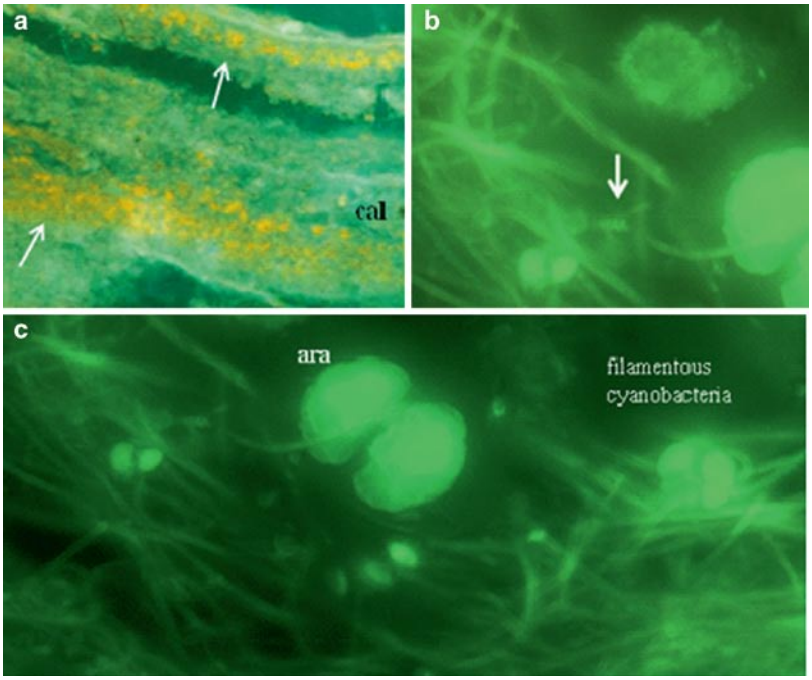

Figure 5. (**a**) Cross section of a microbial mat showing marginal $CaCO_3$ precipitation zones; (**b, c**) randomly precipitated crystals of in situ of calcite (cal) and aragonite (ara); *arrow* in (**b**) points to *Spirulina* sp.

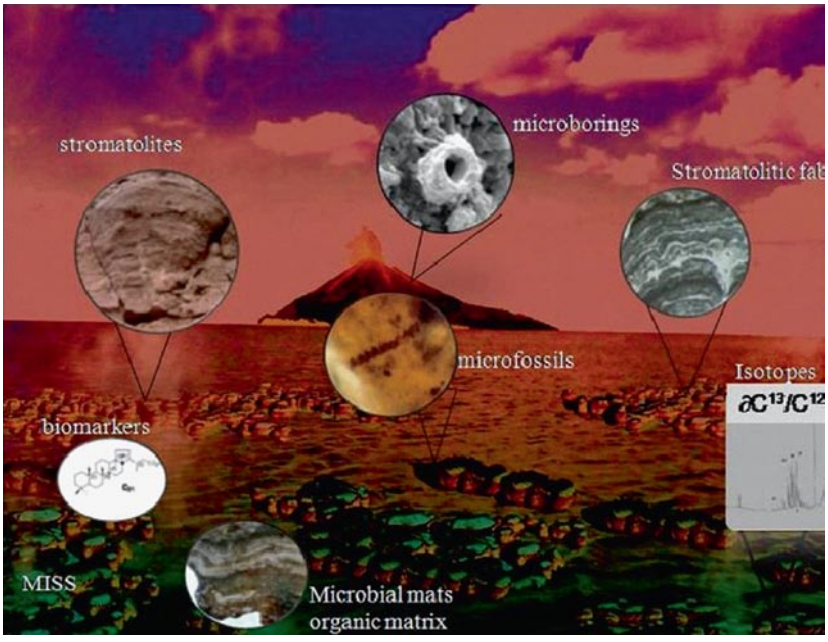

Figure 6. Diversity of potential biosignatures encoded in microbial mats during Precambrian times (Modified from Chacon, 2010).

three main lines of paleontological evidence: stromatolites, microfossils, and stable isotopes (Schidlowski, 1993; Walter, 1994; Cady et al., 2005).

Another excellent window to fossil microbial mats is found as microbially induced sedimentary structures (MISS) preserved in ancient rocks (Noffke et al., 2006). Although morphology is the main criterion for macroscopic biosignatures, a morpho-chemical approach may be required for microscopic biosignatures. An acute awareness in the search of biosignatures is characterizing modern research projects in geosciences, particularly in the emerging field of astrobiology (see for example, Seckbach and Walsh (2009) and references therein).

3. Chemical Biosignatures Embedded in Microbial Mats

Stable isotopes of some elements as C, H, O, or N react at different rates producing an isotopic fractionation that can be used as signatures to trace biochemical or biogeochemical pathways by measuring their relative abundances in living organisms and in rocks. Although the chemical behavior of two isotopes is qualitatively similar, their differences in mass lead to differences in both reaction rate and bond strength, and therefore their physical behavior is quantitatively different: the reaction products will be relatively enriched in the lighter isotope (Libes, 1992). Since the incorporation of carbon compounds into the biosphere is one of the most ancient biogeochemical pathways (Schidlowski, 1993), their isotopic fractionation constitutes a good biosignature in recent and fossil material. One fine example of the utility of isotopes as biosignatures is the study of the compound-specific method known as ^{13}C-phospholipid fatty acid analysis. This method involves the organic extraction of phospholipids using polar solvents in a phosphate buffer followed by a filtration and solid-phase extraction (Zelles, 1997). The isolated fraction of phospholipids is saponified and methylated to fatty-acid methyl esters that can be injected into a gas chromatograph (GC) where compounds are separated and introduced into an isotope ratio mass spectrometer. The resulting pattern of peaks is then compared with standard peaks from a reference library to identify the general class of organisms that took up the ^{13}C-labeled substrate.

The other type of chemical biosignatures known as molecular biomarkers are organic compounds derived from living organisms that retain their species-specific carbon skeleton through geological time (Brocks and Pearson, 2005); this is possible because recent and fossil microbial communities contain a good amount of organic matter (Pratt et al., 1992). Fossil biomarkers are individual organic compounds contained in sedimentary rocks that derive from biological precursors (Ourisson et al., 1982); they can also be extracted within the solvent-soluble (lipid) portion of the derived organic matter from sediments and multiple biological materials. Although the preservation potential of each biomolecule varies with its chemical structure, sedimentary environment, and diagenetic history, a group of taxonomically relevant biomolecules has been isolated, identified, and

characterized (Summons and Walter, 1990; Summons et al., 2008). Biomarkers are indicative of either environmental factors acting on specific organisms or constitutive components that are species-specific (Ourisson et al., 1987). Some of the biochemical information contained in a microbial mat may be unraveled by isolating the soluble organic fraction. This analysis has yielded solid results in terms of establishing minimal ages for clades and phylogenetic evolution (Eigenbrode, 2008), a critical area for the search of biosignatures. It is generally known that biomarkers are species-specific and distributed accordingly (Rohmer et al., 1984). Through the fossil record, geohopanoids are among the most common biomarkers in sediments. Geohopanoids derive from hopanoids, which are the most abundant lipids in the membranes of bacteria, although not all bacteria contain hopanoids (Duvold and Rohmer, 1999). Hopanoids are organic compounds built by four pentacyclic triterpenoids and a cyclopentane E-ring whose name derives from the plant genus *Hopea*, after isolating it as a resin component (http://www.lipidlibrary.co.uk). Summons et al. (1999) reported the presence of 2-MeOH hopanoids (aromatic compounds containing a tetrapyrrole ring with a methyl-group (CH_3) in their C_{28} position) as cyanobacterial biomarkers in sediments as old as 2.7–2.8 Ga, indicating a minimal age for their appearance. Although new findings have demonstrated that such biomarkers can also be derived from anoxygenic phototrophic bacteria (Rashby et al., 2007), cyanobacteria do represent the major source of C-2 methylated hopanoids (Talbot et al., 2008). But again, cyanobacteria emergence continues to be an open issue (Knoll, 2008). Even when isotopes and stromatolites might suggest that cyanobacteria may have evolved by 3.5 Ga, or by the Neoarchean at least (Beukes and Lowe, 1989), molecular data questions this hypothesis as to whether the change toward an oxygenic atmosphere was a global event exclusively triggered by cyanobacteria (Blank, 2004). The geological interpretations of the hematite-rich chert from the 3.46 billion years old rocks favors a well-oxygenated atmosphere by that time (Ohmoto et al., 2004); in any case, the global atmospheric oxygenation was preceded by a well-established photosynthesis (Buick, 2008), and under the assumption that oxygen was exclusively released by cyanobacteria.

On the other hand, some species of cyanobacteria isolated from modern hydrothermal springs present different lipid biomarkers concentrations, suggesting once more an underestimation of the real microbial diversity at the biomarker level too (Jahnke et al., 2004) and confirming previous observations on the microbial biochemical diversity (Ward, 1998; Zundel and Rohmer, 1985; Pearson et al., 2003). Finally, other chemical signatures derived from complex polymeric material such as EPS and DNA derived from any type of microbial mat may yield novel information. The EPS represent the infrastructure on which 3D-community assemblages interact (Decho et al., 2008). On the other hand, metagenomics or the genomics of environmental communities will soon provide valuable insights into the genetics, physiology, and biochemistry of microorganism populations in their natural habitats.

4. Morphological Biosignatures

4.1. MACROSCOPIC

Stromatolites, thrombolites, microbially induced sedimentary structures (MISS), or massive bacterial calcifications are but a few examples of the macroscopic manifestation of mineral–microbial interactions over time. These examples of microbialites put in evidence that the study of interphase interactions among organic matter, crystal shapes, and surface processes will be crucial to develop models of interpretation and recognition of biosediments. It is generally assumed that the morphology of stromatolite reflects both the composition of the mat-building community and local environmental conditions (Walter, 1994) that may have a differential influence on stromatolites macrostructure by their relative contribution of sediments and framework growth in certain hemispheroidal and branching stromatolitic morphologies (see Planavsky and Grey, 2008). Stromatolite morphology reflects the interplay between microbial binding and sediment accretion under a given hydrodynamic environment (Altermann, 2008). Ancient stromatolites are morphologically variable (Grotzinger and Knoll, 1999), which may be attributable to the differences in microbial communities, as is the case with the stromatolites from Hamelin Pool (Papineau et al., 2005). Recent reports by Murphy and Sumner (2006) on the morphology of stromatolites from the Neoarchean Carawine Formation in Western Australia indicate that stromatolite morphology may have been controlled by biological reactions in response to a changing environment. However, in those Proterozoic microbialites from deep subtidal environments, morphology may have been influenced by chemical or biological changes that occurred in relation to the surface oxidation of the oceans (Murphy and Sumner, 2006). Stromatolites occur in diverse environments ranging from recent freshwater settings, shallow subtidal and marginal, marine to hypersaline, and transitional environments as geothermal systems (Cohen and Rosenberg, 1989). Stromatolites may occur as bioherms (discrete subspherical, domed, tabular, or individual beds ranging from a few decimeters to several meters in thickness), or as biostromes (laterally linked tabular or domed stromatolitic beds). Their primary mineralogy is carbonate, although siliceous and evaporitic stromatolites also do occur (Walter, 1976 and references therein). Typical stromatolite macrostructures include conical, domal, pseudocolumnar, branched, and cube-shaped forms, among others (Fig. 7).

It has been generally accepted that environmental factors such as paleo-current direction, energy, or sediment supply play a modeling role in the macrostructure (Eckman et al., 2008), while microstructure is more influenced by biological factors. Stromatolites as a whole represent the historical result of microenvironmental conditions in continuous interaction with the microbial mat community. Fine-scale features such as laminae and texture are fundamental units of stromatolites, and they appear to be biologically determined (Grotzinger and Knoll, 1999). The microstructure includes all microscopic textural characters such as lamination,

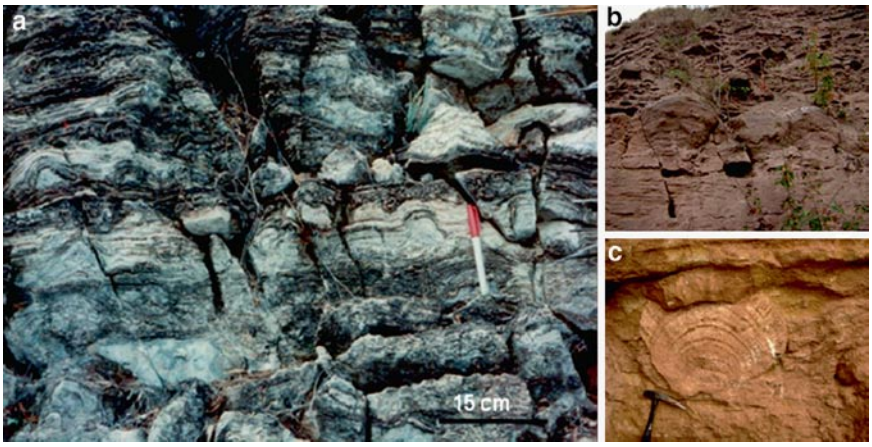

Figure 7. (**a**) Representative Cretaceous stromatolites from the Tarahumara Formation, Sonora, Mexico; (**b, c**) triassic stromatolites first described by Kalkowsky in 1908 from the Heeseberg quarries, Lower Saxony, Germany.

matrix, and sedimentary particles (Bathurst, 1971), among which lamination is the main character to be described. Although not all stromatolites exhibit a well-marked lamination, it is possible to observe a clear alternate lamination in some binding stromatolites. This lamination has been typically attributed to the variations in sediment supply and reflects recurring and periodic changes in sedimentation alternating with episodes of high organic content (Seong-Joo et al., 2000). The dark lamina is rich in organic remains while the light lamina, commonly consisting of precipitated micrite, results from dissolution and precipitation of carbonate grains incorporated into the microbial mat. The contact between each alternating laminae may be sharp, continuous, wavy, or discontinuous. The time required to develop a couple of laminae remains undetermined; millimeter-scale lamination may represent days, months, or years or other periodic cycles (Park, 1976).

The microstructure is a function of the building microbial community and all diagenetic inputs that modify the original texture, including seasonal changes in mat composition and carbonate supersaturation that consequently change the calcification process (Bissett et al., 2008). Lamination is not only an abiogenic feature characteristic of sedimentary rocks, but also a recurrent feature developed in some biological structures. It reflects periodicity (Knoll and Semikhatov, 1998). In stromatolites, lamination may be the result of an accretion process concomitant to crystal nucleation by heterotrophic bacteria (Awramik, 1977; Knoll and Semikhatov, 1998; Reid et al., 2000; Visscher et al., 2000). Figure 8 shows some examples of the Cretaceous stromatolites and their associated chert from the Tarahumara Formation. Thin sections from carbonates and chert show the fine-medium lamination in both textures (Fig. 8d and e). The fossiliferous chert from Huepac has yielded diverse microfossils pertaining to different taxa, of which

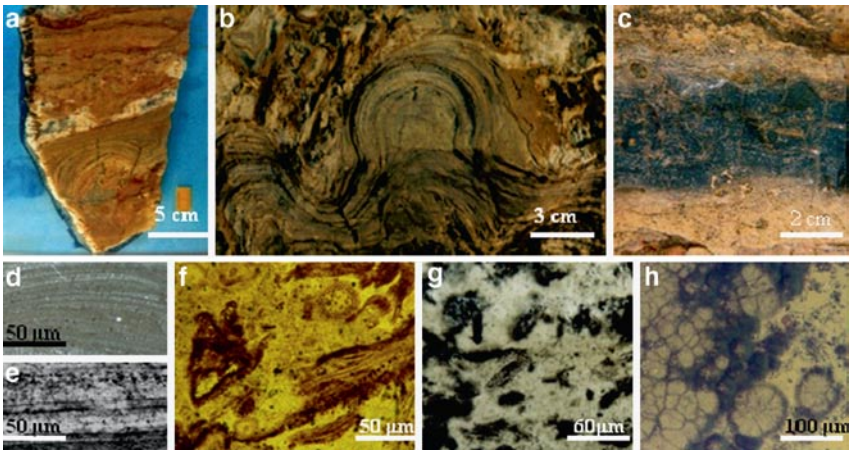

Figure 8. (**a** and **b**) Typical domal stromatolites from Huepac and its associated chert (**c**); (**d** and **e**) carbonate and cherty lamination as seen in petrographic thin sections; (**f**) representative thin section of the Huepac fossiliferous chert; (**g**) partially fragmented and dark-colored pennate diatom frustules; (**h**) chalcedony precipitating in open spaces.

vascular tissue remains predominant (Fig. 8f). Especially conspicuous are worn pennate diatom frustules with dark rims (Fig. 8g). The chert also shows a very constant precipitation of chalcedony (Fig. 8h).

Other types of microbialites in which microbial mats are also involved are thrombolites, which lack lamination and present a characteristic clotted fabric. Thrombolites are commonly found in subtidal areas; frequently, thrombolites reflect an irregular sediment supply and coarse microbial colonization surfaces, where ooids and diatoms are common, as well as other microscale textures as delamination and burrowing.

4.2. MICROSCOPIC BIOSIGNATURES

4.2.1. Microfossils

Microfossils include microscopic cell remains of former microorganisms or parts of organisms such as acritarchs, diatoms, algae, or prokaryotic remains. Microfossils also involve the soft organic remains of a preexisting cell that after diagenesis have been preserved in any type of sediments with enough distinctive features to recognize the general morphology of microbial cells. Such preserved features include shape, size, or patterns of organization of multicellular remains that allow comparison with extant organisms (Knoll and Golubic, 1992). Frequently, the most diagenetically resistant cell material consists of extracellular sheaths and mucilaginous envelopes. Microfossil assemblages may represent

remains of microbial mats where the organic matrix of EPS has minimal chances for preservation, though recent analysis shows that cyanobacterial EPS contains cellulose too (Nobles et al., 2001). Cellulose is a highly structured polymer that provides UV protection, tolerance to desiccation, adhesion to substrates, and gliding motility; theoretically, it should have a relatively good preservation potential as well as other organic fibers secreted by diatoms. Thus, the EPS composition plays a cardinal role during fossilization, providing a mineral matrix to which organic matter can concentrate, adhere, and protect.

Normally, diagenesis tends to homogenize all microbial organic remains during fossilization, adding an extra bias (Allison and Awramik, 1989), and consequently microbial diversity is significantly underestimated. The small size and regular morphology exhibited by other noncyanobacterial prokaryotes limit their identification and interpretation in the fossil record. The taxonomic assignment to a specific morphotype is based on the general morphology and behavior, understanding the orientation and geometrical arrangement preserved in situ for behavior (Lee and Golubic, 1999). Among the microorganisms normally associated with the construction of stromatolites are cyanobacterial morphotypes as *Phormidium*, *Scytonema*, *Oscillatoria*, *Schizothrix*, *Eoentophysalis*, *Glenobotrydon*, *Gunflintia*, *Eomycetopsis*, and *Eosynechococcus*, among others; a complete list is given in Mendelson and Schopf (1992). The cyanobacterial morphotypes may represent remains of microbial mat builders preserved by silicification in stromatolitic cherts dating back from the Precambrian. The most common microfossils found in many fossiliferous cherts from different strata are cell remains found as discrete colonial cells or unicells preserved at different stages of their life cycle (Hofmann, 1976). In the case of certain form-genera, a direct correspondence between the modern species of cyanobacteria and their fossil counterparts or paleospecies has been documented, such as *Polybessurus*, *Eoentophysalis*, and *Archaeoellipsoides*, among many others in excellent detailed works. This literature reporting microfossils description, systematic paleontology, and paleobiological interpretation of a number of fossil assemblages preserved in Proterozoic rocks have provided solid data for the fossil record of cyanobacteria and their associations (Golubic, 1991; Golubic et al., 1995; Hofmann, 1976; Knoll and Golubic, 1979; Schopf, 1983 and references therein; Knoll and Golubic, 1992). The general observations indicate that the fossil record of cyanobacteria is continuous at least from the Neoarchean, and the fact that many Neoarchean and Proterozoic morphotypes share a striking similarity with modern cyanobacterial morphotypes.

Silicification of microfossils is not restricted to Precambrian stromatolites. Figure 9 shows some examples of the microfossils diversity preserved in the Cretaceous chert from Huepac pertaining to different taxonomic groups.

The cretaceous chert from Huepac (Tarahumara Formation), as many other excellent examples throughout the world, contains an outstanding microbial assemblage that can also be considered a *lagerstatten*. The Huepac chert was probably preserved under paleoenvironmental conditions that included a high organic productivity environment where microbial mats developed at the external

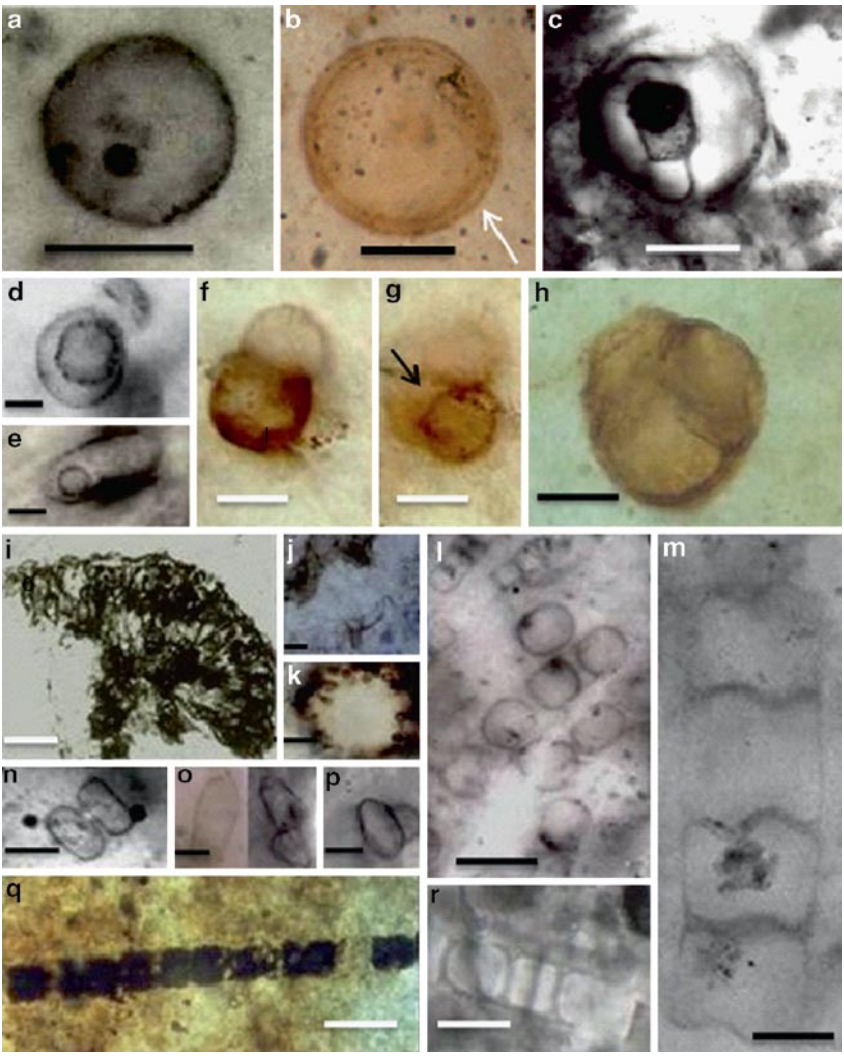

Figure 9. Large acritarchs (**a**–**e** and **o**); diads (**f**–**g**); trispores (**h**); algal remains (**i**); arthropod appendages (**j**); fungal cells within vascular cells (**k**); diatom frustules (**l**, **m**, **r**, and **q**); diasmid remains (**n**); and fungal acritarchs (**p**). Scales = 30 μm in **a**, **b**, **c**, **j**, and **l**; 20 μm in **d**, **e**, **h**, **n**, **o** and **r**; 50 μm in **i** and **q**; and 10 μm in **f**, **k**, **g**, and **m**.

facies of a lake; the continuous carbonate precipitation eventually formed stromatolites, probably under conditions of high volcanic activity. This lake presumably had a high continental input, restricted circulation, and periodic diagenetic events of early silicification (Chacon, 2002). Given the types of diatom morphotypes found in this chert with most of them inhabiting modern lakes nowadays, the

depositional setting could be more related to a lake than to a coastal brackish setting (Chacon et al., 2002). One of the most relevant microfossils findings is the assemblage of diatoms from the genera *Melosira*, *Tabellaria*, and *Fragillaria* preserved *in situ* (Fig. 9l, m and r).

Among the preserved microbiota, the presence of acritarchs is frequent. Acritarchs, defined as "form-taxa," are interpreted here as remnants of whole organisms at one stage of their life cycle that show a high degree of structural integrity. In fact, acritarchs may also be defined as organic-walled microfossils of uncertain taxonomic affinities that consist of a central cavity enclosed by one or more walls and usually regarded as planktonic algae/plant protists (Evitt, 1963; Mendelson and Schopf, 1992). Most of the acritarchs are spheromorphs (lack processes), and include morphs with a single, granular cell wall often with an inclusion (Fig. 9a) or with a double-layered cell wall (arrow in Fig. 9b) and vesicles with a solid, uniform cell wall and dense internal content that are found in groups or solitary (Fig. 9c). The specimen in Fig. 9c shares similarity in morphology, size, and taphonomy with the organically preserved algal microfossils from the late Precambrian Bitter Spring Formation and with some green algae morphotypes reported in silicification experiments (Oehler and Schopf, 1971). Microfossils also include diverse morphologies like the bowl-shaped spore (Fig. 9d), cell within a cell (Fig. 9e), diads or triads with or without envelope (Fig. 9f and g), a well-preserved trispore-like microfossil was found in two different planes (Fig. 9h), showing a thick-layered double wall along divisional planes. This trilete spore with a dense brownish color, suggesting a thick kerogenous cell wall, is comparable with those spores produced by the Lycopodiaceae. Some of these higher taxa acritarchs are represented by a thallus remain, which is solid, thick, and morphologically similar to some representatives of the green algae of modern genera *Chaetomorpha* (Fig. 9i), by arthropod remains preserved as exoskeletal appendages of keratinized segments (Fig. 9j), and by vascular cell tissues bordered by fungal cells (Fig. 9k). A very particular microfossil is a paired organic-walled microfossil (Fig. 9n), whose size and shape resembles the taxonomic descriptions given for modern desmids of the genus *Xantidium* without the lateral spines or other external ornamentation.

Other typical acritarchs in the Huepac chert are represented by the globular vase-shape soft-body microfossil similar to the ventral opening of modern ciliates (Fig. 9o) and by rare morphologies that resemble growing or divisional stages of fungi (Fig. 9p). The occasional and highly localized presence of pyrite crystals replacing filamentous diatom colonies (Fig. 9q) further indicates locally anaerobic conditions derived from bacterial degradative process, since pyrite is rather scarce in continental settings and relatively more common in seawater.

Many of the silicified microfossils exhibit an excellent three-dimensional preservation of their original structures, whereas others show different categories of preservation in the same sample. The finding of several categories of preservation indicates a discontinuous distribution of organic matter. This three-dimensional preservation correlates well with the organic matter content and with fibrous

chalcedony, usually found as radial-fibrous precipitates of varying sizes in the open spaces. The cherty microbiota from Huepac also preserve three-dimensionally filamentous and coccoidal cyanobacterial morphotypes, respectively, that have their precambrian counterparts (Figs. 10 and 11). Some of the most common filamentous populations preserving their original in situ orientation (Fig. 10a and b) have been ascribed to *Eomycetopsis*, which consists of nonseptate uniseriate filamentous sheaths of the type LLP cyanobacteria (Hofmann, 1976). These filaments have also been reported within the cherty microbiota from Bitter Springs and were classified as *Siphonophycus inornatum* (Schopf, 1971); later this morphotype was described from Precambrian sediments of the Sukhaya Tunguska Formation and emended as *Eomycetopsis robusta* (Knoll and Golubic, 1979). *Eomycetopsis* and *Siphonophycus* have been also reported from Neoarchean sediments (Altermann and Schopf, 1995). Less frequently, *Spirulina*-like solitary filaments showing a tight coiling are found by SEM and in petrographic thin section (arrows in Fig. 10c and d).

The tubular empty sheaths shown in Fig. 10b and e are considered as autochthonous building microbiota, and therefore, directly related to the stromatolitic framework. Their constructive role in the stromatolites is suggested by its interwoven orientation, abundance, and geometry in both fossil and recent microbial mats (Knoll et al., 1989). Since they are able to stabilize the trapping and union of small sedimentary particles (Knoll et al., 1991), they are interpreted as microbiota from the supratidal and upper-tidal lagoonal facies. When some of these filaments are individually analyzed, they reveal a tapered filament with a high

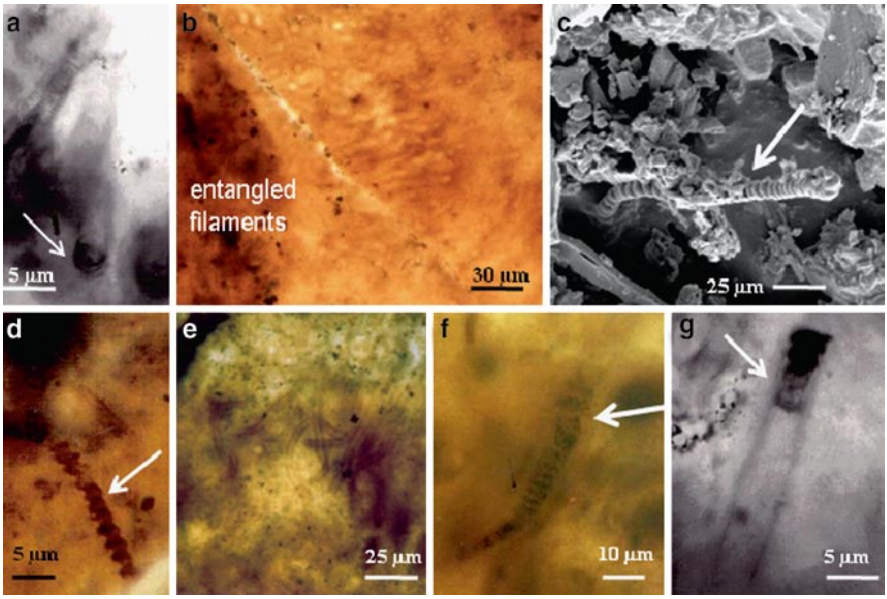

Figure 10. Filamentous microfossils preserved in the Cretaceous stromatolitic chert from Huepac.

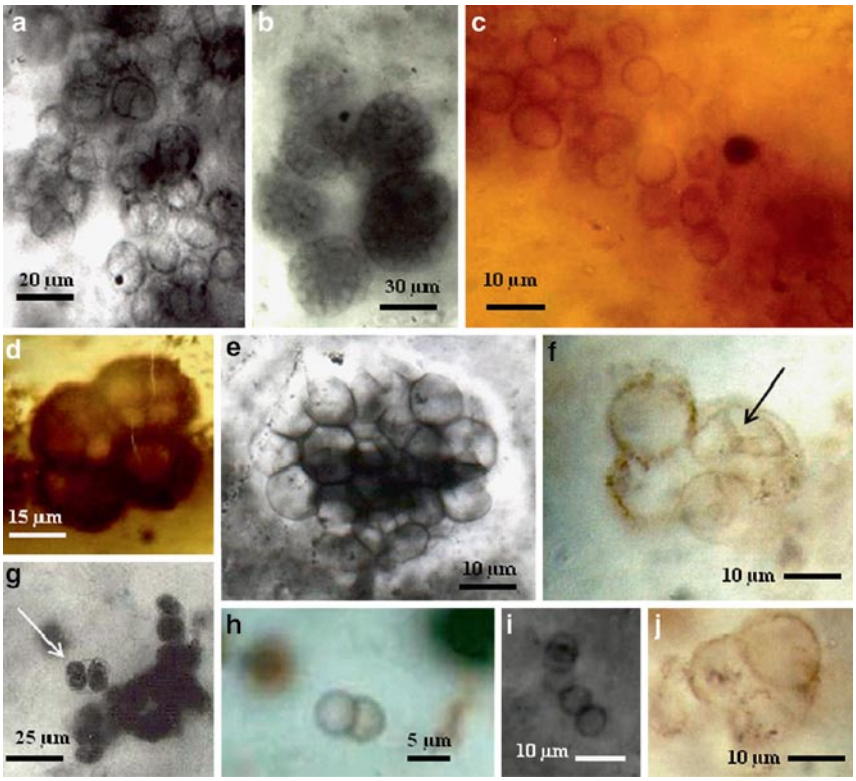

Figure 11. Microfossils preserved in the Huepac chert (Chacon, 2002). (**a**–**c**) Pluricellular aggregates of coccoidal cells in different colonial patterns; (**d**) robust tetrads; (**e**) *Myxococcoides cantabrigensis*; (**f**) hyaline tetrads with a clear division scar (*arrow*); (**g**) colonial *Myxococcoides* where the original common envelope is distinguishable (*arrow*); (**h**) cell bipartition (**i**) filament formed by coccoidal cells; (**j**) hyaline dividing cells.

organic content concentrated in the apical end that closely resembles filaments of modern species of *Calothrix* spp. (Fig. 10f). Some tubular empty filaments concentrate organic matter at the apical ends (arrows in Fig. 10a and g). Coccoidal cyanobacterial remains are also well represented in the Huepac chert, as those pluricellular aggregates showed in Fig. 11.

Multicellular aggregates and other colonial remains resemble the organizational patterns found in the chroococcalean and pleurocapsalean cyanobacteria (Fig. 11a–c, e and g). The thick-walled tetrads illustrated in Fig. 11d share close similarities with some microfossils found in Proterozoic strata as microbenthonic populations of the genera *Sphaerophycus* and *Gloediniopsis* (Schopf, 1968). The most abundant morphotypes are those spheroidal microfossils known as *Myxococcoides*, which are another problematic genus form (Fig. 11c). They exhibit a generalized occurrence through the fossil record, either as solitary cells or as multicellular aggregates.

Its presence is associated with warm carbonate environments of shallow waters and restricted circulation (Allison and Awramik, 1989).

Myxococcoides has been reported from the subtidal facies, along with other vase-shaped microfossils, and is considered as a planktonic allochthonous element of the phytoplankton (Sergeev et al., 1995), whereas those small vesicles also described as *Myxoccocoides* are similar in shape and size to some genus form identified as *Myxococcoides chlorelloidea* and to *Myxococcoides* sp. found in fine-grained carbonate assemblages from the Backlundtoppen Formation and reported as allochthonous material (Knoll et al., 1989). The colony identified as *Myxococcoides cantabrigensis* (Fig. 11e) is identical to the one found in the Duck-Creek Dolomite (Strother et al., 1983). Proterozoic microfossils belonging to these genera are often placed in *incertae sedis* and considered either cyanobacteria or green or red algae (Green et al., 1989).

The differential pigmentation or degradational pattern is essential in the evaluation of biosignatures (Hofmann, 2004). The preserved microbial remains correspond to soft and robust cell remains. Through the fossil record, it is possible to find this quality of preservation, especially in high-productivity environments with reducing conditions and restricted circulation (Knoll, 1985). The microfossils of Huepac represent diverse taxa, including fossilized fruits, roots, and seeds from *Haloragacea* (Hernández-Castillo and Cevallos, 1999); the organic matter content and the high preservation of plant remains indicate a shallow basin proximal to the coastal line, where organisms from different habits intermix prior to early diagenetic process inducing fossilization. The preservation of microfossils in chert is not only fortuitous but also inhomogeneous. It has been suggested that aqueous fluids that promote homogeneous or heterogeneous nucleation may occur in hydrothermal systems and stratified lake basins (Konhauser et al., 2003).

At the microscopic level, microbial cell walls provide multiple sites for the binding of cations exposing their negative charges. Some of the early processes of fossilization may include the collapse of cell organelles and shrinkage by water loss, mainly associated with an early fossilization (Knoll and Golubic, 1979). A change in the electric potential of surrounding membranes promotes mineral replacement by cations (Gilbert et al., 2005); other preservation mechanisms involve adhesion mechanisms of cell-surface hydrophobicity during a selective biofilm formation (López-Cortes, 1999), since cyanobacteria surfaces are hydrophobic (Bartley et al., 2000). Postmortem fossilization of microorganisms favors the formation of fine-grained mineral matrices, exposing more reactive sites for mineral nucleation once the microorganism starts to decay (Leo and Barghoorn, 1976). If silica emplacement occurs rapidly enough during early diagenesis, even the finest anatomical details can be beautifully retained (Knoll, 1985).

4.2.2. Microbial Ichnofossils

Minerals produced by prokaryotic cells are quite common in microbial communities. In contrast with eukaryotes, prokaryotic cells do gain energy when they

mineralize (Gilbert et al., 2005). According to their relation with the substrate, cyanobacteria and other microorganisms may be classified in euendoliths when they actively penetrate rocks, cryptoendoliths when they dwell in rocks crevices and pores without dissolution of the substrate, and in chasmoendoliths if they invade preexisting holes and cracks (Golubic et al., 1981). Certainly, mineralogical precipitates, bores, and holes left by endolithic microorganisms may be regarded as traces of their boring activity, and therefore can be considered as microbial ichnofossils. The oldest fossil record of endolithic cyanobacterial dates back to 2,000 Ma sediments, where preserved *Eohyella campbellii* has been found within the Hebrei Formation from China (Zhang and Golubic, 1987). Other non-cyanobacterial microfossils have recently been identified in the Archean pillow lavas from the 3.35 Ga Euro Basal of the Pilbarra Craton in Western Australia (Banerjee et al., 2007) and from Barberton, South Africa, by Furnes et al. (2007). These biosignatures are tubular structures of titanite quite similar to modern microbial ichnofossils found in modern basalts, ophiolites, and greenstone belts (Perry et al., 2007). The identification of microbial endoliths emphasizes the tolerance and persistence of microbial life to colonize rocks; this ability may have been fundamental during the early evolution of life. A recent review suggests that simple endolithic microbial ecosystems may be associated to ancient hydrothermal systems (Walker and Pace, 2007). Endoliths are ubiquitous, resistant, ancient, and their traces may be characterized relatively easily; particularly the sedimentary traces produced by some species of endolithic cyanobacteria hold a great potential as biosignatures (McLoughlin et al., 2007). Typical endoliths "fingerprints" are left in the sediment as micritic envelopes in several types of substrates, including lithified fossil microbial mats. These micro-ichnofossils can be identified in petrographic thin sections too. Micrite envelopes are associated with micrite-filling of borings and it has been recognized as a diagenetically primary process (Perry and Macdonald, 2002). Micritic constructive envelopes are also associated with biofilms composed of cyanobacterial mucilage, bacteria, and diatoms. They represent a biological-mediated precipitation of micrite and its associated trapping of carbonate mud and fine-grained sediments (Margolis and Rex, 1971). The calcification of filaments around the external grain-surfaces has been also reported in fossil sediments (Kobluk and Risk, 1977) showing characteristic rims in ooids in microbial mats (Chafetz and Buczynski, 1992) and in other karstic systems (Jones and Kahle, 1995) during the micritization of carbonate grains. The more familiar microbial fingerprints of euendolithic cyanobacteria are the bores and holes in the three-dimensional infrastructure of some carbonates, as illustrated in Fig. 12e and f. Microbial ichnofossils may be found as rims or micritic envelopes, as single crystals reprecipitated simultaneously with their boring activity (Fig. 12a and c). They can also be identified as white coverings around boreholes (Fig. 12b), as a bored surface showing a regular pattern of round traces (Fig. 12e), or as collapsed remains of the apical cell (Fig. 12f). Other early or contemporaneous signatures commonly found in bored substrates are loose packages

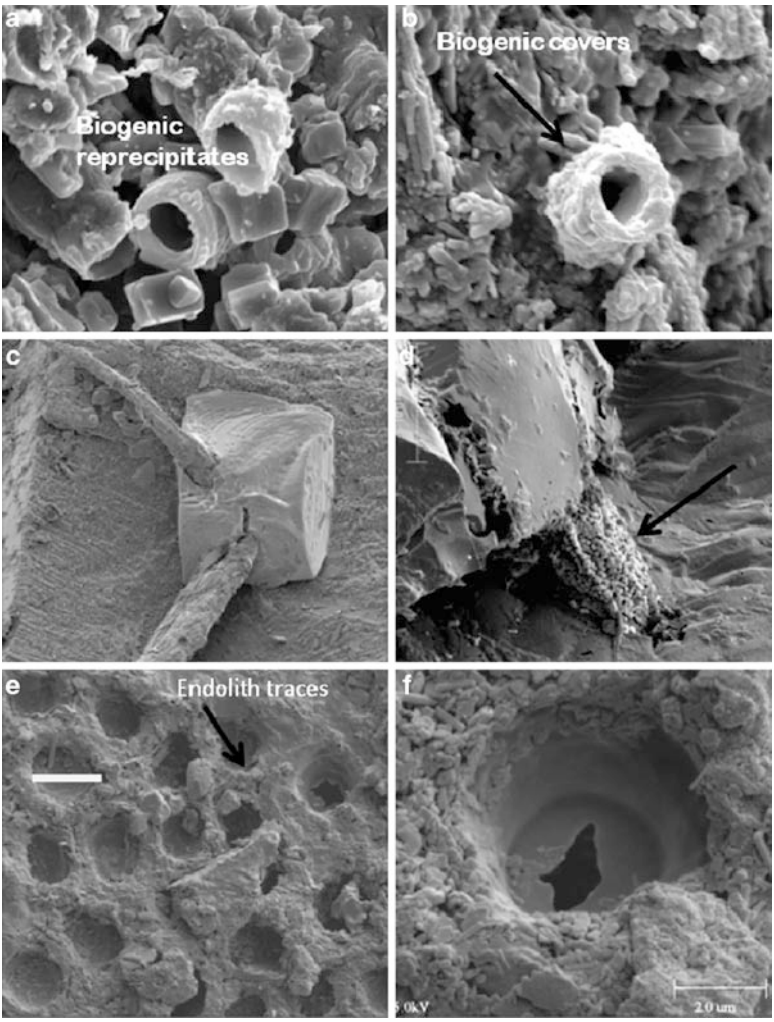

Figure 12. Microbial ichnofossils from recent endolithic cyanobacteria. (**a**) Bioprecipitation of eudral carbonates crystals in situ on boring cyanobacteria; (**b**) typical white coverings in recent endolithic cyanobacteria; *arrow* points to precipitated small granules; (**c**) in situ reprecipitation on a filamentous boring cyanobacteria; (**d**) boring filaments between two bored surfaces (*arrows* point to regular round precipitates along the filament); (**e**) surface pattern on a bored clam shell by endolithic cyanobacteria; (**f**) colapse scar of the apical cell of boring cyanobacteria ((**b**) and (**f**) were modified from Chacon et al., 2006). Scale bar in (**a**, **b**, and **e**) is 10 μm; in (**c** and **d**) is 5 μm, and in (**f**) is 2 μm.

of aragonite needles and spots of micritization zones (Chacon et al., 2006) and reprecipitates of microborings concurrent with endolithic activity, although their distinction is not straightforward and recrystallization has been reported (Reid and Macintyre, 2000).

5. Concluding Remarks

In the early 1970s, Jacques Monod, a scientist famous for his outstanding contribution to the operon model in prokaryotes, speculated on the distinction between the "natural" and the "artificial," detailing all the implicit difficulties encountered solely in drawing this difference (Monod 1970). In spite of the great scientific and technological advances of the last 3 decades, his reflections are still valid and they are even more relevant in astrobiology where biogenicity criteria are under continuous evaluation. Never before have the search for biosignatures been as intense and interdisciplinary, but also as unifying as today. We keep facing confusions to establish reliable standards onto which biosignatures should be categorized and classified: morphology, preserved 3D-shape, complexity, performance, microscopic irregularities in stromatolitic lamination, and so on. And certainly, at every scale of analysis, interpretation may be challenging. Among the well-established biosignatures are microfossils, chemical and molecular biomarkers, stromatolites and microfabrics, and microbial ichnofossils that certainly may deserve a more detailed testing and null-hypothesis formulation than other biosignatures. Probably living or lithified microbial mats still preserve other informative encoded elements to extract. Through much of the history of the Earth, microbial mats have prevailed as vital contributors to the evolution of our biosphere. They represent some of the earliest ecosystems that thrived in a number of environmental settings. As synergic biosystems where "the whole is greater than the sum of its parts," microbial mats represent communities with a higher complexity than previously estimated. The information contained within microbial mats is not only structural but also genetic and environmental. In addition to the complex biogeochemistry going on within the microbial mat matrix, there is a wide range of physical, chemical, and biological elements that if preserved, could render elemental biosignatures. Biogenic minerals in mats may represent, for example, a specific biological response to microgradients, to the genetic information from environmental DNA, to the compositional diversity of EPS, or to the extracellular matrix according to their relative position within the mat. Macroscopically, microbial mats also hold good potential for lithification and preservation in the sedimentary record (Schieber et al., 2007). The study of interface interactions among organic matter, crystal shapes, and surface processes is crucial to develop models of interpretation and recognition of biosediments, along with the recognition of genetic–environmental regulation of microbial communities.

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7. References

Abed, R.M.M., Kohls, K., Schoon, R., Scherf, A.K., Schacht, M., Palinska, K.A., Al-Hassani, H., Hamza, W., Rullkötter, J. and Golubic, S. (2008) Lipid biomarkers, pigments and cyanobacterial diversity of microbial mats across intertidal flats of the arid coast of the Arabian Gulf (Abu Dhabi, UAE). FEMS Microbiol. Ecol. **65**: 449–462.

Allison, W.C. and Awramik, S.M. (1989) Organic-walled microfossils from earliest Cambrian or latest Proterozoic Tindir Group rocks, Northwest Canada. Precambrian. Res. **43**: 253–294.

Allwood, A.C., Walter, M.R., Kamber, B.S., Marshall, C.P. and Burch, I.W. (2006) Stromatolite reef from the Early Archean era of Australia. Nature **441**: 714–717.

Aloisi, G. (2008) The calcium carbonate saturation state in cyanobacterial mats throughout Earth's history, Geochim. Cosmochim. Acta **72**: 6037–6060.

Aloisi, G., Gloter, A., Krüger, M., Wallmann, K., Guyot, F. and Zuddas, P. (2006) Nucleation of calcium carbonate on bacterial nanoglobules. Geology **34**: 1017–1020.

Altermann, W. and Schopf, J.W. (1995) Microfossils from the Neoarchean Campbell Group, Griqualand West Sequence of the Transvaal Supergroup, and their paleoenvironmental and evolutionary implications. Precambrian Res. **75**: 65–90.

Altermann, W. and Kazmierczak, J. (2003) Archean microfossils: a reappraisal of early life on Earth. Res. Microbiol. **154**: 611–617.

Altermann, W., Kazmierczak, J., Oren, A. and Wright, D.T. (2006) Cyanobacterial calcification and its rock-building potential during 3.5 billion years of earth history. Geobiology **4:** 147–166.

Altermann, W. (2008) Accretion, trapping and binding of sediment in Archean stromatolites – morphological expression of the antiquity of life. Space Sci. Rev. **135**: 55–79.

Andres, M.S. and Reid, P.R. (2006) Growth morphologies of modern marine stromatolites: a case study from Highborne Cay, Bahamas. Sed. Geol. **185**: 319–328.

Arp, G., Reimer, A. and Reitner, J. (1999) Calcification in cyanobacterial biofilms of alkaline salt lakes. Eur. J. Phycol. **34**: 393–403.

Arp, G., Reimer, A. and Reitner, J. (2003) Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. J. Sed. Res. **73**: 105–127.

Awramik, S.M. (1977) Paleobiology of stromatolites, In: C. Ponnamperuma (ed.) *Chemical Evolution of the Early Precambrian*. Academic, New York, pp. 111–131.

Banerjee, N.R., Simonetti, A., Furnes, H., Muehlenbachs, K., Staudigel, H., Heaman, L. and van Kranendonk, M.J. (2007) Direct dating of Archean microbial ichnofossils. Geology **35**: 487–490.

Bartley, J.K., Knoll, A.H., Grotzinger, J.P. and Sergeev, V.N. (2000) Lithification and fabric genesis in precipitated stromatolites and associated peritidal carbonates, Mesoproterozoic Billyakh Group, Siberia, In: J.P. Grotzinger and N.P. James (eds.) *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. SEPM Sp. Publication **67**, Tulsa, pp. 59–73.

Bathurst, R.G.C. (1971) *Carbonate Sediments and Their Diagenesis*, *Developments in Sedimentology 12*. Elsevier, Amsterdam.

Benzerara, K., Menguy, N., López-García, P., Yoon, T.H., Kazmierczak, J., Tyliszczak, T., Guyot, F. and Brown, G.E. (2006) Nanoscale detection of organic signatures in carbonate microbialites. Proc. Natl. Acad. Sci. USA **103**: 9440–9445.

Bertrand-Sarfati, J. and Moussine-Pouchkine, A. (1985) Evolution and environmental conditions of the Conophyton associations in the Atar Dolomite (Upper Proterozoic, Mauritania). Precambrian Res. **29**: 207–234.

Beukes, N.J. and Lowe, D.R. (1989) Environmental control on diverse stromatolite morphologies in the 3000 Myr Pongola Supergroup, South Africa. Sedimentology **36**: 383–397.

Bissett, A., Reimer A., de Beer, D., Shiraishi, F. and Arp, G. (2008) Metabolic microenvironmental control by photosynthetic biofilms under changing macroenvironmental temperature and pH conditions. Appl. Environ. Microbiol. **74**: 6306–6312.

Black, M. (1933) The algal sediments of Andros Island, Bahamas. R. Soc. Lond. Philos. Trans. Ser. B **122**: 165–192.

Blank, C.E. (2004) Evolutionary timing of the origin of mesophilic sulfate reduction and oxygenic photosynthesis, a phylogenetic approach. Geobiology **2**: 1–20.

Bontognali, T.R.R., Vasconcelos, C., Warthmann, R J., Dupraz, C., Bernasconi, S.M. and McKenzie, J.A. (2008) Microbes produce nanobacteria-like structures avoiding cell entombment. Geology **36**: 663–666.

Breitbart, M., Hoare, A., Nitti, A., Siefert, J., Haynes, M., Dinsdale, E., Edwards, R., Souza, V., Rohwer, F. and Hollander, D. (2008) Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Cienegas, Mexico. Environ. Microbiol. **11**: 16–34.

Brocks, J.J. and Pearson, A. (2005) Building the biomarker tree of life. Rev. Min. Geochem. **59**: 233–258.

Buick, R. (1992) The antiquity of oxygenic photosynthesis, evidence from stromatolites in sulfate-deficient archaean lakes. Science **255**: 74–77.

Buick, R. (2008) When did oxygenic photosynthesis evolve? Philos. Trans. R. Soc. B **363**: 2731–2743.

Cady, S.L., Farmer, J.D., Grotzinger, J.P., Schopf, W. and Steele, A. (2005) Morphological biosignatures and the search for life on Mars. Astrobiology **3**: 351–368.

Chacon, B.E. (2002) *Estudio Biogeoquimico de los Estromatolitos de la Formación Tarahumara en Sonora, Mexico*. PhD Thesis, Universidad Nacional Autonoma de Mexico, Mexico D.F., 130 pp.

Chacon, B.E. (2010) Microbial signatures from the Precambrian Files, In: V. Basiuk (ed.) *Astrobiology, Emergence, Search and Detection of Life*. American Scientific Publishers, Valencia, CA, pp. 315–340.

Chacon, B.E., Beraldi, H.C., Cevallos, F.S.R.S., Knoll, A.H. and Golubic, S. (2002) 70 Ma nonmarine diatoms from northern Mexico. Geology **30**: 279–281.

Chacon, B.E., Barrendero, E. and Garcia-Pichel, F. (2006) Biogeological signatures of microboring cyanobacterial communities in marine carbonates from Cabo Rojo, Puerto Rico. J. Sed. Geol. **185**: 215–228.

Chafetz, H.S. and Buczynski, C. (1992) Bacterially induced lithifications of microbial mats. Palaios **7**: 277–293.

Cohen, Y. and Rosenberg, E. (eds.) (1989) *Microbial Mats Physiological Ecology of Benthic Microbial Communities*. American Society of Microbiology, Washington, DC.

Costerton, J.W., Lewandowski, Z., Caldwell D.E., Korber, D.R. and Lappin-Scott, H.M. (1995) Microbial biofilms. Ann. Rev. Microbiol. **49**: 711–745.

Decho, A., Visscher, P.T., Braissant, O., Dupraz, C., Norman, R.S., Reid, P.R. and Stolz, J.F. (2008) An emerging framework for understanding marine stromatolites formation, In: J. Reitner, N.V. Queric and M. Reich (eds.) *Geobiology of Stromatolites*. International Kalkowsky-Symposium, Göttingen Abstract Volume. Universitätsdrucke Göttingen, Göttingen, pp. 21–22.

Dittrich, M., Müller, B., Mavrocordatos, D. and Wehrli, B. (2003) Induced calcite precipitation by cyanobacterium *Synechococcus*. Acta Hydrochim. Hydrobiol. **31**: 162–169.

Dittrich, M. and Sibler, S. (2005) Cell surface groups of two picocyanobacteria strains studied by zeta potential investigations, potentiometric titration and infrared spectroscopy. J. Colloid Interface Sci. **286**: 487–495.

Dittrich, M. and Sibler, S. (2006) Influence of H^+ and calcium ions on surface functional groups of *Synechococcus* PCC 7942 cells. Langmuir **22**: 5435–5442.

Douglas, A. E. and Raven, J.A. (2003) Genomes at the interface between bacteria and organelles. Philos. Trans. R Soc. Lond. B Biol. Sci. **358**: 5–18.

Dupraz, C., Visscher, P.T., Baumgartner, L.K. and Reid, R.P. (2004) Microbe–mineral interactions: early $CaCO_3$ precipitation in a recent hypersaline lake (Eleuthera Islands, Bahamas). Sedimentology **51**: 745–765.

Dupraz, C. and Visscher, P.T. (2005) Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol. **13**: 429–438.

Duvold, T. and Rohmer, M. (1999) Synthesis of ribosylhopane, the putative biosynthetic precursor of bacterial triterpenoids of the hopane series. Tetrahedron **55**: 9847–9858.

Eckman, J.E., Andres, M.S., Marinelli, R.L., Bowlin, F., Reid, R.P., Aspden, R.J. and Paterson, D.M. (2008) Wave and sediment dynamics along a shallow subtidal sandy beach inhabited by modern stromatolites. Geobiology **6:** 21–32.

Eigenbrode, J.L. (2008) Fossil lipids for life-detection: a case study from the early earth record. Space Sci. Rev. **135**: 161–185.

Evitt, W.R. (1963) A discussion and proposals concerning fossil dinoflagellates, hystrichospheres, and acritarchs. Proc. Nat. Acad. Sci. USA **49**: 158–164.

Falkowski, P.G. (2006) Tracing oxygen´s imprint on earth´s metabolic evolution. Science **311**: 1724–1725.

Fenchel, T., King, G.M. and Blackburn, T.H. (1998) *Bacterial Biogeochemistry.* Academic Press, San Diego, CA.

Furnes, H., Banerjee, N. R., Staudigel, H., Muehlenbachs, K., McLoughlin, N., de Wit, M. and van Kranendonk, M. (2007) Comparing petrographic signatures of bioalteration in recent to Mesoarchean pillow lavas: tracing subsurface life in oceanic igneous rocks. Prec. Res. **158**: 156–176.

Gerdes, G. (2008) Structures left by modern microbial mats in their hosts sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, O. Catuneanu and W. Altermann (eds.) *An Atlas of Microbial Mats Features Preserved Within the Siliciclastic Rock Record.* Elsevier, Amsterdam, pp. 1–56.

Gilbert, P.U.P.A., Abrecht, M. and Frazer, B.H. (2005) The organic mineral interface in biominerals. Rev. Mineral. Geochem. **59**: 157–185.

Golubic, S. (1991) Modern stromatolites – a review, In: R. Riding (ed.) *Calcareous Algae and Stromatolites*. Springer, New York, pp. 541–561.

Golubic, S., Friedmann, I.E. and Schneider, J. (1981) The lithobiontic ecological niche, with special reference to microorganisms. J. Sed. Petrol. **51**: 475–478.

Golubic, S., Sergeev, V.N. and Knoll, A.H. (1995) Mesoproterozoic akinetes (Archaeoellipsoides), fossil evidence of heterocystous cyanobacteria. Lethaia **28**: 285–298.

Green, J.W., Knoll, A.H. and Swett, K. (1989) Microfossils from silicified stromatolitic carbonates of the Upper Proterozoic Limestone-dolomite series, central East Greenland. Am. J. Bot. **74**: 928–940.

Grotzinger J.P. and Knoll A.H. (1999) Precambrian stromatolites: evolutionary milestones or environmental dipsticks? Ann. Rev. Earth Planet. Sci. **27**: 313–339.

Hernández-Castillo, G. and Cevallos, F.S.R. (1999) Reproductive and vegetative organs with affinities to *Haloragaceae* from the Upper Cretaceous Huepac Chert locality of Sonora, México. Am. J. Bot. **86**: 1717–1734.

Hoffman, P.F. (1974) Shallow and deep-water stromatolites in lower Proterozoic platform-tobasin facies change, Great Slave Lake, Canada. AAPG Bull. **58:** 856–867.

Hofman, N.B. (1826) Om confervernes nytte i naturens husholdning. Kgl Danske Vidensk. Selsk. **4**: 207–220.

Hofmann, H. (1976) Precambrian microflora, Belcher Islands, Canada: significance and systematics. J. Paleontol. **50**: 1040–1073.

Hofmann, H. (2000) Archean stromatolites as microbial archives, In: R. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Berlin, pp. 315–327.

Hofmann, H. (2004) Archean microfossils and abiomorphs. Astrobiology **4**: 135–136.

Jahnke, L.L., Tsegereda E., Hope, J., Turk, K.A., van Zuilen, M., Des Marais, D., Farmer, J.D. and Summons, R.E. (2004) Lipid biomarker and carbon isotopic signatures for stromatolite-forming, microbial mat communities and *Phormidium*. Geobiology **2**: 31–47.

Jones, B. and Kahle, C.F. (1995) Origin of endogenetic micrite in karst terrains – a case-study from the Cayman Islands. J. Sed. Res. **65**: 283–293.

Jones, B. and Renaut, R. (2006) siliceous spicules in acidic hot springs, Waiotapu Geothermal Area, North Island, New Zealand. Palaios **21**: 406–423**.**

Kah, L.C., Bartley, J.K. and Stagner, A.F. (2009) Reinterpreting a Proterozoic Enigma, *Conophyton-Jacutophyton* stromatolites of the Mesoproterozoic Atar Group, Mauritania, In: P.K. Swart, G.P. Eberli and J.A. McKenzie (eds.) *Perspectives in Carbonate Geology, A Tribute to the Career of Robert Nathan Ginsburg.* IAS Special Publication 41. Wiley-Blackwell, Chichester.

Kasting, J.F. and Siefert, J. (2002) Life and the evolution of Earth´s atmosphere. Science **296**: 1066–1068.

Kempe, S. and Kazmierczak, J. (1990) Calcium carbonate supersaturation and the formation of in situ calcified stromatolites, In: V. Ittekkot, S. Kempe, W. Michaelis and A. Spitzy (eds.) *Facets of Modern Biogeochemistry.* Springer, Berlin, pp. 255–278.

Kazmierczak, J. and Altermann, W. (2002) Neoarchean biomineralization by benthic cyanobacteria. Science **298**: 2351–2351.

Kazmierczak, J., Altermann, W., Kremer, B., Kempe, S. and Eriksson, P.G. (2009) Mass occurrence of benthic coccoid cyanobacteria and their role in the production of Neoarchean carbonates of South Africa, Prec. Res. (PRECAM30651–14), doi:10.1016/j.precamres.2009.02.002.

Knoll, A.H. (1985) Exceptional preservation of photosynthetic organisms in silicified carbonates and silicified peats. Philos. Trans. R. Soc. Lond. B **311**: 111–122.

Knoll, A.H. (2008) Cyanobacteria and earth history, In: A. Herrero and E. Flores (eds.) *The Cyanobacteria, Molecular Biology, Genetics and Evolution*. Caister, Academic, Norfolk, UK, pp. 1–20.

Knoll, A.H. and Golubic, S. (1979) Anatomy and taphonomy of a Precambrian algal stromatolite. Precambrian Res. **10**: 115–151.

Knoll, A.H. and Golubic, S. (1992) Proterozoic and living stromatolites, In: M. Schidlowski (ed.) *Early Organic Evolution, Implication for Mineral and Energy Sources.* Springer, New York, pp. 450–462.

Knoll, A.H., Swett, K. and Burkhardt, E. (1989) Paleoenvironmental distribution of microfossils and stromatolites in the Upper Proterozoic Backlundtoppen Formation, Spitsbergen. J. Paleontol. **63**: 129–145.

Knoll, A.H., Swett, K. and Mark, J. (1991) Paleobiology of a neoproterozoic tidal flat/lagoonal complex, the Draken Conglomerate Formation, Spitsbergen. J. Paleontol. **64**: 531–570.

Knoll, A.H. and Semikhatov, M.A. (1998) The genesis and time distribution of two distinctive Proterozoic stromatolites microstructures. Palaios **13**: 408–422.

Kobluk, D.R. and Risk, M.J. (1977) Rate and nature of infestation of carbonate substratum by a boring algae. J. Exp. Mar. Biol. Ecol. **27**: 107–115.

Konhauser, K.O., Jones, B., Reysenbach, A.L. and Renaut, R.W. (2003) Hot spring sinters, keys to understanding Earths earliest life forms. Can. J. Earth Sci. **40**: 1713–1724.

Kremer, B., Kazmierczak, J. and Stal, J. (2008) Calcium carbonate precipitation in cyanobacterial mats from sandy tidal flats of the North Sea. Geobiology **6**: 46–56.

Krumbein, W.E. (1983) Stromatolites: the challenge of a term in space and time. Precambrian Res. **20**: 493–531.

Krumbein, W.E. (2008) Biogenerated rock structures. Space Sci. Rev **135**: 81–94.

Krumbein, W.E., Paterson, D.M. and Zavarzin, G.A. (2003) *Fossil and Recent Biofilms – A Natural History of Life on Earth.* Kluwer, Dordrecht, The Netherlands.

Lee, S.J. and Golubic, S. (1999) Microfossils populations in the context of synsedimentary micrite deposition and acicular carbonate precipitation, Mesoproterozoic Gaoyuzhuang Formation, China. Precambrian Res. **96**: 183–208.

Leo, R.F. and Barghoorn, E.S. (1976) Silicification of wood. Bot. Mus. Leaflts Harvard Univ. **25**: 1–46.

Levit, W.E. (2008) Is there an adequate terminology of biofilms and microbial mats, In: W.E. Kumbrein, D.M. Paterson, D.M. and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms – A Natural History of Life on Earth*. Kluwer, Dordrecht, The Netherlands, pp. 333–341.

Liang, M.C., Hartman, H., Kopp, R.E. and Kirschvink, J.N. (2006) Production of hydrogen peroxide in the atmosphere of a Snowball Earth and the origin of oxygenic photosynthesis. Proc. Natl. Acad. Sci. USA **103**: 18896–18899.

Libes, S.M. (1992) *Marine Biogeochemistry*. Wiley, New York.

López-Cortes, A. (1999) Paleobiological significance of hydrophobicity and adhesion of phototrophic bacteria from microbial mats. Precambrian Res. **96**: 25–39.

Lowenstam, H.A. and Weiner, S. (1989) *On Biomineralization*. Oxford University Press, Oxford.

Ludwig, R., Al-horani, F.A., De Beer, D. and Jonkers, H.M. (2005) Photosynthesis-controlled calcification in a hypersaline microbial mat. Limnol. Oceanogr. **50**: 1836–1843.

Margolis, S. and Rex, R.W. (1971) Endolithic algae and micrite envelope formation in Bahamian oolites as revealed by scanning electron microscopy. Geol. Soc. Am. Bull. **82**: 843–852.

McLoughlin, N., Brasier, M.D., Wacey, D., Green, O.R. and Perry, R.S. (2007) On biogenicity criteria for endolithic microborings on early Earth and beyond. Astrobiology **7**: 10–26.

Mendelson, C.V. and Schopf, J.W. (1992) Proterozoic and selected early Cambrian microfossils and micorfossils-like objects, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge University Press, Cambridge, pp. 865–951.

Merz-Preiß, M. and Riding, R. (1999) Cyanobacterial tufa in fresh water streams, ambient environment, chemical threshold and biological properties. Sed. Geol. **126**: 103–124.

Monod, J. (1970) *Le Hasard et la Nécessité,* Edtions du Seuil, Paris.

Murphy, M. and Sumner, D. (2006) Variations in Neoarchean microbialite morphologies, clues to controls on microbialite morphologies through time. Sedimentology **53**: 465–480.

Narbonne, G.M. and James, N.P. (1996) Mesoproterozoic deep-water reefs from Borden Peninsula, Arctic Canada. Sedimentology **43**: 827–848.

Nobles, D.R., Romanovicz, D.K. and Brown, R.M. (2001) Cellulose in cyanobacteria. Origins of vascular plant cell synthase. Plant Physiol. **127**: 529–542.

Noffke, N. and Paterson, D. (2008) Microbial interactions with physical sediment dynamics, and their significance for the interpretation of Earth's biological history. Geobiology **6**: 1–4.

Noffke, N., Beukes, N. and Hazen, R. (2006) Microbially induced sedimentary structures in the 2.9 Ga old Brixton Formation, Witwatersrand Supergroup, South Africa. Precambrian Res. **146**: 35–44.

Obst, M., Gasser, P., Mavrocordatos, D. and Dittrich, M. (2005) TEM-specimen preparation of cell/mineral interfaces by Focused Ion Beam milling. Am. Mineral. **90**: 1270–1277.

Oehler, J.H. and Schopf, J.W. (1971) Artificial microfossils: experimental studies of permineralization of blue-green algae in silica. Science **174**: 1229–1231.

Ohmoto, H. Watanabe, Y. and Kumazawa, K. (2004) Evidence from massive siderite beds for a CO_2-rich atmosphere before ~ 1.8 billion years ago. Nature **429**: 395–399.

Ørsted, A.S. (1842) Beretning om en excursion til Trindelen, Naturhist. Tidsskrift **3:** 552–569.

Ourisson, G., Albrecht, P. and Rohmer, M. (1982) Predictive microbial biochemistry: from molecular fossils to procaryotic membranes. Trends Biochem. Sci. **148**: 77–79.

Ourisson, G., Rohmer, M. and Poralla, K. (1987) Prokaryotic hopanoids and other polyterpenoid sterol surrogates. Ann. Rev. Microbiol. **41**: 301–333.

Papineau, D., Walker, J.J., Mojzsis, S.J. and Pace, N.R. (2005) Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl. Environ. Microbiol. **71**: 4822–4832.

Park, R. (1976) A note on the significance of laminations in stromatolites. Sedimentology **23**: 379–393.

Paterson, D.M., Aspden R.J., Visscher, P.T., Andres, M.S., Decho, A.W., Stolz, J. and Reid, R.P. (2008) Light-dependent biostabilisation of sediments by stromatolite assemblages. PLoS ONE **3**(9): e3176.

Pearson, A., Budin, M. and Brocks J.J. (2003) Phylogenetic and biochemical evidence for sterol synthesis in the bacterium *Gemmata obscuriglobus*. Proc. Natl. Acad. Sci. USA **100**: 15352–15357.

Perry, C.T. and Macdonald, I.A. (2002) Impacts of light penetration on the bathymetry of reef microboring communities: implications for the development of microendolithic trace assemblages. Palaeogeogr. Palaeoclimatol. Palaeoecol. **186**: 101–113.

Perry, R.S., McLoughlin, N., Lynne, B.Y., Sephton, M., Oliver, J., Perry, C.C., Campbell, K., Engel, M.H., Farmer, J.D., Brasier, M.D. and Staley, J.T. (2007) Biominerals and organominerals direct and indirect indicators of life. Sed. Geol. **201**: 157–179.

Planavsky, N. and Grey, K. (2008) Stromatolite branching in the Neoproterozoic of the Centralian Superbasin, Australia, an investigation into sedimentary and microbial control of stromatolite morphology. Geobiology **6**: 33–45.

Pratt, L.M., Comer, B. and Brassell, S.C. (1992) *Geochemistry of Organic Matter in Sediments and Sedimentary Rocks*. SEPM Short Course 27, Tulsa, Oklahoma.

Rashby, S.E., Sessions, A.L., Summons, R. and Newman, D.K. (2007) Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. Proc. Natl. Acad. Sci. USA **104**: 15099–15104.

Reid R.P. and Macintyre, I.G. (2000) Microboring versus recrystallization: further insight into the micritization process. J. Sed. Res. **70**: 24–28.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, I.G., Pinckney, J., Paerl, H.W., Prufert-Bebout, L., Steppe, T.F. and DesMarais, D.J. (2000) The role of microbes in accretion, lamination and lithification in modern marine stromatolites. Nature **406**: 989–992.

Reid, R.P., Dupraz, C., Visscher, P.T., Decho, A.W. and Sumner, D.Y. (2003) Microbial processes forming modern marine stromatolites, microbe–mineral interactions with a three-billion-year rock record, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms – A Natural History of Life on Earth*. Kluwer, Dordrecht, The Netherlands, pp. 103–118.

Riding, R. (2000) Microbial carbonates: The geological record of calcified bacterial-algal mats and biofilms. Sedimentology **47**: 179–214.

Rohmer, M., Bouvier-Nave, P. and Ourisson, G. (1984) Distribution of hopanoid triterpenes in prokaryotes. J. Gen. Microbiol. **130**: 1137–1150.

Sanchez-Roman, M., Vasconcelos, C., Schmid, T., Dittrich, M., Mckenzie, J.A., Zenobi, R. and Rivadeneyra, M.A. (2008) Aerobic microbial dolomite at the nanometer scales: implications for the geological record. Geology **36**: 879–882.

Schidlowski, M. (1993) The initiation of biological processes on Earth, In: M.H. Engel and S.A. Macko (eds.) *Organic Geochemistry*. Plenum, New York, pp. 639–655.

Schieber, J., Bose, P.J., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann W. and Catuneanu O. (eds.) (2007) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geosciences 2*. Elsevier, Amsterdam, 311 pp.

Schneider, J. and LeCampion-Alsumard, T. (1999) Construction and destruction of carbonates by marine and freshwater cyanobacteria. Eur. J. Phycol. **34**: 417–426.

Schopf, J.W. (1968) Microflora of the Bitter Springs Formation, Late Precambrian, Central Australia. J. Paleontol. **42**: 651–668.

Schopf, J.W. (1971) Fossil algae. Sci. Mag. **174**: 1229–1231.

Schopf, J.W. (ed.) (1983) *The Earth's Earliest Biosphere.* Princeton University Press, Princeton, NJ.

Seckbach, J. and Walsh, M. (eds.) (2009) *From Fossils to Astrobiology: Records of Life on Earth and the Search for Extraterrestrial Biosignatures.* Springer, Dordrecht, The Netherlands, 546 pp.

Seong-Joo, L., Browne, K.M. and Golubic, S. (2000) On stromatolite lamination, In: R. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Berlin, pp. 16–24.

Sergeev, V.N., Knoll, A.H. and Grotzinger, J.P. (1995) Paleobiology of the Mesoproterozoic Billyakh Group, Anabar Uplift, Northern Siberia. J. Paleontol. Memoir **39**: 1–37.

Stal, L.J. (2000) Cyanobacterial mats and stromatolites, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria, Their Diversity in Time and Space*. Kluwer, Dordrecht, The Netherlands, pp. 61–120.

Stal, L J. (2001) Coastal microbial mats, the physiology of a small-scale ecosystem. South African J. Bot. **67**: 399–410.

Stal, L.J. (2007) Cyanobacteria, diversity and versatility, clues to life in extreme environments, In: J. Seckbach (ed.) *Algae and Cyanobacteria in Extreme Environments*, Cellular Origin, Life in Extreme Habitats and Astrobiology, Vol. 11. Springer, Dordrecht, The Netherlands, pp. 659–680.

Stal, L.J., van Gemerden, H. and Krumbein, W.E. (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol. Ecol. **31**: 111–125.

Stal, L.J., Albertano, A., Bergman, B., von Bröckel, K., Gallon, J.R., Hayes, P.K., Sivonen, K. and Walsby, A.E. (2003) Baltic Sea Cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea – responses to a changing environment. Cont. Shelf Res. **23**: 1695–1714.

Stephens, E.A, Braissant, O. and Visscher, P.T. (2008) Spirochetes and salt marsh microbial mat geochemistry: Implications for the fossil record, Carnets de Geol. **2008/09**: CG2008 A09, 1–11.

Stolz, J. F. (2000) Structure of microbial mats and biofilms, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Berlin, pp. 1–8.

Stolz, J.F., Feinstein, T. N., Salsi, J., Visscher, P.T. and Reid, R.P. (2001) TEM Analysis of microbial mediated sedimentation and lithification in modern marine stromatolites. Am. Mineral. **86**: 826–833.

Stoodley, P., Sauer, K., Davies, D.G. and Costerton, J.W. (2002) Biofilms as complex differentiated communities. Ann. Rev. Microbiol. **56**: 187–209.

Strother, P.K., Knoll, A.H. and Barghoorn, E.S. (1983) Microorganisms from the Late Precambrian Narssarssuk Formation, northwestern Greenland. Paleontology **26**: 1–32.

Summons, R.E. and Walter, M.R. (1990) Molecular fossils and microfossils of prokaryotes and protists from Proterozoic sediments. Am. J. Sci. **290-A**: 212–244.

Summons, R.E., Janhke, L.L., Hope, J.M. and Logan, G.A. (1999) 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature **400**: 554–557.

Summons, R.E., Albrecht, P., McDonald, G. and·Moldowan, J.M. (2008) Molecular biosignatures. Space Sci. Rev. **135**: 133–159.

Talbot, H.M., Summons, R.E., Jahnke, L.L., Cockell, C.S., Rohmer, M. and Farrimond, P. (2008) Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. Org. Geochem. **39**: 232–263.

Tomitani, A., Okada, K., Miyashita, H., Matthijs, H.C.P., Phno, T. and Tanaka, A. (1999) Chlorophyll *b* and phycobilins in the common ancestor of cyanobacteria and chloroplasts. Nature **400**: 159–162.

Tourney, J. and Ngwenya, B.T. (2009) Bacterial extracellular polymeric substances (EPS) mediate $CaCO_3$ morphology and polymorphism. Chem. Geol. **262**: 138–146.

van Gemerden, H. (1993) Microbial mats: a joint venture. Mar. Geol **113**: 3–25.

van Kranendonk, M.J. (2008) Stromatolite morphology as an indicator of biogenicity for Earth's oldest fossils from the 3.5–3.4 Ga Pilbarra Craton, Western Australia, In: J. Reitner, N.V. Queric and M. Reich (eds.) *Geobiology of Stromatolites*, International Kalkowsky-Symposium, Göttingen Abstract Volume. Universitätsdrucke Göttingen, Göttingen, pp. 33–37.

Vasconcelos, C., Warthmann, R., McKenzie, J.A., Visscher, P.T., Bittermann, A.G. and van Lith, Y. (2006) Lithifying microbial mats in Lagoa Vermelha, Brazil, Modern Precambrian Relicts? Sed. Geol. **185**: 175–183.

Verrecchia, E P., Freytet, P., Verrecchia, K.E. and Dumont, J.L. (1995) Spherulites in calcrete laminar crusts, biogenic $CaCO_3$ precipitation as a major contributor to crust formation. J. Sed. Res. **A65**: 690–700.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., Macintyre, I.G. and Thompson, J. Jr. (1998) Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am. Mineral. **83**: 1482–1491.

Visscher, P.T., Reid, R.P. and Bebout, B.M. (2000) Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology **28**: 919–922.

Walker, J.J. and Pace, N.R. (2007) Endolithic microbial ecosystems. Ann. Rev. Microbiol. **61**: 331–347.

Walter, M.R. (1994) Stromatolites, the main source of information on the evolution of the early benthos, In: S. Bengtson (ed.) *Early Life on Earth.* Columbia University Press, New York, pp. 270–286.

Walter, M.R. (ed.) (1976) *Stromatolites*. Elsevier, Amsterdam, 790 pp.

Ward, D.M. (1998) A natural species concept for prokaryotes. Curr. Opin. Microbiol. **1**: 271–277.

Ward, D.M, Bateson, M.M, Ferris, M.J., Kühl, M., Wieland, A., Koeppel, A. and Cohan, F.M. (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. Philos. Trans. R. Soc. B **361**: 1997–2008.

Weckesser J. and Drews, G. (1979) Lipopolysaccharides of photosynthetic prokaryotes. Ann. Rev. Microbiol. **33**: 215–239.

Xiong, J., Fischer, W.M., Inoue, K., Nakahara, M. and Bauer, C.E. (2000) Molecular evidence for the early evolution of photosynthesis. Science **289**: 1724–1730.

Zavarzin, G.A. (2001) Diversity of cyanobacterial mats, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria, Their Diversity in Time and Space*. Kluwer, Dordrecht, The Netherlands, pp. 141–150.

Zelles, L. (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere **35**: 275–294.

Zhang, Y. and Golubic, S. (1987) Endolithic microfossils (Cyanophyta) from early Proterozoic stromatolites, Hebei, China. Acta Micropaleontol. Sin. **4**: 1–12.

Zundel, M. and Rohmer, M. (1985) Prokaryotic triterpenoids. 1. 3 β-Methylhopanoids from *Acetobacter* species and *Methylococcus capsulatus*. Eur. J. Biochem. **150**: 23–27.

Biodata of **Stefan J. Green** and **Linda L. Jahnke**, authors of ***"Molecular Investigations and Experimental Manipulations of Microbial Mats: A View to Paleomicrobial Ecosystems"***

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MOLECULAR INVESTIGATIONS AND EXPERIMENTAL MANIPULATIONS OF MICROBIAL MATS: A VIEW TO PALEOMICROBIAL ECOSYSTEMS

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1. Evolution of Microbial Mats

Modern microbial mats, laminated microbial communities encased within thick organic matrices composed of excreted polymers, are present in a variety of hypersaline, marine, and freshwater environments (e.g., Krumbein et al., 1977). Recent research has strongly indicated the widespread development of structurally similar biogenic, laminated, reef-like structures in shallow marine environments at least as early as 3.4 Ga (Allwood et al., 2006). This extensive geological history suggests that microbial mats are highly stable but adaptable communities, having survived billions of years of environmental change. The greatest abundance of microbial mats and stromatolites was during the Proterozoic (2.5–0.57 Ga), and their widespread presence suggests that mat communities were a dominant sedimentary feature during this period. The widespread distribution of mats during this period has been attributed to a number of possible factors: development of oxygenic photosynthesis (using water as an electron donor), evolution of novel metabolic pathways (nitrogen fixation), and environmental stability (Awramik et al., 1976; Des Marais, 1997). The success of microbial mat communities in the Proterozoic should be considered in context of significant differences in oceanic and atmospheric conditions at that time. High UV irradiance limited microbial communities to a subaqueous environment shield by dissolved Fe^{2+}-rich seawater (Pierson, 1994). Anion concentrations varied widely from modern levels – Archean and early Proterozoic seawater was oversaturated with respect to calcium carbonate (Grotzinger, 1994). Seawater bicarbonate levels were also much higher than in the modern ocean – dissolved concentrations were constrained at perhaps 70 mM by relatively high dissolved Fe^{2+} (40–120 μM) and higher atmospheric CO_2 (pCO_2 = 0.03–0.2 atm) – and seawater pH has been estimated to have been near neutral (Grotzinger and Kasting, 1993; Canfield et al., 2006). The sulfur cycle of ancient seawater would have been constrained by dissolved Fe^{2+} and a minimal supply of bioavailable sulfate (<1 mM) primarily derived from atmospheric photolysis of

reduced sulfur species (Canfield, 1998; Canfield et al., 2006). Low continental mass resulted in much saltier seas with chloride perhaps twice that of today (Knauth, 1998) but on a geologic time scale would have varied with rising and falling sea levels. The primary production of the earliest microbial communities depended on anoxygenic photosynthesis fueled by H_2 or Fe^{2+}. Little molecular oxygen would have been available for biosynthetic reactions, and even after the evolution of oxygenic photosynthesis by the cyanobacteria, free O_2 would be removed by reaction with abundant reduced species from the mantle (Canfield et al., 2006). These reactions most likely delayed the oxygenation of the atmosphere and oceans to 2.45 Ga (Holland, 2006). The abundance of microbial mats and stromatolites diminished drastically after the Proterozoic, concomitant with the development of metazoal life. However, decreasing carbon dioxide and increasing oxygen in the atmosphere also may have influenced the prevalence of mats.

Hoehler et al. (2001) explored the production of reduced gases by modern microbial mats and inferred a global-scale impact of ancient microbial mats. Modern mats produce CO, H_2, and CH_4 at various stages during the diurnal cycle, and the production of these gases appears tied to oxygenic photosynthesis (Hoehler et al., 2001). Initially, oxygenic photosynthesis drastically increased microbial productivity, including that of microbial mats – on the order of 100–1,000 fold (Des Marais, 2003), resulting in the increased production of fixed carbon and reduced gases. Since modern mats with defined aerobic zones are net producers of reduced gases, mats in the early Proterozoic with limited potential for oxidation of these reduced compounds would have been significant sources. An important theoretical development of this study is perhaps counterintuitive – namely, that oxygenic photosynthesis most likely dramatically increased the efflux of reduced gases from the mat. Hoehler et al. (2001) suggested that the oxidation of the exterior of the Earth could not have occurred without burial or escape of reducing equivalents, and that a plausible escape could be attributed to molecular hydrogen loss to outer space. In modern mats, even under artificially lowered sulfate conditions, methane production is quite limited − most likely the result of the lack of access to labile organic matter by oxygen-sensitive methanogens (Bebout et al., 2004).

Oxygen production caused fundamental changes to the environment and to microbial mat ecosystems. Initially, while the atmosphere and ocean were still essentially anoxic (perhaps, 2.7–2.2 Ga; Canfield et al., 2000) oxygenic photosynthesis would have established temporary aerobic zone between anoxic zones above and below. The lack of an oxidizing water column would no doubt have limited oxidation of reduced gases prior to escape from the mat. Ultimately, however, the great energy yields from oxygenic photosynthesis resulted in an increase in the levels of oxygen in ocean and atmosphere, and indirectly allowed the development of macroeukaryotes capable of culling the growth of microbial communities. Thus, the mats have been relegated to small niches of the modern environment, where environmental conditions (such as salinity and sulfate) diverge significantly from those that would have been present in

the earliest mats, and from those in the early Proterozoic. In the section on Manipulation Experiments of Hypersaline Microcoleus Mats (Section 4) we consider the results and implications of experimental manipulations of salinity and sulfate to approximate oceanic conditions during the early Proterozoic. Here, we consider what is known about microbial populations in a variety of natural environments across a range of salinities from observational and molecular analyses.

2. Dominant Microorganisms in Hypersaline Microbial Mats

Modern microbial mats are highly diverse ecosystems, particularly abundant in marginal marine environments (Javor, 1989; Pierson et al., 1992). Coastal intertidal zones are conducive to the development of restrictive conditions such as hypersalinity that support the growth of visible, laminated microbial communities. Supratidal sabkhas, restricted lagoons fed directly by the sea or lakes fed by percolation through natural barriers, provide a habitat for the accumulation of diverse microbial mat types. In general, a very limited eukaryotic presence is found – diatoms (present at the surface of the mats) and nematodes are the predominant eukaryotic organisms that do persist (Minz et al., 1999a; Feazel et al., 2008; Fig. 1). Commonly, the surface layers are composed of unicellular or filamentous cyanobacteria that are the dominant primary producers; such halophilic

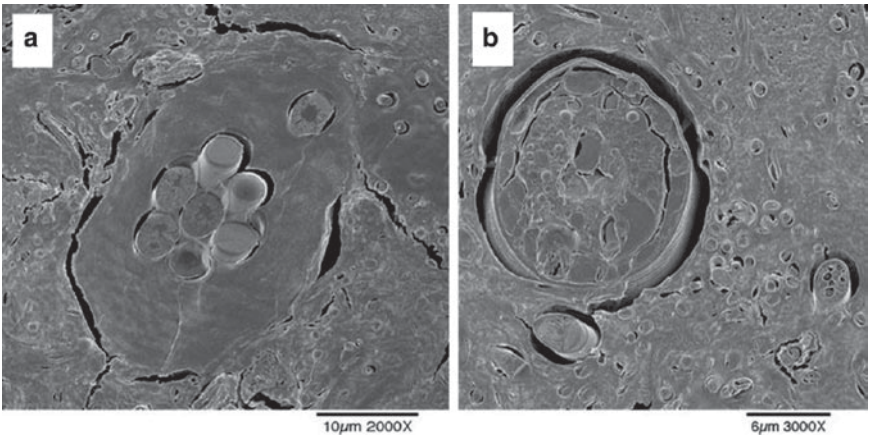

Figure 1. Cryoscanning electron micrographs of a transverse section of the surface (0–1 mm) of a microbial mat from Solar Lake, Sinai, Egypt. (**a**) The image contains a bundle of *Microcoleus chthonoplastes* filaments and associated sheath material that is devoid of other bacteria. Just outside of the sheath are a host of unidentified microorganisms. (**b**) The image contains an unidentified eukaryote, presumably a nematode, in association with a large number of microorganisms. Preparation of the samples was as described elsewhere (Green et al., 1999) (Courtesy of Green, S.J., Minz, D., Stahl, D.A., Chen, Y., Lavin, C.A., and Cohen, Y.).

cyanobacteria survive and grow across a broad range of salinities reaching over 200 parts per thousand (‰) (Golubic, 1980). Historically, the first best-studied systems were the Gavish Sabkha and Solar Lake, on the shore of the Red Sea, Sinai Peninsula (Friedman and Krumbein, 1985). Similar physical environments are also represented on the extensive intertidal flats and supratidal sabkhas of Laguna Guerrero Negro, on the Pacific coast of the Baja California peninsula, Mexico. Here, microbial mats develop in salinities ranging from normal seawater to the hypersalinity of sabkha environments. Laguna Guerrero Negro is also the location for one of the largest man-made, solar evaporation pond systems.

The filamentous nonheterocystous *Microcoleus chthonoplastes* is the dominant cyanobacterium in most marine microbial mats (Stal et al., 1985; Garcia-Pichel et al., 1996; Martinez-Alonso et al., 2004). A morphological trademark of *M. chthonoplastes* is twisted filament bundles enclosed in a common polysaccharide sheath (Fig. 1). The finely laminated mats most often studied as analogs for ancient stromatolites are built by this cyanobacterium. The predominance of *M. chthonoplastes* in a variety of different hypersaline microbial mats (Prufert-Bebout and Garcia-Pichel, 1994) has demonstrated the adaptability of this organism to the fluctuating and toxic conditions found in a diurnal cycle in a typical hypersaline mat. In part, *M. chthonoplastes* appear to be successful due to these physiological characteristics: tolerance of a wide range of salinities (Karsten, 1996; Nübel et al., 1999), potential to operate sulfide-insensitive oxygenic photosynthesis concurrent with sulfide-dependent anoxygenic photosynthesis (Cohen et al., 1986; Cohen, 1989), motility (Whale and Walsby, 1984; Garcia-Pichel et al., 1996), fermentation and sulfur reduction (Moezelaar et al., 1996), and production of UV protectants (Karsten, 2002). Although *M. chthonoplastes* is considered a monophyletic, coherent group of organisms (e.g., Garcia-Pichel et al., 1996), many functional variants have been noted (e.g., Karsten, 1996; Lodders et al., 2005). Recently, a draft genome of a representative member of this species has been generated (PCC 7420; Tandeau de Marsac et al., 2007). The genome of this organism is quite large; currently the draft genome is greater than 8.5 Mb, at the higher end of the range of cyanobacteria (Fogel et al., 1999). The large genome of some cyanobacteria has been attributed to an ancient genome duplication event, with a resulting potential for the expansion of the cell's metabolic capabilities (Sugaya et al., 2004). Previously, no nitrogen fixation capability had been identified for *M. chthonoplastes* (e.g., de Wit et al., 2005); however, the genome sequence of *M. chthonoplastes* PCC7420 (isolated from a salt marsh in Woods Hole, MA, USA) appears to contain a nitrogen-fixing pathway (e.g., GenBank accession number EDX77768 for *nifH*, nitrogenase reductase gene; www.ncbi.nih.gov). Whether this is a universal feature of the species is not known, but if so, it would represent another physiological capability of this versatile organism.

The cyanobacteria represent only the most visible microbial element of these ecosystems, and prior to the development of molecular tools, great morphological diversity had been observed (e.g., Ehrlich and Dor, 1985; Gerdes et al., 1985). The genetic diversity of hypersaline microbial mats has only recently begun to be

reasonably explored. In part, this appears to be a result of the extraordinarily high diversity of these mats systems, limiting standard molecular approaches such as the generation of clone libraries. Prior to the advent of truly high-throughput DNA sequencing technology, there was a surprising dearth of cultivation-independent, total microbial community analysis. Molecular techniques, when employed to microbial community analysis, tended to focus on key functional groups such as cyanobacteria and nitrogen-fixing bacteria (e.g., Nübel et al., 1997, 1999, 2000; Neilan et al., 2002; Omoregie et al., 2004; Yannarell et al., 2006; Abed et al., 2007; Green et al., 2008), sulfate-reducing bacteria (SRB; e.g., Risatti et al., 1994; Teske et al., 1998; Minz et al., 1999a, b), and to a lesser extent, *Chloroflexus* spp. (e.g., Nübel et al., 2001). In some cases, community profiling techniques such as denaturing gradient gel electrophoresis (DGGE) or terminal restriction fragment length polymorphism (T-RFLP) were used to characterize these systems, but in retrospect, the high microbial diversity and the often predominant presence of the cyanobacterium *M. chthonoplastes* limited such approaches for the characterization of total diversity.

More recently, with the advent of high-throughput sequence analyses, there has been a resurgent interest in a wider characterization of the mat microbial community (Spear et al., 2003; Ley et al., 2006; Liu et al., 2007; Feazel et al., 2008; Robertson et al., 2009). In general, mats have been vertically sectioned on a millimeter scale, with the daytime aerobic zone typically from 2 to 3 mm below the surface. The application of high-throughput sequence analysis of microbial mat community structure has utilized several advances in sequencing technology. The first sizable clone library of bacterial 16S rRNA genes (~1,600 clones over ten depths) using standard cloning and sequencing was generated from a Guerrero Negro mat (Ley et al., 2006). More recently, however, it has been demonstrated that short regions of the 16S rRNA gene, roughly 100–200 bases, are suitable for identification purposes down to a genus or species level (Wang et al., 2007). This bioinformatic advance was coupled with newly developed sequencing platforms that can generate large numbers of short (100–250 base reads) sequences (e.g., pyrosequencing; Ronaghi et al., 1998). This has enabled the generation of large numbers of short sequences from various depths in microbial mats to better characterize the diversity of these systems (Liu et al., 2007). In addition, metagenomic data generated from ten different depths in a Guerrero Negro mat has been recently published (Kunin et al., 2008).

What then have these cultivation-independent analyses provided for in the way of understanding the diversity, distribution, and activity of hypersaline microbial mat communities? To begin, they have expanded our understanding of functional diversity of these mat communities to a genetic diversity. The link between these has not yet been gapped because the ecosystem function of these uncultivated microorganisms is not yet known. Traditionally, the key constituents of hypersaline microbial mats have been considered to be: oxygenic phototrophs (cyanobacteria and diatoms), aerobic heterotrophic bacteria (e.g., Bacteroidetes, Proteobacteria), colorless sulfide-oxidizing bacteria (e.g., *Beggiatoa*), anoxygenic

photosynthetic bacteria (e.g., *Chromatium*, *Chloroflexus*), sulfate-reducing bacteria (SRB) (e.g., *Desulfonema*), and methanogens (e.g., Methanosarcinales). These organisms had been thought to distribute spatially in relation to the oxygen and sulfide gradients according to their major metabolic function: Aerobic phototrophs and aerobic heterotrophs in the surface of the mat, removed from the sulfidic, anoxic layer; colorless sulfur bacteria at the base of the oxygen chemocline at the interface between sulfide and oxygen; anoxygenic photrophic bacteria below the oxygen chemocline provided light of appropriate wavelengths penetrates; SRB and possibly methanogens even deeper (e.g., van Gemerden, 1993; Des Marais, 2003). This paradigm has been altered by the detection of a much broader distribution of the SRB in hypersaline mats, including the very surface, and the measurement of extremely high rates of sulfate-reduction in the aerobic surface of these mats (Canfield and Des Marais, 1991; Risatti et al., 1994; Minz et al., 1999a, b; Fike et al., 2008).

Despite the functional flexibility of some SRB, the oxygen chemocline plays a crucial role in the microbial community structure. Small clone libraries, community profiling analyses, and northern hybridization studies of extracted RNAs previously demonstrated the importance of the oxygen chemocline on the microbial community, and accompanying migration of microorganisms relative to this interface during the diurnal cycle (Teske et al., 1998; Minz et al., 1999a; Fourçans et al., 2006, 2008; Villanueva et al., 2007). High-throughput community analyses of microbial mats have allowed a deeper understanding of the relationship between the mat geochemistry and microbial population structure. Ley et al. (2006) and Liu et al. (2007) have both employed 16S rRNA gene sequence data to generate community relationship dendograms. This approach has provided a statistically defensible demonstration of the significant differences in bacterial community structure above and below the oxygen chemocline. Ley et al. (2006) defined three biological and geochemical zones: 0–2 mm (oxic), 2–6 mm (low sulfide), and 6–60 mm (high sulfide). We note, however, that this characterization of microbial community structure by depth and geochemistry can miss ecological zones that exist at a much finer resolution (Minz et al., 1999a, b; Fike et al., 2008).

This transition is corroborated with functional gene data produced in a metagenomic study by Kunin et al. (2008). Genes involved in photosynthesis were heavily represented in the surface 2 mm, and genes for chaperones also tracked the oxygen gradient and were abundant in the surface of the mat (Kunin et al., 2008). Consistent with other studies, cyanobacterial genes predominated in the surface of the mat, but were 4–6% of the total detected genes below a depth of 3.5 mm (Kunin et al., 2008 – supplementary information). Previous work has shown that a significant photosynthetic potential resides in the deep mat (Jørgensen et al., 1988), and we have detected active ribosomal RNAs from *M. chthonoplastes* to a depth of 1 cm (Green and Jahnke, unpublished data). Furthermore, the metagenome data are consistent northern hybridization studies performed on rRNA extracted from Solar Lake mats (Minz et al., 1999a).

In both studies, Deltaproteobacteria (specifically, deltaproteobacterial SRB in the Minz et al., 1999a study) were observed to represent roughly 10% of the total recovered genes from the metagenome at all depths (Kunin et al., 2008 – supplementary information). These metagenome data also present information for the development of new paradigms. For example, Kunin et al. (2008) show an increase in the relative abundance of genes encoding for enzymes involved in sugar degradation with depth.

In addition to examining the microbial community structure with depth, high-throughput sequencing analyses have also expanded on the full microbial diversity found in these hypersaline mats. However, we appear to still be far from fully characterizing the diversity in these systems. In the metagenome data generated by Kunin et al. (2008), only 61 ribosomal RNA genes were recovered. For comparison, in the study by Ley et al. (2006), a broad diversity of microorganisms was detected in a clone library of nearly 1,600 sequences. Ley et al. (2006) defined 752 operational taxonomic units (OTUs) that were identified using 97% sequence similarity as a cut-off value. These OTUs were classified into 42 bacterial phyla, including 15 previously unidentified phyla. Sequences of organisms from the phylum Chloroflexi were the most abundant detected in the overall library, and composed roughly 20–40% of the clone libraries at each depth. Since many of these sequences were deeply branching and distant from sequences of cultured organisms, it is difficult to infer physiological properties from the sequence data alone. Using fluorescent in situ hybridization (FISH), some Chloroflexi were observed in direct contact with cyanobacterial filaments in the surface and in direct contact with polysaccharides deeper in the mat (Ley et al., 2006). This study also revealed sequences significantly divergent from known phyla, and determined 15 novel phyla based on sequence data alone.

High-throughput sequencing has also allowed us to consider more closely the extensive diversity of aerobic heterotrophic microorganisms that have generally escaped detection in studies dedicated to cyanobacteria, SRB, sulfide-oxidizing bacteria, and anoxygenic phototrophic bacteria. Villanueva et al. (2007) found that aerobic heterotrophic bacteria were the major components of the microbial community in the surface of the mat, and these organisms were primarily from the phylum Bacteroidetes or from the class Gammaproteobacteria. In the recent metagenomic study of Guerrero Negro mats, Kunin et al. (2008) detected bacteria from the phylum Bacteroidetes in great abundance below 2 mm, with a peak at 3 mm, and a great abundance of Alphaproteobacteria, particularly in the top 2 mm of the mat. Previously, Jonkers and Abed (2003) identified a variety of aerobic, heterotrophic Alphaproteobacteria from microbial mats using combined cultivation-independent (i.e., DGGE and sequencing) and cultivation approaches. Some of these organisms were capable of glycolate consumption; glycolate has been posited as a key carbon source in the surface of these hypersaline (and other) mats, exuded by cyanobacteria during the daytime as a photorespiration product to alleviate CO_2 limitation (Tolbert, 1980; Bateson and Ward, 1988; Fründ and Cohen, 1992).

3. Community Structure of Microbial Mats as Revealed by Lipid Biomarker Analysis

The microfossil record indicates that cyanobacteria-dominated microbial communities were the most pervasive ecosystem throughout the vast majority of Earth's history. The organic geochemical record derives from the primary productivity of this group of oxygenic phototrophs and the subsequent network of secondary and terminal microbial processes. Studies of these finely laminated mats provide insights into the processes of diagenesis and preservation of this organic matter.

With the exception of porphyrins, most sedimentary organic biomarkers derive from lipids derived from the formation and integrity of microbial cytoplasmic membranes. The types of lipid molecules biosynthesized by microorganisms are extensive, and when a lipid is specific to a species or other taxonomic group, it is referred to as a biomarker. Complex polar lipids, amphiphilic molecules that form the backbone of membrane bilayers can often serve as biomarkers. Membrane lipids are generally composed of a polar moiety consisting of a glycerol molecule linked via the *sn*-1 or *sn*-3 hydroxyl group to a variety of polar head groups. The hydrophobic moieties such as fatty acyl (bacterial) or isopranyl (archaeal) chains are linked to the remaining hydroxyl groups by ester or ether bonds, respectively. Some hydrocarbon chain structures offer attribution to specific groups of organisms but their major ecological importance is in the estimation of viable community biomass (White et al., 1997). After cell death, lipases immediately begin the enzymatic degradation of polar lipids. Polar head groups and acyl moieties are cleaved. Free fatty acids are released to the environment and serve as growth substrates for heterotrophic microorganisms. The ether bond is somewhat more stable and isopranylglycerols (e.g., archaeol) are more resistant to microbial degradation. An important distinction in defining a lipid biomarker is the potential for survival of the carbon skeleton within the sedimentary environment. Macromolecular substances that survive during the processes of sedimentation and diagenesis are referred to as "selectively preserved." de Leeuw and Largeau (1993) have reviewed this topic in detail. The mass of sedimentary organic carbon forms a highly resistant, polymeric macromolecule named kerogen. A small fraction of sedimentary carbon is recovered as free hydrocarbons or by cleavage of specific chemical bonds. These latter molecules compose the lipid biomarker record (Summons and Walter, 1990; Brocks and Summons, 2005).

The geochemical fate of cyanobacterially derived organic matter is considered central to the interpretation of the Precambrian sedimentary organic record. Thus, the survival of various classes of branched acyclic and cyclic hydrocarbons and some functionalized molecules, such as polyhydroxylated hopanoids and sterols, has been the focus of most lipid studies of microbial mats. Boon and de Leeuw (1987) reviewed results from early organic geochemical studies of microbial marine mats. A joint study of Solar Lake and the Gavish Sabkha mats found lipid compositions were generally consistent. Analyses of the extractable, free lipid fraction (often referred to as neutral lipid) identified various

hopanoid and steroid compounds, methyl-branched alkanes, primarily 8-methylhexadecane, and cyclopropane fatty acids as characteristic components. Carotenoids representative of cyanobacteria and purple photosynthetic bacteria were extensive, but archaeal isoprenoid and diatom lipids were limited. In the Solar Lake mat, extended hopanoids, identified as hopanetetrols, were present in the top mat and in the underlying, 70 cm deep, sediment. Most noteworthy, the hopanetetrol and co-occurring carotenoids recovered were estimated to have survived burial for 2,500 years. More in-depth analyses of various lipid classes have been addressed in later works. Pigment studies of Guerrero Negro salina mats indicated that carotenoids such as myxoxanthophyll, zeaxanthin, echinenone, and γ-carotene are representative of the photosynthetic community and are better preserved than chlorophylls in recent sediments (Palmisano et al., 1989). Kenig et al. (1995) characterized a variety of mono- and dimethylalkanes, cyanobacterial biomarkers extensively studied in hot spring mats (Robinson and Eglinton, 1990; Shiea et al., 1990), in coastal marine mats from an Abu Dhabi sabkha. The methylation patterns and C-chain lengths were considerably more diverse than the 8-methylhexadecane generally identified in *Microcoleus*-dominated mats. A number of salina microbial mat studies have also focused more broadly on lipid composition in an attempt to equate community composition with hypersaline depositional environments (Barbe et al., 1990; Grimalt et al., 1992). Grimalt et al. (1992) were the first to characterize mat lipids at the millimeter scale relevant to the steep chemical microgradients and shifts in community composition found in such mats. Cyanobacterial lipids were clearly recognized in the surface mat but only a few distinct contributions representative of cyanobacterial productivity, *n*-heptadecane, *n*-heptadecene, 8-methylhexadecane, and diploptene were identified below 3-mm depth indicating rapid post-depositional changes in microbial community and organic matter. In this study, Grimalt and colleagues also used innovative enrichment strategies to support fatty acid attribution. The *iso*- and *anteiso*-pentadecanoic and heptadecanoic acids were found more abundant in enrichments for sulfate-reducing and sulfur-oxidizing bacteria, whereas the purple photosynthetic bacteria correlated well with the cyclopropylnonadecanoic and Δ11 octadecenoic acids.

The simultaneous use of phylogenetic molecular and lipid biomarker tools for in situ analysis of bacterial composition allows an additional degree of attribution, and provides a valuable perspective to characterize environmental influence on community structure and reconstructions related to residual organic biosignatures. Using this approach, Fourçans et al. (2004) were able to correlate various lipid biomarkers to functional groups of bacteria. Though the topmost millimeter was dominated by cyanobacteria, both T-RFLP and fatty acid profiles clearly demonstrate a highly diverse bacterial population in this surface layer. The abundant polyunsaturated fatty acids, $C_{16:2}$ and $C_{18:2}$, and the Δ9 octadecenoic acid isomer are cyanobacterial products, as well as *n*-heptadecane, *n*-heptadecenes, and 8-methyhexadecane being the most abundant hydrocarbons identified. Abundant *iso*- and *anteiso*-pentadecanoic acids, the Δ11 octadecenoic acid isomer, and

relatively large amounts of 10-methylhexadecanoic and cyclopropylnonadecanoic acids were also identified and reflect the diverse community. The archaeal population of a 130-mm core from *Microcoleus*-dominated mat has also been studied using this simultaneous approach (Jahnke et al., 2008; Orphan et al., 2008). These studies have confirmed the low abundance of Archaea relative to bacteria in this hypersaline mat community. With the exception of the lowest sedimentary horizon (>100 mm), Bacteria vastly outnumber Archaea. The most prevalent group in the surface is the halophilic Archaea; however, Thermoplasmatales is the primary archaeal group recovered throughout the underlying sedimentary horizon. Distinct zonal distribution of archaeal diversity documented by fluorescence in situ hybridization (FISH) images is observed throughout the core and correlates well to changes in molecular and archaeal lipid compositions. Higher relative abundance of archaeol and clones representative of methylotrophic methanogens resulted from enrichment of core sections with trimethylamine. In the enrichment of an upper sedimentary horizon, greater numbers of *Methanolobus* clones directly correlate to a higher abundance of the hydrocarbon biomarker pentamethylicosene (PMI). Most striking is the consistent high ratio of archaeols to tetraether dibiphytanyl lipids and the almost complete absence of cyclic biphytanyl moieties as a representative biosignature for hypersaline environmental organic matter.

4. Manipulation Experiments of Hypersaline Microcoleus Mats

As the geographic distribution of microbial mats is limited in the modern environment, extrapolation to ancient microbial ecosystems can be difficult. To overcome some of these limitations, hypersaline microbial mats from salterns operated by the salt-producing company, Exportadora de Sal SA de CV, located in Guerrero Negro (Baja, California Sur, Mexico), were returned to a greenhouse facility at the NASA Ames Research Center (ARC) in Moffett Field, CA (Bebout et al., 2002) and maintained under experimentally modified systems (Bebout et al., 2004; Kelley et al., 2006). The study of microbial mats subject to salinity and sulfate manipulations has been a long-term effort at the NASA ARC. Decrease of sulfate concentration below 1 mM significantly impacts mat biogeochemistry, and acetate concentration (significantly increased; Kelley et al., 2006), carbon isotope values of DIC and methane (significantly more positive; Bebout et al., 2004; Kelley et al., 2006), rates of sulfate reduction (significantly lowered; Bebout et al., 2004), and rates of methanogenesis and concentrations of methane (significantly increased; Bebout et al., 2004; Kelley et al., 2006) have been observed to change. Decreased sulfate concentrations have not been shown to significantly affect the rates of oxygenic photosynthesis (Bebout et al., 2004). Elsewhere, research into hypersaline microbial mats has indicated that salinity, often varying as a function of desiccation, is a crucial factor in shaping community structure (Garcia-Pichel et al., 1999; Nübel et al., 2000; Rothrock and Garcia-Pichel, 2005; Abed et al., 2007). Salinity is a key factor in determining microbial community structure (e.g., Lozupone and Knight, 2007), and the microbial response to the hypersalinity

in microbial mat systems is of great interest. One of the most insightful finding of the recently published metagenome data set is the observation that the inferred proteins from the entire community appear to be enriched in acidic amino acids. This type of modification also has been shown in halophilic Archaea as a response to hypersaline conditions (Oren, 2002).

It is clear that these salinity and sulfate manipulations can have a substantial impact of key metabolic processes in microbial mats. The earlier studies examined the microbial community function, and did not explicitly examine the shifts in microbial community associated with these manipulations. Recently, these experiments have been supplemented with molecular analyses examining total bacterial community, cyanobacteria (Green et al., 2008), methanogens (Smith et al., 2008), and sulfate-reducing bacteria (Green et al., 2006). Here, we consider these previously published studies, and some unpublished data regarding the effects of these manipulations on the dominant lipids extracted from these mats as well as additional nucleic-acids-based analyses.

4.1. LONG-TERM EFFECTS OF EXPERIMENTALLY LOWERED SALINITY

Increased salinity, even on a short-term scale, can have a substantial negative impact on photosynthesis via direct and indirect effects, and truly dramatic decreases in gross photosynthesis begin above salinities of 100% (Javor and Castenholz, 1984; Garcia-Pichel et al., 1999). In long-term (ca. 1 year) salinity manipulations of hypersaline microbial mats returned to NASA-ARC in Moffett Field, CA from Guerrero Negro, Baja, Mexico, revealed only a limited effect of decreased salinity on microbial community structure and function (Bebout et al., 2004; Green et al., 2008). Additionally, Bebout et al. (2002) demonstrated either no or limited effect on oxygen fluxes and oxygen microelectrode profiles as a result of long-term incubation of microbial mats at native and elevated (120%) salinity. Salinity does not appear to have been a determinative factor in the shifts associated with sulfate-reducing and methanogenic microbial communities associated with long-term manipulations of sulfate and salinity (Green et al., 2006; Smith et al., 2008). For methanogens, an effect associated with salinity is confounded by the substantially lower sulfate levels in the low salinity, low-sulfate treatment (ca. 0.2 mM) as opposed to the native salinity, low-sulfate treatment (ca. 0.9 mM; Kelley et al., 2006; Smith et al., 2008). The dominant cyanobacteria in these mats, *M. chthonoplastes* and a second, uncultivated oscillatorian organism (termed "Cluster 5"; Green et al., 2008) were essentially unaffected by either salinity or sulfate manipulations. A few cyanobacterial populations were detected developing under lowered salinity conditions, and by sequence analysis these organisms were most closely related to other filamentous, nonheterocystous hypersaline microbial cyanobacteria (Green et al., 2008). We also explored the effects of a more significant salinity reduction, to 20%, on cyanobacterial community structure, and found very similar results (Green and Jahnke, unpublished data). The dominant cyanobacteria were unaffected, and their depth distribution remained unchanged. In the top layers of these mats (0–0.5 and 0.5–2 mm), the lowered salinity did result in

a relative increase of different cyanobacteria, as assayed by cyanobacterial-specific PCR-DGGE analyses. However, as with the mats maintained at 35‰ salinity, these organisms were most closely related to other hypersaline cyanobacteria. This result suggests a limitation of this experimental approach, namely that the native hypersaline conditions are lethal to many organisms that could be highly competitive at the marine and brackish salinities tested in these studies. Coupled with the highly flexible physiology of the dominant cyanobacteria, there is limited opportunity to see dramatic shifts in cyanobacterial community structure. The minor changes in cyanobacterial community structure corroborate the very limited effects of the manipulations on the gross photosynthetic rates of these mats (Bebout et al., 2004).

Our polar lipid analysis of the 20% salinity treatment indicated no significant differences in the microbial populations represented by the ether-*O*-alkyls or the archaeal ether-*O*-isoprenyls moieties, either in abundance or relative distribution over the 0.5–15 mm depth analyzed (Table 1). However, the mass of bacterial population is represented by the polar ester-acyls (i.e., fatty acids) that are considered

Table 1. Effect of low-sulfate and low-salinity manipulations on abundance and distribution of lipid moieties and short-chain alkanes (μg.g−1 TOC) with core depth.

	Polar lipid moieties[b]						Alkane (C_{14-18})[c]	
	Ester acyl			Archaeols				
Condition depth[a] (mm)	Σ	10-Me[d]	Ether alkyl	C_{20}	C_{25}	Tetraether (C_{40})	Σ	% MMe
85‰-Low sulfate								
0.5–2	21,195	834	63	28	10	nd	676	3.1
2.5	12,459	592	7	47	19	nd	1,980	13.5
5	5,292	135	6	54	15	nd	1,169	13.8
10	3,298	63	15	31	1.3	nd	75	4.8
15	2,739		33	33	3.5	1.4[5]	89	4.8
85‰-Normal sulfate								
0.5–2	14,700	256	116	16	0.4	0.3	3,410	9.3
2.5	11,074	159	26	8.7	0.5	0.4	150	13.0
5	5,426	103	23	15	0.4	0.7	632	7.6
10	4,316	146	72	22	0.8	0.9	287	5.3
15	–	–	33	23	1.2	1.7	79	8.6
20%-Normal sulfate								
0.5–2	17,822	128	125	9.0	0.1	0.1	2,810	3.1
2.5	17,907	139	19	10	0.1	0.2	4,427	7.6
5	7,092	109	50	14	0.4	0.6	4,035	2.9
10	7,920	230	14	16	0.2	0.6	955	6.6
15	3,075	97	39	26	1.1	1.2	556	4.6

[a]0–0.5 mm, surface brownish-orange flocculent material, not analyzed.

[b]nd = none detected.

[c]Major alkanes are heptadecane, heptadecenes, and 8-methylheptadecane (MMe = monomethyl).

[d]10-Me = 10-methylhexadecanoate.

[e]Primarily monocyclic C_{40}.

a measure of total cell numbers (Jahnke et al., 2008). Relative to the 85‰ control, fatty acid abundance increased by ~40% in the low salinity experimental mat summed through the entire 0.5–15 mm depths. In the surface (0.5–2.5 mm) layers, this apparent increase in cell numbers was accompanied by increased synthesis of the Δ11 octadecenoic acid isomer generally considered a biomarker for gram-negative bacteria. Additionally, though overall fatty acid compositions were generally similar, the relative proportion of cyclopropylnonadecanoic acid decreased to 1% of that of the 85% mats, an expected response to lower salinity (Monteoliva-Sanchez et al., 1993). Remarkably, the abundance of cyanobacterial alkanes increased more than twofold throughout the core. This increase could indicate cyanobacterial growth or a physiological response to lower salinity. The functional role of alkanes in cyanobacteria is unknown; however, they are presumed to have a membrane function, interacting with the hydrophobic acyl chain in a manner similar to that proposed for Archaea (Haines, 2001). Since microbial mats tend to accrete new biomass very slowly (e.g., Cohen et al., 1977), increased rates of photosynthesis under reduced salinity may result in increased photorespiration and exudation of labile organic matter for heterotrophic bacteria. At the depths with the greatest increases in cyanobacterial alkanes (2–5 mm), a very limited change in the cyanobacterial community structure was observed, and *M. chthonoplastes* was the predominant organism detected in both salinities (Green and Jahnke, unpublished data). The increased levels in cyanobacterial alkane, coupled with DNA- and RNA-based studies, indicate that the *M. chthonoplastes* population is metabolically active and capable of cellular adaptation even at depths below the photic zone. The mats retain metabolically active cyanobacteria below the photic zone and the oxygen chemocline; for *M. chthonoplastes* this survival is most likely the result of mixed-acid fermentation under dark, anaerobic conditions (Jørgensen et al., 1988; Moezelaar et al., 1996).

4.2. LONG-TERM EFFECTS OF LOWERED SULFATE

Sulfate-manipulation experiments did not have a strong effect of the cyanobacterial community structure (Green et al., 2008). These modifications, however, had a much more significant effect on mat biogeochemistry and on the population structure of sulfate-reducing and methanogenic microorganisms (Bebout et al., 2004; Green et al., 2006; Kelley et al., 2006; Smith et al., 2008). The geochemical shifts associated with sulfate removal (final sulfate concentrations discussed vary from 80 to 1,000 μM) are stark: these include a dramatic decrease in sulfate reduction (a conservative estimate of a threefold decrease at a concentration of 80 μM; Bebout et al., 2004), a continuous increase in methane production by low-sulfate mats (Bebout et al., 2004), a near two order of magnitude increase in powerwater methane (roughly 300–900 μM sulfate; Kelley et al., 2006; Smith et al., 2008), and a substantial increase in porewater acetate (roughly fourfold to eightfold increase; Kelley et al., 2006; Smith et al., 2008). These geochemical shifts were accompanied

by a shift in methanogen microbial community structure. In general, methanogens consuming so-called noncompetitive substrates (e.g., methylated amines, methylated sulfides) are expected in hypersaline environments (Orphan et al., 2008; Smith et al., 2008). This appears due to a few factors, including (a) the lack of competi tion for substrate from sulfate-reducing bacteria, (b) the higher free energy yield per mol substrate from these compounds (Oren, 1999), (c) competition for molecular hydrogen from sulfate-reducing microorganisms, and (d) the absence of acetoclastic methanogens at such high salinities (Oren, 1999). Despite high-sulfate levels in native hypersaline microbial mats, elevated molecular hydrogen levels may still exist, and Hoehler et al. (2001) suggested that this level of hydrogen would relieve thermodynamic inhibition of methanogenesis. Bebout et al. (2004) observed, however, that even in low-sulfate treatments, the role of methanogenesis in the carbon cycle within the mats was still tiny when compared to that from sulfate reduction. They attributed this to the inhibition of methanogens in the surface of the mat by oxygen and the inability to access fermentation products in this zone. Still, removal of sulfate did yield a shift in the methanogen community structure. Smith et al. (2008) observed that long-term sulfate depletion of intact mats lead to the appearance of three novel clades of putatively H_2-consuming methanogens of the order *Methanomicrobiales*. These organisms were presumably present at very low levels in the native mats, and developed to detectable levels after enrichment under low-sulfate conditions. Such a shift in methanogen community structure suggests that sulfate depletion alleviates competitive pressure for H_2 from SRB in these mats. It is assumed that the *Methanomicrobiales* are at least in part responsible for the increased rates of methanogenesis observed.

Despite the increased detection of H_2-consuming *Methanomicrobiales* in the low-sulfate treatments, methylotrophic methanogens also appear to be stimulated by the low-sulfate conditions and may contribute substantially to the observed increase in methane production. Methylotrophic methanogens of the *Methanosarcinales* are mainly composed of isoprenoidal diethers such as archaeol, whereas members of the *Methanomicrobiales* contain acylic tetraether-bound biphytanes (Koga et al., 1998). In lipid analysis of the low-sulfate–high-salinity treatment (Table 1), while polar archaeol lipid increased more than twofold relative to the high-sulfate control, the tetraether associated biphytanyl actually decreased and was not detected in most depth intervals. Archaeol increased throughout the 0.5–15 mm depth; however, the 0.5–5 mm horizon was marked by a major increase in archaeols containing the regular C_{25} isoprenoid, sesterterpane (Jahnke et al., 2008). Though the biphytanyls were not detected in upper sediment horizons, the small amount of tetraether observed in the lowest 10–15 mm was distinct containing one unsaturated biphytanyl moiety with a retention time consistent with one cyclopentane ring. The lipid data are consistent with an increase in methylotrophic methanogens in the sulfate-depleted treatments. Similar to methylotrophic methanogens, which feed on dumped osmolytes (King, 1984; Oren, 1990), Smith et al. (2008) and Orphan et al. (2008) observed that methylotrophic methanogens closely related to the genus *Methanolobus*,

abundant in native sulfate mats, increased in microcosm experiments in which methylamines were added to sliced mat cores. Orphan et al. (2008) observed a more diverse community of methanogens in enrichment "microcosom" experiments (*Methanolobus*, *Methanohalophilus*, *Methanococcoides*), and an interesting vertical zonation of methanogens. *Methanolobus* were abundant below the oxygen chemocline, whereas members of the genus *Methanococcoides* were detected in the underlying sediments beneath the mat.

In microbial mats subjected to long-term sulfate depletion, acetate accumulates in the porewater (Kelley et al., 2006; Smith et al., 2008). Two processes may be considered to account for this (a) lower consumption of acetate by complete oxidizing sulfate-reducing microorganisms such as members of the routinely observed *Desulfobacteraceae* (e.g., Minz et al., 1999a; Fike et al., 2008) and (b) production of acetate by homoacetogenic bacteria (Kelley et al., 2006). Acetoclastic methanogens are absent in the high salinity native mats, most likely as a result of the low free energy yield from this metabolic reaction (Oren, 1999). However, even under conditions suitable for acetoclastic methanogens (e.g., low salinity, elevated acetate) in the mat manipulations, these organisms were never detected. As in the salinity manipulation studies with marine cyanobacteria, the native environmental conditions are most likely lethal to certain organisms, and even when these conditions ameliorate, they are unable to develop. The SRB community structure was impacted by the sulfate manipulations as well (Green et al., 2006). The effect was less salient, in part due to the tremendously high diversity of these organisms in the mat. SRB are a critical functional group in native microbial mats, and are found distributed throughout the aerobic zone, particularly the oxygenic chemocline, and in the deeper, perpetually anaerobic zone (e.g., Minz et al., 1999a, b). The low-sulfate treatment resulted in a major reduction in a lipid class, the alkylglycerolethers (Table 1), that have been associated with sulfate-reducing bacteria found in consortia with methanogens responsible in some sedimentary environments for anaerobic methane oxidation (ANMOX) (Hinrichs et al., 2000). Most of the etheralkyl moiety was recovered in the 0.5–2.5 mm horizon and the major component was equivalent to a 10-methylhexadecanyl moiety as obtained by Hinrichs et al. (2000). The methanogens associated with ANMOX have not been detected in the native mat or enrichments (Orphan et al., 2008) or by use of *mcr* primers in the analysis of the low-sulfate treatments (Smith et al., 2008). The role of the putative SRB group associated with the biosynthesis is unknown, but their relevance to a low-sulfate mat environment during the Archean eon is probably minimal.

In the surface of the native mats, bacteria from the Deltaproteobacterial family *Desulfobacteraceae* are particularly abundant (Teske et al., 1998; Minz et al., 1999a, b; Fike et al., 2008). These organisms are complete-oxidizing bacteria, capable of acetate utilization, and are localized to the surface of the mat (e.g., 0–5 mm; Green et al., 2006). Sulfate manipulations did not appear to immediately impact the community structure of sulfate-reducing microorganisms; depth was the primary determinant of the community composition (Green et al., 2006). After nearly 1 year of depleted sulfate conditions, the sulfate-reducing community in the

surface of the mat (0–5 mm), as assayed by DGGE and sequences analyses of the dissimilatory sulfite reductase gene (*dsrB*; Wagner et al., 1998), diverged significantly from other surface samples (Green et al., 2006). This appears to be due in part to the disappearance of key members of the *Desulfobacteraceae* in the lowered sulfate treatments (Green et al., 2006). In this regard, the significant increase (greater than threefold) in ester-linked 10-methylhexadecanoic acid (Table 1), normally considered a robust biomarker for this SRB group (White et al., 1997 and references therein), in the surface 0.5–2.5 mm layer appears inconsistent with molecular results. Unfortunately, DGGE analyses are not quantitative, and represent relative abundance within the taxonomic group targeted by the PCR primers used. Furthermore, the potential for the synthesis of the 10-methylhexadecanoate by some yet to be described group cannot be negated, particularly as major bacterial growth is documented by increased polar lipid in this upper mat horizon.

Unlike the surface, the deep mat (5–30 mm) appears more resilient to the sulfate depletion, and some of the detected SRB may represent organisms capable of alternate metabolic activity, such as syntrophy (e.g., Imachi et al., 2006). The sulfate-reducing community, while certainly affected by the depletion of sulfate, appears to be less sensitive to sulfate manipulations than might be expected. Bebout et al. (2004) suggested that the compact nature of the mats, with aerobic and anaerobic zones in such tight proximity, favors diffusion and may allow sulfate to be resupplied to the surface of the mats. This could, coupled with tight coupling of sulfate reduction and sulfide oxidation in the surface of the mats, account for the longer time required to observe shifts in the community structure. As Bebout et al. (2004) observed, even after the long-term depletion of sulfate in these mats, methanogenesis was insignificant in comparison with sulfate reduction as an anaerobic terminal electron acceptor used for the oxidation of organic carbon. Furthermore, in a recent review, Muyzer and Stams (2008) opined that low-sulfate conditions favor sulfate-reducing microorganisms capable of oxidizing hydrogen, lactate, and ethanol, but not organic acids such as acetate. Under low-sulfate conditions, syntrophic communities begin to degrade these compounds, and sulfate-reducing microorganisms persist by sulfate reduction coupled to hydrogen oxidation.

The experimental sulfate and salinity manipulations of hypersaline microbial mats have revealed intriguing effects on mat biogeochemistry. The major geochemical effects of these manipulations, primarily a result of sulfate depletion, appear to decrease sulfate reduction, increase methanogenesis, and increase pore-water acetate and methane concentrations. The major biological effects of these manipulations appear to be the development of molecular hydrogen consuming methanogens at depth and the disappearance of some complete-oxidizing sulfate-reducing bacteria from the surface. Salinity manipulations appear to have a more modest effect, and though lowered salinity does allow the development of additional cyanobacteria in the surface of the mat, the dominant cyanobacteria are unaffected. The effects of salinity are more visible in lipid analyses, in which the documentation of increased cyanobacterial alkane below the photic zone clearly points to the metabolic versatility and potential longevity of *Microcoleus* in the sedimentary

environment. A few caveats should be considered. The native high salinity, high-sulfate conditions appear to preclude the development of more mesophilic microorganisms even when the conditions are experimentally manipulated. Second, the continued presence of oxygen production in these mats limits the access of many anaerobic microorganisms from the labile organic carbon produced in the photic zone. These experimental caveats limit our ability to understand the biogeochemistry of ancient microbial mat ecosystems. We may be fairly certain, however, that the evolution of reduced gases from microbial mats in the early Proterozoic was substantially higher than in the modern mat equivalents.

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6. References

Abed, R.M.M., Kohls, K. and de Beer, D. (2007) Effect of salinity changes on the bacterial diversity, photosynthesis and oxygen consumption of cyanobacterial mats from an intertidal flat of the Arabian Gulf. Environ. Microbiol. **9**: 1384–1392.

Allwood, A.C., Walter, M.R., Kamber, B.S., Marshall, C.P. and Burch, I.W. (2006) Stromatolite reef from the Early Archean era of Australia. Nature **441**: 714–718.

Awramik, S.M., Margulis, L. and Barghoorn, E.S. (1976) Evolutionary processes in the formation of stromatolites, In: M.R. Walter (ed.) *Stromatolites*. Elsevier, Amsterdam, pp. 149–162.

Barbe, A., Grimalt, J.O., Pueyo, J.J. and Albaiges, J. (1990) Characterization of model evaporitic environments through the study of lipid components. Org. Geochem. **16**: 815–828.

Bateson, M.M. and Ward, D.M. (1988) Photoexcretion and fate of glycolate in a hot-spring cyanobacterial mat. Appl. Environ. Microbiol. **54**: 1738–1743.

Bebout, B.M., Carpenter, S.P., Des Marais, D.J., Discipulo, M., Embaye, T., Garcia-Pichel, F., Hoehler, T.M., Hogan, M., Jahnke, L.L., Keller, R.M., Miller, S.R., Prufert-Bebout, L.E., Raleigh, C., Rothrock, M. and Turk, K. (2002) Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating Earth's present and past field environments. Astrobiology **2**: 383–402.

Bebout, B.M., Hoehler, T.M., Thamdrup, B., Albert, D., Carpenter, S.P., Hogan, M., Turk, K. and Des Marais, D.J. (2004) Methane production by microbial mats under low sulphate concentrations. Geobiology **2**: 87–96.

Boon, J.J. and de Leeuw, J.W. (1987) Sedimentary hydrocarbons, biomarkers for early life, In: P. Fay and C. Van Baalen (eds.) *The Cyanobacteria*. Elsevier, Amsterdam, pp. 471–492.

Brocks, J.J. and Summons, R.E. (2005) Sedimentary hydrocarbons, biomarkers for early life, In: W.H. Schlesinger (ed.) *Biogeochemistry*. Elsevier, Amsterdam, pp. 63–116.

Canfield, D.E. (1998) A new model for Proterozoic ocean chemistry. Nature **396**: 450–453.

Canfield, D.E. and Des Marais, D.J. (1991) Aerobic sulfate reduction in microbial mats. Science **251**: 1471–1473.

Canfield, D.E., Habicht, K.S. and Thamdrup, B. (2000) The Archean sulfur cycle and the early history of atmospheric oxygen. Science **288**: 658–661.

Canfield, D.E., Rosing, M.T. and Bjerrum, C. (2006) Early anaerobic metabolisms. Philos. Trans. R. Soc. B. **361**: 1819–1834.

Cohen, Y. (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, pp. 22–36.

Cohen, Y., Krumbein, W.E. and Shilo, M. (1977) Solar Lake (Sinai). 2. Distribution of photosynthetic microorganisms and primary production. Limnol. Oceanogr. **22**: 609–620.

Cohen, Y., Jørgensen, B.B., Revsbech, N.P. and Poplawski, R. (1986) Adaptation to hydrogen-sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl. Environ. Microbiol. **51**: 398–407.

de Leeuw, J.W. and Largeau, C. (1993) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation, In: M.H. Engel and S.A. Macko (eds.) *Organic Geochemistry, Principles and Applications*. Plenum, New York, pp. 23–72.

Des Marais, D.J. (1997) Long-term evolution of the biogeochemical carbon cycle, In: J. Banfield and K. Nealson (eds.) *Geomicrobiology*. Mineralogical Society of America, Washington, DC, pp. 429–445.

Des Marais, D.J. (2003) Biogeochemistry of hypersaline microbial mats illustrates the dynamics of modern microbial ecosystems and the early evolution of the biosphere. Biol. Bull. **204**: 160–167.

de Wit, R., Falcon, L.I. and Charpy-Roubaud, C. (2005) Heterotrophic dinitrogen fixation (acetylene reduction) in phosphate-fertilised *Microcoleus chthonoplastes* microbial mat from the hypersaline inland lake 'la Salada de Chiprana' (NE Spain). Hydrobiologia **534**: 245–253.

Ehrlich, A. and Dor, I. (1985) Photosynthetic microorganisms of the Gavish Sabkha, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems: The Gavish Sabkha.* Springer, Berlin, pp. 296–321.

Feazel, L.M., Spear, J.R., Berger, A.B., Harris, J.K., Frank, D.N., Ley, R.E. and Pace N.R. (2008) Eucaryotic diversity in a hypersaline microbial mat. Appl. Environ. Microbiol. **74**: 329–332.

Fike, D.A., Gammon, C.L., Ziebis W. and Orphan, V.J. (2008) Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. ISME J. **2**: 749–759.

Fogel, G.B., Collins, C.R., Li, J. and Brunk, C.F. (1999) Prokaryotic genome size and SSU rDNA copy number: estimation of microbial relative abundance from a mixed population. Microb. Ecol. **38**: 93–113.

Fourçans, A., De Oteyza, G., Wieland, A., Sole, A., Diestra, E., van Bleijswijk, J., Grimalt, J.O., Kühl, M., Esteve, I., Muyzer, G., Caumette, P. and Duran, R. (2004) Characterization of functional bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue, France). FEMS Microbiol. Ecol. **51**: 55–70.

Fourçans, A., Sole, A., Diestra, E., Ranchou-Peyruse, A., Esteve, I., Caumette, P. and Duran, R. (2006) Vertical migration of phototrophic bacterial populations in a hypersaline microbial mat from Salins-de-Giraud (Camargue, France). FEMS Microbiol. Ecol. **57**: 367–377.

Fourçans, A., Ranchou-Peyruse, A., Caumette, P. and Duran, R. (2008) Molecular analysis of the spatio-temporal distribution of sulfate-reducing bacteria (SRB) in Camargue (France) hypersaline microbial mat. Microb. Ecol. **56**: 90–100.

Friedman, G.M. and Krumbein, W.E. (eds.) (1985) *Hypersaline Ecosystems. The Gavish Sabkha.* Springer, Berlin.

Fründ, C. and Cohen, Y. (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl. Environ. Microbiol. **58**: 70–77.

Garcia-Pichel, F., Prufert-Bebout, L. and Muyzer, G. (1996) Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. Appl. Environ. Microbiol. **62**: 3284–3291.

Garcia-Pichel, F., Kühl, M., Nübel, U. and Muyzer, G. (1999) Salinity-dependent limitation of photosynthesis and oxygen exchange in microbial mats. J. Phycol. **35**: 227–238.

Gerdes, G., Spira, K. and Dimentman, C. (1985) The fauna of the Gavish Sabkha and the Solar Lake – a comparative study, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems*, *The Gavish Sabkha*. Springer, Berlin, pp. 323–345.

Golubic, S. (1980) Halophily and halotolerance in cyanophytes. Origins Life Evol. B. **10**: 169–183.

Green, S.J., Minz, D., Stahl, D.A., Chen, Y., Lavin, C.A. and Cohen, Y. (1999) Filamentous cyanobacteria in a microbial mat. American Society of Microbiology Education Instructional Library. http://www.microbelibrary.org/Bacteria/details.asp?id=361&Lang=

Green, S.J., Smith, J.M., Hogan, M., Jahnke, L.L., Kelley, C., Bebout, B.M. and Bebout, L.E. (2006) Effect of salinity and sulfate manipulations on the community composition and structure of sulfate-reducing prokaryotes in a hypersaline microbial mat. American Society for Microbiology General Meeting.

Green, S.J., Blackford, C., Bucki, P., Jahnke, L.L. and Prufert-Bebout, L. (2008) A salinity and sulfate manipulation of hypersaline microbial mats reveals stasis in the cyanobacterial community structure. ISME J. **2**: 457-470.

Grimalt, J.O., de Wit, R., Texidor, P. and Albaiges, J. (1992) Lipid biogeochemistry of *Phormidium* and *Microcoleus* mats. Org. Geochem. **19**: 509–530.

Grotzinger, J.P. (1994) Trends in precambrian carbonate sediments and their implication for understanding evolution, In: S. Bengtson (ed.) *Early Life on Earth*. Columbia University Press, New York, pp. 245–258.

Grotzinger, J.P. and Kasting, J.F. (1993) New constraints on Precambrian ocean composition. J. Geol. **101**: 235–243.

Haines, T.H. (2001) Do sterols reduce proton and sodium leaks through lipid bilayers? Prog. Lipid Res. **40**: 299–324.

Hinrichs, K.U., Summons, R.E., Orphan, V., Sylva, S.P. and Hayes, J.M. (2000) Molecular and isotopic analysis of anaerobic methane-oxidizing communities in marine sediments. Org. Geochem. **31**: 1685–1701.

Hoehler, T.M., Bebout, B.M. and Des Marais, D.J. (2001) The role of microbial mats in the production of reduced gases on the early Earth. Nature **412**: 324–327.

Holland, H.D. (2006) The oxygenation of the atmosphere and oceans. Philos. Trans. R. Soc. B. **361**: 903–915.

Imachi, H., Sekiguchi, Y., Kamagata, Y., Loy, A., Qiu, Y.L., Hugenholtz, P., Kimura, N., Wagner, M., Ohashi, A. and Harada, H. (2006) Non-sulfate-reducing, syntrophic bacteria affiliated with *Desulfotomaculum* cluster I are widely distributed in methanogenic environments. Appl. Environ. Microbiol. **72**: 2080–2091.

Jahnke, L.L., Orphan, V.J., Embaye, T., Turk, K.A., Kubo, M.D., Summons, R.E. and Des Marais, D.J. (2008) Lipid biomarker and phylogenetic analyses to reveal archaeal biodiversity and distribution in hypersaline microbial mat and underlying sediment. Geobiology **6**: 394–410.

Javor, B.J. (1989) *Hypersaline Environments: Microbiology and Biogeochemistry.* Springer, Berlin.

Javor, B.J. and Castenholz, R.W. (1984) Productivity studies of microbial mats, Laguna Guerrero Negro, Mexico, In: Y. Cohen, R.W. Castenholz, and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites*. Alan R. Liss, New York, pp. 149–170.

Jonkers, H.M. and Abed, R.M.M. (2003) Identification of aerobic heterotrophic bacteria from the photic zone of a hypersaline microbial mat. Aquatic Microb. Ecol. **30**: 127–133.

Jørgensen, B.B., Cohen, Y. and Revsbech, N.P. (1988) Photosynthetic potential and light-dependent oxygen-consumption in a benthic cyanobacterial mat. Appl. Environ. Microbiol. **54**: 176–182.

Karsten, U. (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (cyanobacteria) from benthic microbial mats: response to salinity change. J. Phycol. **32**: 501–506.

Karsten, U. (2002) Effects of salinity and ultraviolet radiation on the concentration of mycosporine-like amino acids in various isolates of the benthic cyanobacterium *Microcoleus chthonoplastes*. Phycol. Res. **50**: 129–134.

Kelley, C.A., Prufert-Bebout, L.E. and Bebout, B.M. (2006) Changes in carbon cycling ascertained by stable isotopic analyses in a hypersaline microbial mat. J. Geophys. Res.-Biogeo. **111**: G04012.

Kenig, F., Damste, J.S.S., Dalen, A.C.K., Rijpstra, W.I.C., Huc, A.Y. and de Leeuw, J.W. (1995) Occurrence and origin of monomethylalkanes, dimethylalkanes, and trimethylalkanes in modern and Holocene cyanobacterial mats from Abu-Dhabi, United-Arab-Emirates. Geochim. Cosmochim. Acta **59**: 2999–3015.

King, G.M. (1984) Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. Appl. Environ. Microbiol. **48**: 719–725.

Knauth, L.P. (1998) Salinity history of the Earth's early ocean. Nature **395**: 554–555.

Koga, Y., Morii, H., Akagawa-Matsushita, M. and Ohga, I. (1998) Correlation of polar lipid composition with 16S rRNA phylogeny in methanogens. Further analysis of lipid component parts. Biosci. Biotech. Biochem. **62**: 230–236.

Krumbein, W.E., Cohen, Y. and Shilo, M. (1977) Solar Lake (Sinai). 4. Stromatolitic cyanobacterial mats. Limnol. Oceanogr. **22**: 635–656.

Kunin, V., Raes, J., Harris, J.K., Spear, J.R., Walker, J.J., Ivanova, N., Von Mering, C., Bebout, B.M., Pace, N.R., Bork, P. and Hugenholtz, P. (2008) Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol. Syst. Biol. **4**: 198.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

Liu, Z.Z., Lozupone, C., Hamady, M., Bushman, F.D. and Knight, R. (2007) Short pyrosequencing reads suffice for accurate microbial community analysis. Nucleic Acids Res. **35**: e120**.**

Lodders, N., Stackebrandt, E. and Nübel, U. (2005) Frequent genetic recombination in natural populations of the marine cyanobacterium *Microcoleus chthonoplastes*. Environ. Microbiol. **7**: 434–442.

Lozupone, C.A. and Knight, R. (2007) Global patterns in bacterial diversity. Proc. Natl. Acad. Sci. USA **104**: 11436–11440.

Martinez-Alonso, M., Mir, J., Caumette, P., Gaju, N., Guerrero, R. and Esteve, I. (2004) Distribution of phototrophic populations and primary production in a microbial mat from the Ebro Delta, Spain. Int. Microbiol. **7**: 19–25.

Minz, D., Fishbain, S., Green, S.J., Muyzer, G., Cohen, Y., Rittmann, B.E. and Stahl, D.A. (1999a) Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl. Environ. Microbiol. **65**: 4659–4665.

Minz, D., Flax, J.L., Green, S.J., Muyzer, G., Cohen, Y., Wagner, M., Rittmann, B.E. and Stahl, D.A. (1999b) Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl. Environ. Microbiol. **65**: 4666–4671.

Moezelaar, R., Bijvank, S.M. and Stal, L.J. (1996) Fermentation and sulfur reduction in the mat-building cyanobacterium *Microcoleus chthonoplastes*. Appl. Environ. Microbiol. **62**: 1752–1758.

Monteoliva-Sanchez, M., Ramos-Cormenzana, A. and Russell, N.J. (1993) The effect of salinity and compatible solutes on the biosynthesis of cyclopropane fatty-acids in *Pseudomonas halosaccharolytica*. J. Gen. Microbiol. **139**: 1877–1884.

Muyzer, G. and Stams, A.J.M. (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat. Rev. Microbiol. **6**: 441–454.

Neilan, B.A., Burns, B.P., Relman, D.A. and Lowe, D.R. (2002) Molecular identification of cyanobacteria associated with stromatolites from distinct geographical locations. Astrobiology **2**: 271–280.

Nübel, U., Garcia-Pichel, F. and Muyzer, G. (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microbiol. **63**: 3327–3332.

Nübel, U., Garcia-Pichel, F., Kühl, M. and Muyzer, G. (1999) Spatial scale and the diversity of benthic cyanobacteria and diatoms in a salina. Hydrobiologia **401**: 199–206.

Nübel, U., Garcia-Pichel, F., Clavero, E. and Muyzer, G. (2000) Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ. Microbiol. **2**: 217–226.

Nübel, U., Bateson, M.M., Madigan, M.T., Kühl, M. and Ward, D.M. (2001) Diversity and distribution in hypersaline microbial mats of bacteria related to *Chloroflexus* spp. Appl. Environ. Microbiol. **67**: 4365–4371.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004) Comparison of diazotroph community structure in *Lyngbya* sp. and *Microcoleus chthonoplastes* dominated microbial mats from Guerrero Negro, Baja, Mexico. FEMS Microbiol. Ecol. **47**: 305–318.

Oren, A. (1990) Formation and breakdown of glycine betaine and trimethylamine in hypersaline environments. Antonie van Leeuwenhoek **58**: 291–298.

Oren, A. (1999) Bioenergetic aspects of halophilism. Microbiol. Mol. Biol. Rev. **63**: 334–348.

Oren, A. (2002) *Halophilic Microorganisms and Their Environments*. Kluwer, Dordrecht, The Netherlands.

Orphan, V.J., Jahnke, L.L., Embaye, T., Turk, K.A., Pernthaler, A., Summons, R.E. and Des Marais, D.J. (2008) Characterization and spatial distribution of methanogens and methanogenic biosignatures in hypersaline microbial mats of Baja California. Geobiology **6**: 376–393.

Palmisano, A.C., Cronin, S.E., D'Amelio, E.D., Munoz, E. and Des Marais, D.J. (1989) Distribution and survival of lipophilic pigments in a laminated microbial mat community near Guerrero Negro, Mexico, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, pp. 138–152.

Pierson, B.K. (1994) The emergence, diversification, and role of photosynthetic eubacteria, In: S. Bengtson (ed.) *Early Life on Earth*. Columbia University Press, New York, pp. 159–180.

Pierson, B.K., Bauld, J., Castenholz, R.W., D'Amelio, E.D., Des Marais, D.J., Farmer, J.D., Grotzinger, J.P., Jørgensen, B.B., Nelson, D.C., Palmisano, A.C., Schopf, J.W., Summons, R.E., Walter, M.R. and Ward, D.M. (1992) modern mat-building microbial communities: a key to the interpretation of proterozoic stromatolitic communities, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere, A Multidisciplinary Study*. Cambridge University Press, Cambridge, pp. 245–340.

Prufert-Bebout, L. and Garcia-Pichel, F. (1994) Field and cultivated *Microcoleus chthonoplastes*: the search for clues to its prevalence in marine microbial mats, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats*. Springer, Berlin, pp. 111–116.

Risatti, J.B., Capman, W.C. and Stahl, D.A. (1994) Community structure of a microbial mat – the phylogenetic dimension. Proc. Natl. Acad. Sci. USA **91**: 10173–10177.

Robertson, C.E., Spear, J.R., Harris, J.K. and Pace, N.R. (2009) Diversity and stratification of Archaea in a hypersaline microbial mat. Appl. Environ. Microbiol. **75**: 1801–1810.

Robinson, N. and Eglinton, G. (1990) Lipid chemistry of Icelandic hot-spring microbial mats. Org. Geochem. **15**: 291–298.

Ronaghi, M., Uhlen, M. and Nyren, P. (1998) A sequencing method based on real-time pyrophosphate. Science **281**: 363–365.

Rothrock, M.J. and Garcia-Pichel, F. (2005) Microbial diversity of benthic mats along a tidal desiccation gradient. Environ. Microbiol. **7**: 593–601.

Shiea, J., Brassell, S.C. and Ward, D.M. (1990) Midchain branched monomethyl and dfimethyl alkanes in hot-spring cyanobacterial mats – a direct biogenic source for branched alkanes in ancient sediments. Org. Geochem. **15**: 223–231.

Smith, J.M., Green, S.J., Kelley, C.A., Prufert-Bebout, L. and Bebout, B.M. (2008) Shifts in methanogen community structure and function associated with long-term manipulation of sulfate and salinity in a hypersaline microbial mat. Environ. Microbiol. **10**: 386–394.

Spear, J.R., Ley, R.E., Berger, A.B. and Pace, N.R. (2003) Complexity in natural microbial ecosystems: the Guerrero Negro experience. Biol. Bull. **204**: 168–173.

Stal, L.J., van Gemerden, H., and Krumbein, W.E. (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol. Ecol. **31**: 111–125.

Sugaya, N., Sato, M., Murakami, H., Imaizumi, A., Aburatani, S. and Horimoto, K. (2004) Causes for the large genome size in a cyanobacterium *Anabaena* sp. PCC7120. Genome Inform **15**: 229–238.

Summons, R.E. and Walter, M.R. (1990) Molecular fossils and microfossils of prokaryotes and protists from Proterozoic sediments. Am. J. Sci. **290A**: 212–244.

Tandeau de Marsac, N., Ferriera, S., Johnson, J., Kravitz, S., Beeson, K., Sutton, G., Rogers, Y.-H., Friedman, R., Frazier, M. and Venter, J.C. 2007. *Microcoleus chthonoplastes* PCC7420 draft genome sequence. http://www.moorefoundation.org/microgenome/detail.aspx?id=146

Teske, A., Ramsing, N.B., Habicht, K., Fukui, M., Küver, J., Jørgensen, B.B. and Cohen, Y. (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. **64**: 2943–2951.

Tolbert, N.E. (1980) Photorespiration, In: D.D. Davis (ed.) *The Biochemistry of Plants,* Vol. 2. Academic, New York, pp. 487–523.

van Gemerden, H. (1993) Microbial mats: a joint venture. Mar. Geol. **113**: 3–25.

Villanueva, L., Navarrete, A., Urmeneta, J., White, D.C. and Guerrero, R. (2007) Analysis of diurnal and vertical microbial diversity of a hypersaline microbial mat. Arch. Microbiol. **188**: 137–146.

Wagner, M., Roger, A.J., Flax, J.L., Brusseau, G.A. and Stahl, D.A. (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J. Bacteriol. **180**: 2975–2982.

Wang, Q., Garrity, G.M., Tiedje, J.M. and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. **73**: 5261–5267.

Whale, G.F. and Walsby, A.E. (1984) Motility of the cyanobacterium *Microcoleus chthonoplastes* in mud. Brit. Phycol. J. **19**: 117–123.

White, D.C., Pinkart, H.C. and Ringelberg, D.B. (1997) Biomass measurement: biochemical approaches, In: C.J. Hurst (ed.) *Manual of Environmenal Microbiology*. American Society for Microbiology, Washington, DC, pp. 91–101.

Yannarell, A.C., Steppe, T.F. and Paerl, H.W. (2006) Genetic variance in the composition of two functional groups (diazotrophs and cyanobacteria) from a hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 1207–1217.

Biodata of **Joachim Reitner**, author of ***"Architecture of Archaeal-Dominated Microbial Mats from Cold Seeps in the Black Sea (Dnjepr Canyon, Lower Crimean Shelf)"***

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ARCHITECTURE OF ARCHAEAL-DOMINATED MICROBIAL MATS FROM COLD SEEPS IN THE BLACK SEA (DNJEPR CANYON, LOWER CRIMEAN SHELF)

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1. Introduction

The Black Sea is an excellent area to study cold methane seeps in various water depths and environments. The upper part of the water column is oxygenated down to a depth of ca. 120 m and is anaerobic below. Abundant small seepage structures within the oxygenated environment are recognizable by dense white mats of sulfide-oxidizing bacteria, within the anaerobic water column forest-like carbonate tower fields (e.g., GHOSTDABS-field), and huge mud volcanoes are common within deeper areas of the Black Sea (Greinert et al., 2006; Michaelis et al., 2002; Reitner et al., 2005a, b). The cold seeps with large methane-related carbonate build-ups in the area of Dnjepr Canyon were discovered in 1989 (Pimenov et al., 1997). For the first time, samples of carbonates were taken in the years 1993–1994 (Peckmann et al., 2001; Thiel et al., 2001). These important observations led to the approval of a big project cooperation of the German Ministry of Education and Research (BMBF): *GHOSTDABS: Gas Hydrates, Occurrence, Stability, Transformation, Dynamic and Biology in the Black Sea* within the framework of the "Geotechnologien Programm" of the BMBF and the German Research Council (DFG). Extensive investigations were made in the years 2001 and 2004 at the cold seeps sites of the so-called GHOSTDABS-field using the Russian research vessel "Professor Logachev," the German research vessel "Poseidon," and the German research submarine "Jago." The central objects of our study were the formation of methane-related authigenic carbonates and the associated microbial communities (Michaelis et al., 2002, Reitner et al., 2005a, b; Heller et al., 2008, Wrede et al., 2008). The aims of the investigations are a better understanding of the process of *Anaerobic Oxidation of Methane* (AOM) and the role of AOM communities regulating the processes of the microbial biomineralization.

There is a consensus that AOM can be mediated by consortia of methane-oxidizing Archaea and sulfate-reducing bacteria (SRB), although the precise pathway(s) and the roles of the respective microbial components are not yet fully elucidated (Valentine, 2002 for review; Nauhaus et al., 2004; Heller et al., 2008).

AOM increases the carbonate alkalinity and sulfide formation according to the overall reactions:

1. $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$
2. $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$
3. $CH_4 + 2H_2O \rightarrow CO_2 + 4H_2$
4. $SO_4^{2-} + 4H_2 \rightarrow S^{2-} + 4H_2O$

Therefore, methane-related C-isotopic light calcium carbonate deposits are typically found as remnants of contemporary and ancient methane seepage (e.g., Peckmann and Thiel, 2004; Peckmann et al., 1999; Reitner et al., 2005a, b). A large part of the bicarbonate ions is carrying the light ^{12}C carbon from methane and a minor part is carrying the heavier ^{13}C carbon isotope signal from the open water column. Mean $\delta^{13}C$ values from methane-related carbonates from the studied sites are between −18‰ and −41‰, respectively (Reitner et al., 2005b).

2. Materials and Methods

Samples were collected from a methane seep area on the lower Crimean shelf during a German−Russian−Ukrainian expedition (GHOSTDABS) in 2001. The samples were collected from the GHOSTDABS-field (44°46.510'N, 31°59.570'E) at about 230 m water depth. For sampling, the manned submersible Jago was used. Samples were fixed with 4% buffered formaldehyde and stored in 70% ethanol or in 50% PBS (phosphate buffered saline). Samples for transmission electron microscopy (TEM), conventional scanning electron microscopy (SEM), and field emission SEM (FE-SEM) were fixed with 4% buffered glutardialdehyde and postfixed with 2% OsO_4. To avoid drying artifacts, glutardialdehyde-fixed samples were dried with HMDS (hexamethyl-disilazane; Polysciences, USA). Thin sections of microbial mats were cut using a Leica hardpart microtome. Image stacks with a Z-spacing of 0.5 or 0.25 µm were obtained using a piezomover (Physik Instrumente GmbH & Co, Waldbronn) attached to a Zeiss Axioplan microscope equipped with a Zeiss "Plan-Apochromat" 63x-objective (NA = 1.4). Detailed methods are described in Reitner et al. (2005a). Paraffin sections of decalcified samples were stained with various histochemicals, namely toluidine blue O (RNA, oligonucleotides, proteins, and glycosaminoglycans) and 4′,6-diamidino-2-phenylindole (DAPI; double-stranded DNA). For details of the staining procedures, see Hoffmann et al. (2003), Manz et al. (2000), and Romeis (1989). Prior to fluorescence *in situ* hybridization (FISH, explanation in Manz et al., 2000), the samples were stained with DAPI to check the cell numbers and vitality of the microbial communities. The probes were purchased 5′-labelled with the indocarbocyanine dye Cy3 (Amersham Pharmacia Biotech) and Oregon Green (Molecular Probes) from Biometra (Göttingen, Germany), Metabion (Planegg-Martinsried, Germany), and were stored in TE buffer (10 mM Tris/1 mM EDTA, pH 7.5) at −20°C. To minimize mismatching of the oligonucleotide probes, several sections of each mat sample were hybridized using varied buffer stringencies (formamide concentrations) from 35% to 60% at set hybridization and washing temperatures. When using bacterial together

with Archaeal probes, best results were obtained with a hybridization solution of 40% stringency. The following 16S rRNA oligonucleotide probes for fluorescence *in situ* hybridization were applied: EUB338 (Amann et al., 1990a), ARCH915 (Amann et al., 1990b), DSS658 (Manz et al., 1998), ANME-1 (Hinrichs et al., 1999), EelMS932 (ANME-2, Boetius et al., 2000), and Cren499 (Burggraf et al., 1994). Uncoated samples were investigated by FE-SEM using a LEO 1530 Gemini instrument at less than 1 kV. Energy dispersive X-ray spectrometry (EDX) was performed on carbon-coated samples using the same instrument operated at 15 kV. TEM investigations were carried out with a Zeiss EM 10 instrument at 60–80 kV. Carbonate samples for stable carbon isotope analysis were prepared using a hand-held microdrill. CO_2 was generated by reaction with orthophosphoric acid and analyzed with a Finnigan MAT 252 mass spectrometer at the University of Erlangen (Dr. M. Joachimski). The $\delta^{13}C$ values are reported relative to the V-PDB standard (precision of values is ±0.05‰). Cathodoluminescence investigations were carried out with a Citl 8200 MK3A cold cathode mounted on a Zeiss Axiolab microscope. Mapping of key elements (Mg, Sr, Mn) was carried out with a JEOL JXA 8900RL electron microprobe housed in the Center for Geosciences at the University of Göttingen (Department of Geochemistry).

3. Principal Build-up and Community Structure of the Archaeal-Dominated AOM Microbial Mats

Depending on the water depth, different microbial communities at the cold seep sites can be distinguished. Within the oxic zone till a water depth of about 130 m, cold seeps are characterized by complex white mats of *Beggiatoa*/*Thioploca* (sulfide oxidizing, thread-like sulfur bacteria) on top of the seep sites. The prominent characteristic of these zones is that no growing formation of methane carbonate is found in the free water column. In the anoxic zones below 130 m water depth, carbonates concretions are often precipitated within the sediment in areas of microseepage of methane. But on spots of enormous methane discharges (macroseepage), it leads to the formation of meter-sized tower-like build-ups of methane-related carbonates (Michaelis et al., 2002; Reitner et al., 2005b) (Fig. 1).

Principally, the microbial mats have a very characteristic community structure and it is possible to distinguish three general layers, an outer black layer, a central orange to pink layer, and an innermost green layer. The innermost green layer is only present within the central portions of mature carbonate towers and is not a topic of this presentation.

4. The External Black Microbial Mat

The outer external zone is black to gray. At the interface to the ambient water, this area is intensive black. To the inside, the color changes from light gray to colorless depending on the rates of the sulfate reduction in this zone. The boundary to the central orange layer is sharp and not diffuse.

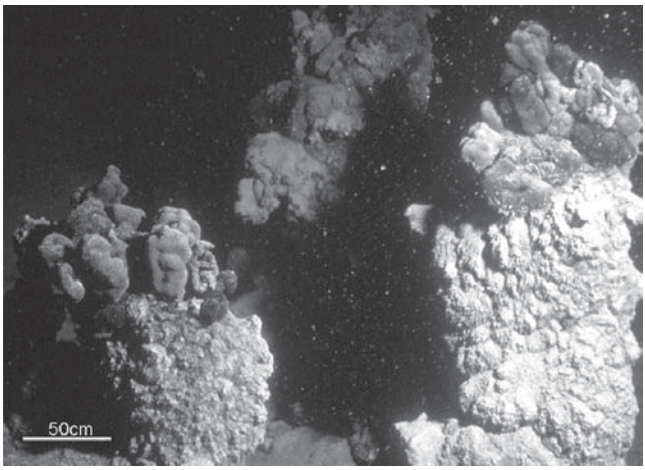

Figure 1. Tower-like carbonate build-ups of the so-called GHOSTDABS field (44°46.510'N; 31°59.570'E) in 230 m water depth. The carbonate towers reach a height up to 4 m and are formed through massive methane blow-outs.

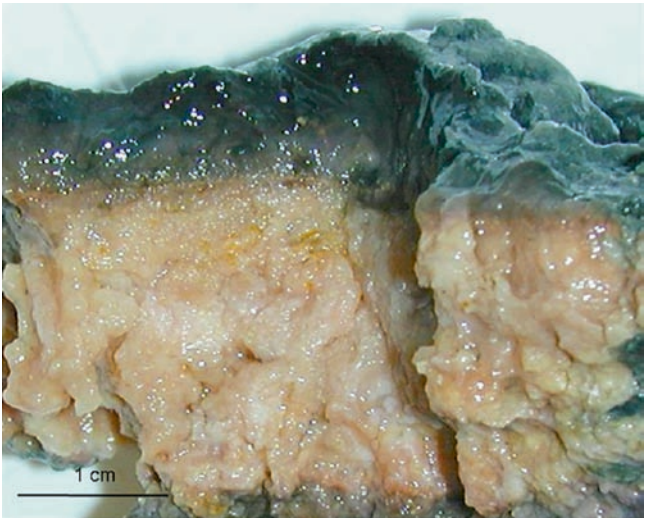

Figure 2. The entire microbial mat structure of a mature carbonate tower. The outer black mat is formed by ANME2 and greigite-bearing sulfate-reducing bacteria (GSRB). The high amounts of iron sulfide colors this mat part gray to black. The inner portion is formed by the orange/pink mat, which is dominated by ANME1.

The black zone is mostly formed by means of clearly delimitated colonies of a size of some 100 μm of ANME2 Archaea (*ANME*: *an*aerobic *me*thane oxidizers) (Figs. 2 and 3a), which are closely related to the methanogenic taxon

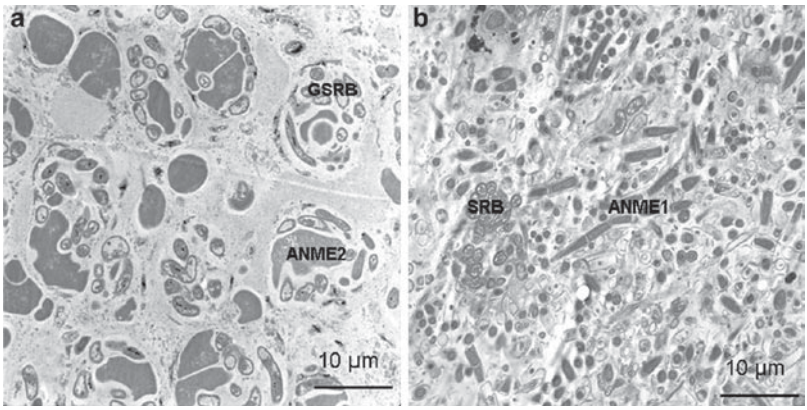

Figure 3. (**a**) Tight mutualistic relationship between the ANME2 Archea and the greigite-bearing sulfate-reducing bacteria (GSRB) of the black microbial mat. TEM micrograph; (**b**) Cylindrical ANME1 Archaea (Euryarchaeota) of the orange/pink zone that form multicellular aggregates in robust sheaths. A connection to sulfate-reducing bacteria (SRB) is visible, but does not show the close symbiotic relationship, like in the external black zone. TEM micrograph.

Methanosarcinales that are tightly surrounded by Deltaproteobacteria of the group *Desulfosarcina-Desulfococcus* (DSS) (Fig. 3a). These Deltaproteobacteria form intracellular magnetosomes in which greigite is formed ("G-SRB"). Greigite ($Fe^3Fe^2{}_3S_4$) is an unstable magnetic Fe-sulfide that in early diagenesis stage is transformed into pyrite (FeS_2). This kind of mutualized relation between ANME2 and G-SRB has been found only in this AOM-community until now. This also explains the different intensities of the black or gray coloration in this system. Besides, the greigite magnetosomes are located vesicles with elementary sulfur and PHA (polyhydroxyalkanoate) in the G-SRB cells (Reitner et al., 2005a). The latter is an important reservoir and can be very well stained with the lipophile Nile-blue A. As a biogeochemical mechanism to the generation of elementary sulfur, the oxidation of H_2S via Fe^{3+} is possible. Fe^{2+} in this process can be fixed via formation of greigite in the magnetosome chain or in pyrite. The reduction of iron is supplying a substantial bigger gain of energy as the sulfate reduction itself though the concentration of Fe^{3+} in the environment and in anoxic area is very low. Some of the marine bacteria synthesize extracellular enzymes when having iron deficiency, the so-called siderophores, which some of them form as micelles to bind the reactive iron in the extracellular environment (Martinez et al., 2003). Laboratory experiments with *Marinobacter* sp. showed that micelles can be reorganized into abundant siderophore marinobactin vesicles via iron absorption that are transported back to the cells (Martinez et al., 2000). It is remarkable that vesicle-like, globular structures often appear in the microbial mats. The vesicles have a diameter of 20–100 µm and are surrounded by a lipid double-layer and correspond to the described siderophore marinobactin vesicles as far as the size and structure are concerned. In fact, the G-SRB shows again and again run-offs

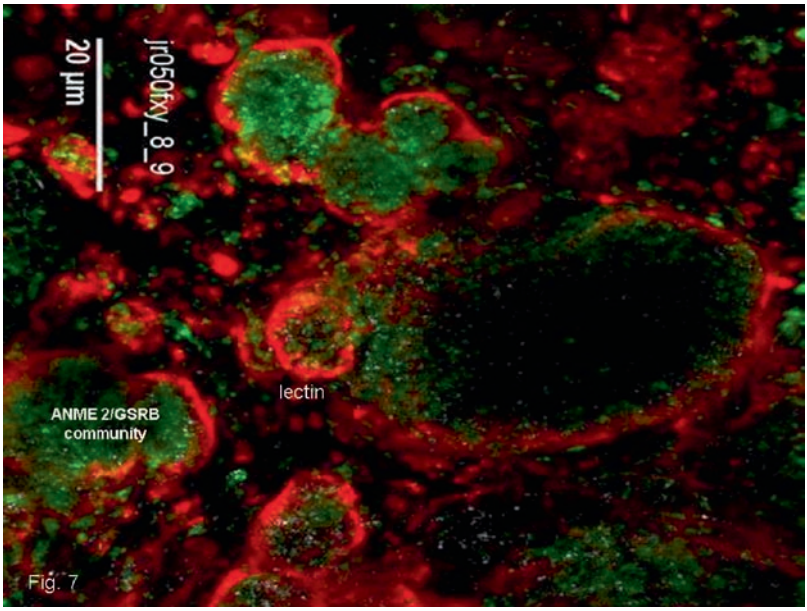

Figure 4. Staining of the black microbial mat with the lectin AAL-A568 (stain cy5 + Sytox green for DNA). AAL binds preferentially to fucose-linked (α-1, 6) *N*-acetylglucosamine, or to fucose-linked (α-1, 3) *N*-acetyllactosamine (*Aleuria aurantia* – Alexa 568; Vector Lab & Molecular Probes) (Micrograph made by Dr. Thomas Neu, UFZ-Magdeburg). All observed mineralization events happen within this lectin enrichment.

in the cellular wall in contact with the globular bodies that obviously show stages of incorporation into the cells. Within the black layer, various bacterial and archaeal biomarkers were extracted. Very common are nonisoprenoidal glycerol diethers, various linear and methyl-branched fatty acids, and sometimes the hydrocarbon *N*-tricosene, which are all biomarkers of sulfate-reducing bacteria (SRB) (Peckmann and Thiel, 2004). Besides the bacterial biomarkers, archaeal isoprenoids are also common. Common are crocetane/crocetene, phytane/phytene, pentamethylicosane, and hydroxyarcheol, which are characteristic for ANME2 (Leefmann et al., 2008). All these compounds are isotopic and very light with values of $\delta^{13}C$ −120‰. The ANME2+G-SRB colonies are often surrounded by dense laminated sheaths of lectins, which strongly separate the ANME2+G-SRB colonies from the ambient environment (Fig. 4).

5. The Internal Orange/Pink Microbial Mat

The internal orange to pink zone is built through another AOM-system (Figs. 2 and 3). The dominating organism is a spherical Euryarchaeon taxon that belongs to the ANME1 group (Fig. 3b). The sheaths of these organisms

consist in a resistant biopolymer. In some areas, concentration of empty sheets covers more than 80% of the whole volume of the ANME1 zone. A TEM survey shows single cells with a diameter of 0.6–1 μm and variable lengths up to 3 μm. Single cells are organized in multicellular filaments, in which the individual cells are separated by means of conspicuous invaginations. SRB of the DSS group also exist, but not as G-SRB and not in close integration as it has been observed in the microbial colonies of the external black zone. Remarkable are the findings that the spherical form cells ANME1 contain areas with internal membrane piles. These structures are not known from Archaea. The methanotrophic bacteria, e.g., *Methylococcus*, localized similar piles of intracytoplasmatic membranes, the C1-metabolism. Chistoserdova et al. (1998) reported that between methanogen Archaea and aerobic, methanotrophic bacteria, a mutual gene code does exist for the C1 transfer enzyme. Therefore, we assume that the observed membranes of the ANME1-Archea from the Black Sea are functionally in relation with their methane metabolism. The immunological localization of the coenzyme M reductase shows a clear spatial resolution within the ANME cells and strongly supports this assumption (Heller et al., 2008). If a green layer is present, the boundary to the orange layer is also sharp and distinctive.

6. Architecture and Structure of Cold-Seep-Related Carbonate Towers

The GHOSTDABS-field is famous for its large carbonate tower "forests" (Michaelis et al., 2002; Reitner et al., 2005a) (Fig. 1). These towers are up to 4–5 m high and exhibit a knobby structure. Bottom currents create often a moat surrounding the bases of the carbonate build-ups. In places, the sediments were completely eroded from the roots of the towers, excavating large disc-shaped basal plates that anchor the towers in the sediment. Very remarkable is the knobby structure of the towers, which is closely related to the entire growth mode of the build-ups. The knobby structure is a result of lithified microbial mat spheres with a mean diameter of 3–5 cm. The entire towers are built up by these spheres and never show a laminated growth mode like real tries. The central part of the tower, however, exhibits an irregular chimney-like opening, which is probably caused by methane-rich fluids, which are moving through the tower supplying the microbial spheres with gas and further nutrients. The initial growth of the towers starts with spherical, centimeter-sized microbial mat constructions displaying a unique architecture.

7. Description of the Microbial Mat Spheres: The "Sponge Model"

The spheres initially exhibit a soft, jelly-like consistency, and are easily compressible. At first, microbial mats are formed on the sediment at the outflow spots of methane. The mats are being arched up through the gas pressure (Figs. 5 and 6).

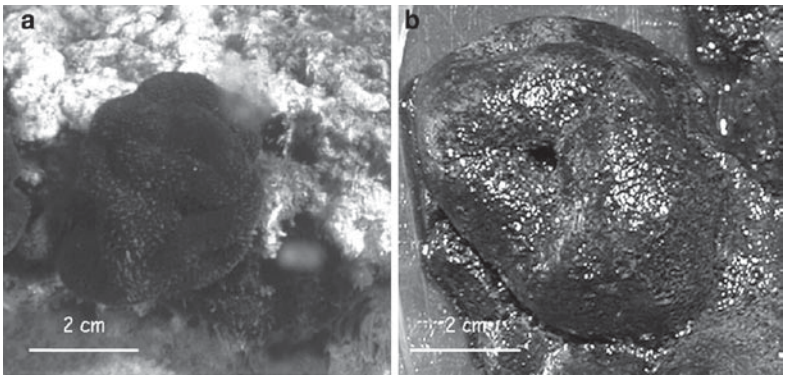

Figure 5. (**a, b**) Initial microbial mat spheres with central opening, which resembles a sponge osculum. Visible is the external black microbial mat. In Fig. 5a, a microbial mat sphere is growing on the sediment surface over a methane release spot. The freshly collected microbial mat sphere exhibits a central opening for gas and fluid release.

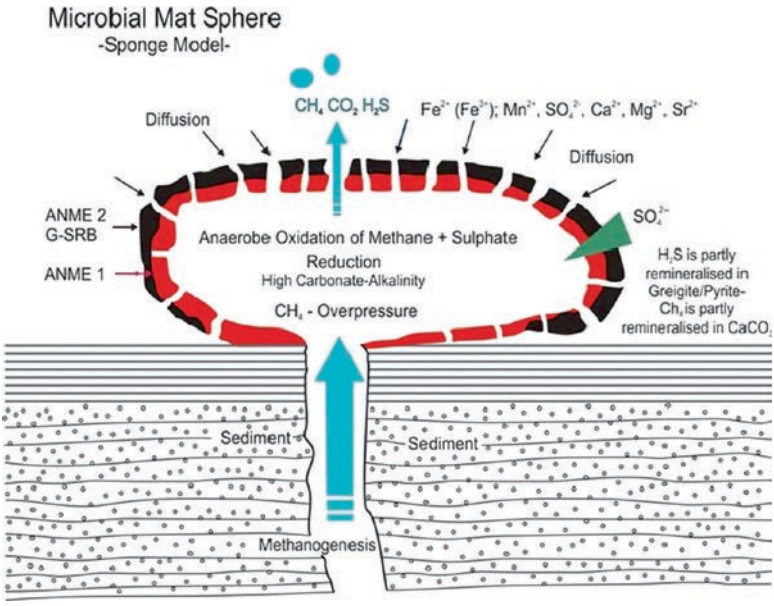

Figure 6. Functional interpretation of a GHOSTDABS-field cold seep microbial mat sphere. Methane is only partly metabolized within the sphere.

Owing to this bulge, spherical form structures arise that are formed through microbial consortia of centimeter thickness. The spherical structures are being filled up with methane, which will be for the larger part metabolized. The nonoxidized methane will be emitted to the water column through a few openings (Fig. 6).

The function of this reactor works as long as the exopolymeric substances (EPS) of the microbial system calcify strongly, so that exchange processes like diffusion are no longer possible. New spheres are produced via budding and they will calcify again through the AOM-process. This process is repeated often and in that way the tower-like build-ups are growing. The entire process resembles strongly the living mode of sponges, especially sphinctozoan heavy calcified sponges (Reitner et al., 2001).

8. Carbonate and Fe-Sulfide Phases

Within the black microbial mats dominated by ANME2/G-SRB community, high-Mg calcite (12–18 mol% $MgCO_3$) is formed, which is coprecipitated with high amounts of Mn. This high Mn amount causes a very strong red-orange cathodoluminescence behavior and, therefore, it is easy to distinguish the very early precipitates within microbial EPS substances. Primary oval to dumbbell-shaped crystal aggregates result from high-Mg calcites of a size of about 100 μm, which are being formed within the EPS of the biofilms. The EPS is rich in lectins and obviously these sugar-binding proteins play a major role in the precipitation of the high-Mg calcite (Fig. 7). Within the orange microbial mat, Sr-rich (ca. 10,000 ppm) nonluminescent aragonite is precipitated. The early formed crystal aggregates are also dumbbell-shaped. However, the EPS has a different chemical

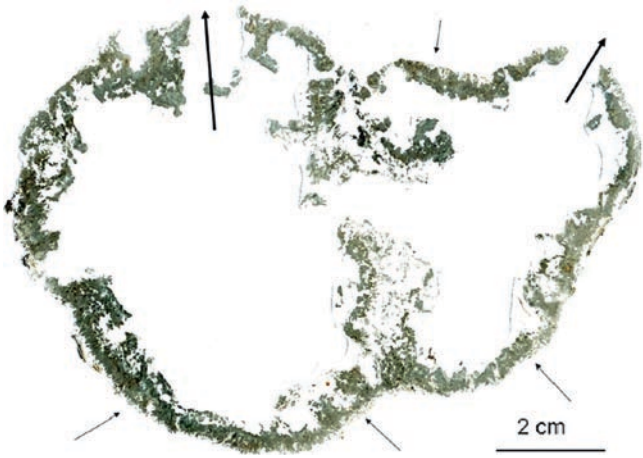

Figure 7. Calcified basis modules of a microbial mat sphere that already show an intensive calcification of the EPS structures. The dark colors are related to Fe-sulfidic material. The large arrows exhibit the outflow direction, the small ones the inflow/diffusion direction. This pattern resembles a sphinctozoan sponge flow pattern.

structure and is dominated mainly by high amounts of polysaccharide and peptides. The mineralogy of the primary mineralization is controlled by the kind of EPS and is therefore dependent on the microorganisms that are composed by the EPS. All carbonates that were analyzed are extremely depleted in ^{13}C. In the carbonate towers, the lowest $\delta^{13}C$ values were observed: −25 to −41‰ for high-Mg calcite phases and −20 to −30‰ for aragonitic phases. The measured $^{87}Sr/^{86}Sr$ relations of about 0.7092 show a seawater signal.

The high-Mg calcite crystal nuclei are formed in the immediate environment of authigenically formed Fe-sulfides, which surround the external sheaths of the ANME2/G-SRB microbial colonies, which are formed by lectins. The Fe-sulfides are mostly greigite crystals classified in the so-called framboid structures. After collapsing with the G-SRB cells, the small magnets are released in the EPS. Owing to their ferrimagnetism, it comes afterward to framboid formation. Owing to further reaction with H_2S, which is common within the ambient environment, the greigite framboids are transformed rapidly into pyrite framboids. Pyrite framboids encounter frequently in sedimentology and are an important feature of anaerobic ecosystems. This kind of coupled mineralization can only be observed in ANME2/G-SRB microbial colonies. In the orange-colored ANME1 zone, no formation of Fe-sulfides was observed.

The observed microbial systems are highly complex and in some parts really well-organized communities, of which their composition locally varies systematically, probably because of organic and biogeochemical gradients. However, only the ANME2/G-SRB microbial consortia and partly the ANME1 community are immediately engaged in the formation of methane carbonate. The interaction between sulfate reduction and iron oxidation during the formation of greigite magnetosomes and the anaerobic methane oxidation obviously provide the necessary high-carbonate alkalinity for the formation of methane carbonates.

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10. References

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R. and Stahl, D.A. (1990a) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. **56**: 1919–1925.

Amann, R.I., Krumholz, L. and Stahl, D.A. (1990b) Fluorescent – oligonucleotide probing of whole cells for determinative phylogenetic and environmental studies in microbiology. J. Bacteriol. **172**: 762–770.

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B.B., Witte, U. and Pfannkuche, O. (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature **407**: 623–626.

Burggraf, S., Mayer, T., Amann, R., Schadhauser, S., Woese, C. and Stetter, K. (1994) Identifying members of the domain Archaea with rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. **60**: 3112–3119.

Chistoserdova, L., Vorholt, J.A., Thauer, R.K. and Lidstrom, M.E. (1998) Transfer enzymes and coenzymes linking methylotrophic bacteria and methanogenic Archaea. Science **281**: 99–102.

Greinert, J., Artemov. Y., Egorov. V., De Batist. M. and McGinnis, D. (2006) 1300-m-high rising bubbles from mud volcanoes at 2080 m in the Black Sea: hydroacoustic characteristics and temporal variability. Earth Plant. Sci. Lett. **244**: 1–15.

Heller, C., Hoppert, M. and Reitner, J. (2008) Immunological localization of coenzyme M reductase in anaerobic methane-oxidizing Archaea of ANME 1 and ANME 2 type. Geomicrobiol. J. **25**: 1–8.

Hinrichs, K.-U., Hayes, J.M., Sylva, S.P., Brewer, P.G. and DeLong, E.F. (1999) Methane-consuming archaebacteria in marine sediments. Nature **398**: 802–805.

Hoffmann, F., Janussen, D., Dröse, W., Arp, G. and Reitner, J. (2003) Histological investigation of organisms with hard skeletons: a case study of siliceous sponges. Biotech. Histochem. **78**: 191–199.

Leefmann, T., Bauermeister, J., Kronz, A., Liebetrau, V., Reitner, J. and Thiel, V. (2008) Miniaturized biosignature analysis reveals implications for the formation of cold seep carbonates at Hydrate Ridge (off Oregon, USA). Biogeosciences **5**: 731–738.

Manz, W., Arp, G., Schumann-Kindel, G., Szewzyk, U. and Reitner, J. (2000) Widefield deconvolution epifluorescence microscopy combined with fluorescence in situ hybridization reveals the spatial arrangement of bacteria in sponge tissue. J. Microbiol. Meth. **40**: 125–134.

Manz, W., Eisenbrecher, M., Neu, T.R. and Szewzyk, U. (1998) Abundance and spatial organization of gram negative sulfate-reducing bacteria in activated sludge investigated by in situ probing with specific 16S rRNA targeted oligonucleotides. FEMS Microbiol. Ecol. **25**: 43–61.

Martinez, J.S., Zhang, G.P., Holt, P.D., Jung, H.-T., Carrano, C.J., Haygood, M.G. and Butler, A. (2000) Self-assembling amphiphilic siderophores from marine bacteria. Science **287**: 1245–1247.

Martinez, J.S., Carter-Franklin, J.N., Mann, E.L., Martin, J.D., Haygood, M.G. and Butler, A. (2003) Structure and membrane affinity of a suite of amphiphilic siderophores produced by a marine bacterium. Proc. Natl. Acad. Sci. USA **100**: 3754–3759.

Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jørgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N.V. and Gulin, M.B. (2002) Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science **297**: 1013–1015.

Nauhaus, K., Treude, T., Boetius, A. and Krüger, M. (2004) Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. Environ. Microbiol. **7**: 98–106.

Peckmann, J. and Thiel, V. (2004) Carbon cycling at ancient methane-seeps. Chem. Geol. **205**: 443–467.

Peckmann, J., Thiel, V., Michaelis, W., Clari, P., Gaillard, C., Martire, L. and Reitner, J. (1999) Cold seep deposits of Beauvoisin (Oxfordian; southeastern France) and Marmorito (Miocene; northern Italy): microbially induced, authigenic carbonates. Int. J. Earth Sci. **88**: 60–75.

Peckmann, J., Reimer, A., Luth, U., Luth, C., Hansen, B.T., Heinicke, C., Hoefs, J. and Reitner, J. (2001) Methane-derived carbonates and authigenic pyrite from the northwestern Black Sea. Mar. Geol. **177**: 129–150.

Pimenov, N.V., Rusanov, I.I., Poglazova, M.N., Mityushina, L.L., Sorokin, D.Y., Khmelenina, V.N. and Trotsenko, Y.A. (1997) Bacterial mats on coral-like structures at methane seeps in the Black Sea. Microbiology (translated from Mikrobiologiya) **66**: 354–360.

Reitner, J., Wörheide, G., Lange, R. and Schumann-Kindel, G. (2001) Coralline Demosponges – a geobiological portrait. Bull. Tohoku Univ. Mus. **1**: 210–226.

Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A. and Thiel, V. (2005a) Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. Palaeogeogr. Palaeoclimatol. Palaeoecol. **227**: 18–30.

Reitner, J., Peckmann, J., Reimer, A., Schumann, G. and Thiel, V. (2005b) Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). Facies **51**: 71–84.

Romeis, B. (1989) *Mikroskopische Technik*. Urban und Schwarzenberg, München.

Thiel, V., Peckmann, J., Richnow, H.H., Luth, U., Reitner, J. and Michaelis, W. (2001) Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat. Mar. Chem. **73**: 97–112.

Valentine, D.L. (2002) Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. Antonie van Leeuwenhoek **81**: 271–282.

Wrede, C., Heller, C., Reitner, J. and Hoppert, M. (2008) Correlative light/electron microscopy for the investigation of microbial mats from Black Sea colds. J. Microsc. Meth. **73**: 85–91.

PART 3:
MARINE, FRESHWATER, AND SOIL BIOFILMS

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Biodata of **David Paterson, Rebecca Aspden**, and **Pamela Reid**, authors of ***"Biodynamics of Modern Marine Stromatolites"***

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BIODYNAMICS OF MODERN MARINE STROMATOLITES

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1. Introduction

Coastal habitats are important global systems owing to the ecosystem services they provide. Some of these services include gas and climate regulation, resilience and resistance, production of oxygen, nutrient cycles, carbon capture through photosynthesis, carbon sequestration via the biological pump, and providing resilience and stability to coastlines. Microbial mats within the sediments are important components of the ecology of these systems that enable these coastal habitats to function (Paterson et al., 2009). Sedimentary microbial communities are diverse including heterotrophs, anoxic phototrophs, and microphytobenthos that can withstand a wide range of conditions from anaerobic (Kruger et al., 2008) to fully oxic. The diverse range of metabolic activities carried out by these microbial assemblages (sediment microbial communities, biofilms, and microbial mats) are integral to the biogeochemistry of the system and give rise to stratified biofilms at the sediment surface (Aspden et al., 2004) (Fig. 1). These coastal biofilms are adapted to survive depositional and highly dynamic environments (Paterson et al., 1998; Yallop et al., 1994). The oldest known representatives of this type of microbial system are likely to be stromatolites (Krumbein et al., 2003). In modern day coastal sediments, transient and permanent biofilms are largely formed by microphytobenthos, the collective term for photosynthetic microbial assemblages including cyanobacteria, diatoms, and euglena living on or in benthic depositional systems. Not only do microphytobenthic biofilms serve as primary producers and provide an important source of autochthonous carbon, they also provide a number of other ecosystem services (Chapin et al., 1997) including the stabilization of cohesive sediment. These communities rely on the ability to trap and retain deposited sediments, thereby enhancing the structural stability of the system (Krumbein, 1994). Most microbes within these assemblages will respond to changes within the immediate environment by migrating within the upper layers of the sediment and placing themselves in an optimum position, in which to

Figure 1. The change in microbial assemblages from algal to diatom to anoxic layers can be discerned in the lamination of this salt marsh sediment.

carry out their metabolic requirements. These cells often produce extracellular polymeric substances (EPS), thereby providing an important source of autochthonous carbon for the surrounding environment (Underwood and Paterson, 2003; Decho et al., 2005). This microbial microcycling creates a very dynamic system (Aspden et al., 2004) with different species occupying or moving between layers. For example, under low light conditions, cyanobacteria will migrate above diatom layers to obtain enough light for photosynthesis. Under high light, cyanobacteria will migrate away from the surface to shade themselves against overexposure to high light (Prufert-Bebout and Garcia-Pichel, 1994). These laminated layers can be seen quite clearly owing to the change in coloration depending on the communities present. Cyanobacterial layers will appear blue-green, diatom layers appear golden brown, and the anoxic layers appear black.

Detailed spatial examination of the layers of microbial mats can be achieved by low-temperature scanning electron microscopy (Fig. 2), confocal microscopy (Decho et al., 2005), and other techniques (Jørgensen et al., 1983; Yallop et al., 1994).

The role of prokaryotes in the initial trapping and binding of sediments owing to the production of polymer or by the physical entrapment by filaments is evident from the microscopic study of these ancient structures (Fig. 3). Despite their presence in marine systems for such a long period, the biomechanical processes involved in the formation of these laminated sedimentary structures are little understood (Paterson et al., 2008).

Bahamian stromatolites are created by sediment trapping and subsequent lithification of the microbial mats (Walter, 1976; Reid et al., 1995; Stoltz et al., 2001) and the initial biological trapping processes are similar to those exhibited by filamentous algae, turfs, and microphytobenthic mats. Modern day stromatolite formation within the Exuma Cays is strongly dependent on this ability of associated microbial mats to bind sediment grains into the structure of the

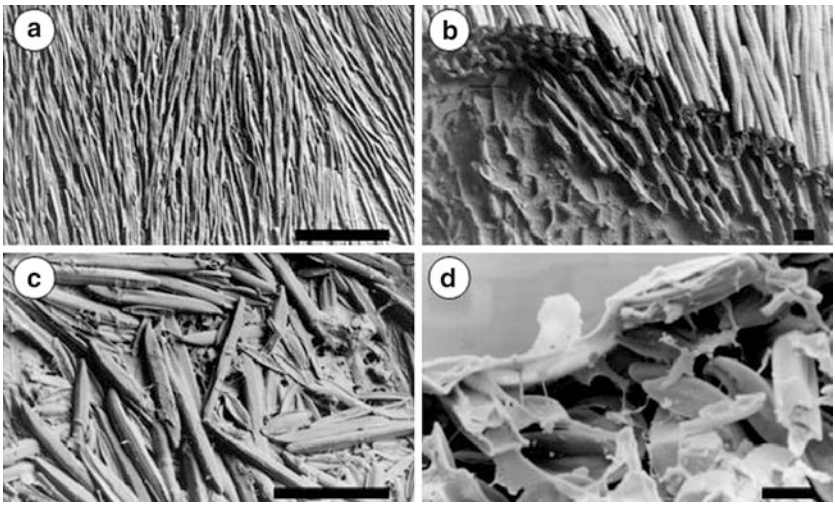

Figure 2. Low-temperature scanning electron micrographs of layered microbial communities. (**a**) The surface of filamentous cyanobacterial assemblage (bar marker: 100 μm). (**b**) Detail of a fracture face through the assemblage (bar marker = 10 μm). (**c**) Surface of a diatom-dominated assemblage (bar marker = 100 μm). (**d**) Detail of a fracture through the diatom assembly (bar marker = 10 μm).

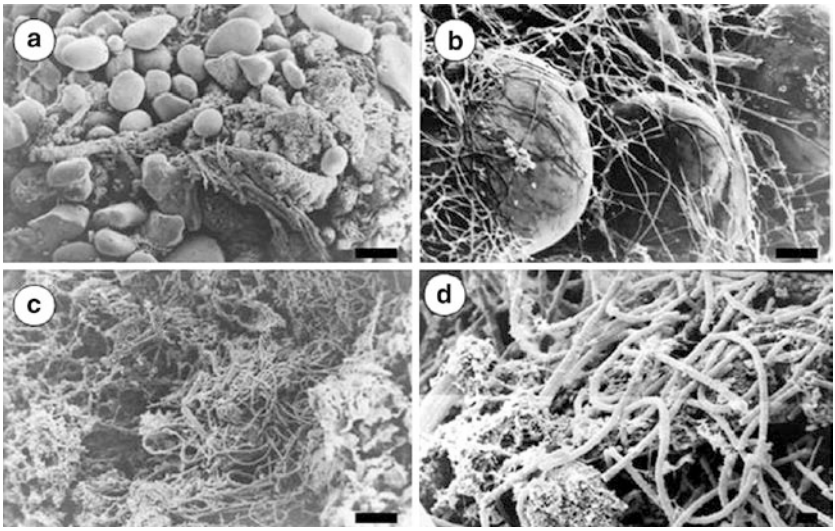

Figure 3. Low-temperature scanning electron micrographs of layered stromatolitic microbial communities. (**a**) The surface of the ooid bed with relatively low colonization (bar marker = 150 μm). Cyanobacterial filaments binding the surface ooids (bar marker = 50 μm). (**b**) Surface of a cyanobacterial-dominated assemblage (bar marker = 10 μm). (**c**) Detail of the cyanobacterial colonization of the stromatolite surface (bar marker = 10 μm). (**d**) Detail of the cyanobacterial colonization of the stromatolite surface (bar marker = 10 μm).

stromatolite, while preventing the erosion of sediment particles due to the wave action and currents. These structures are capable of accumulating sediment through a combination of physical entrapment within the filaments, and binding of the sediment particles by EPS produced by the microbial community present (Scoffin, 1970; Stewart, 1983; Kendrick, 1991; Airoldi and Cinelli, 1996) (Fig. 3). Stromatolites, such as those in the Exuma Cays, allow researchers to determine what processes may have occurred for these intricate assemblages to form. Previous studies have suggested that the structure and formation of the stromatolite assemblages were dependent on physical factors such as sedimentation rates, and the position of the stromatolites within the reef with respect to movement of sand ripples (MacIntyre et al., 1996; Golubic and Browne, 1996). The microbial mats present in modern day stromatolites have been shown to react to varying sedimentation rates by creating the three stromatolite types as described by Reid et al. (2000).

Studies of stromatolite formation have, in the past, largely focused on the cyanobacterial species present; however, within the modern day stromatolites the same functions may also be carried out by other heterotrophs and a variety of autotrophs, particularly diatoms (see Franks et al., this volume). Diatoms are a relatively recent development in the phylogeny of the eukaryotes, but it is fair to assume that they have been associated with stromatolite systems since their emergence. Centric diatoms evolved in the early Cretaceous period (Gersonde and Harwood, 1990) with pennate diatoms following in the late Cretaceous period (Harwood, 1988), and many species found within this time period were morphologically similar to the species found today with around 200,000 extant species (Admiraal, 1984; Mann, 1999). The first pennate diatoms were araphid (nonmotile), and motile forms did not appear in great numbers until the Eocene period (Medlin et al., 1993). These species have a key role to play in the trapping and binding of freshly deposited sediment owing to their growth form (stalked and branching) and the copious production of EPS (Awramik and Riding, 1988; Paterson and Black, 2000; Underwood and Paterson, 2003; Paterson et al., 2008).

2. Recent Biodynamic Studies

Modern stromatolites are clearly structures that are shaped and formed through both biotic and physical processes, but there have been few studies describing the biodynamics of stromatolites. Recent work on biostabilization and particle capture and retention by stromatolites (Paterson et al., 2008) has gone some way to rectifying this situation. Measurements of the engineering capacity, including stabilization, capture, and retention of ooids, by natural stromatolite-forming assemblages under ambient conditions were obtained using the cohesive strength meter (CSM), and a new technique using magnetic particle induction (Larson et al., 2009) to assess the surface retentive capacity of stromatolite material.

2.1. RECONSTITUTION STUDIES

Stromatolites are subjected to dynamic conditions and storm events and as a result may become damaged. Stromatolite material was broken down and any large shell fragments were removed to determine the rate at which the microbial communities could reestablish some form of stabilization (for methods, see Paterson et al., 2008). This work highlights the engineering capacity of the microbial assemblages that constitute stromatolites but does not replicate the formation of stromatolites themselves except perhaps in the event of severe storm damage. Engineering effects were observed to occur within hours of the initial disturbance; however, light was an essential component of the process suggesting that photosynthetic activities of the microbial assemblages present within the system speed up the process of biogenic stabilization (Paterson et al., 2008). Samples maintained under natural light began to stabilize within hours and the stability continued to increase throughout the experiment (Figs. 4 and 5).

The stability of reconstituted material subjected to the light treatment increased significantly over a few days and was significantly greater than stabilization under the dark treatments (Fig. 5). The stability of the control sediment remained unaltered.

Examination of the surface structure of material maintained in dark conditions showed limited microphytobenthic growth, and ooids were loosely packed when compared with those of the material maintained in light conditions, suggesting evidence of higher quantities of cyanobacterial and diatomaceous species. Ooids appeared to be trapped within a matrix of cyanobacterial filaments and this is consistent with the sediments becoming more difficult to erode (Fig. 6a, b). The results obtained from these initial studies suggested that the biostabilization of the ancient stromatolites might become more effective following the evolution of photosynthesis.

Figure 4. Restructured stromatolite material after 156 h in light conditions.

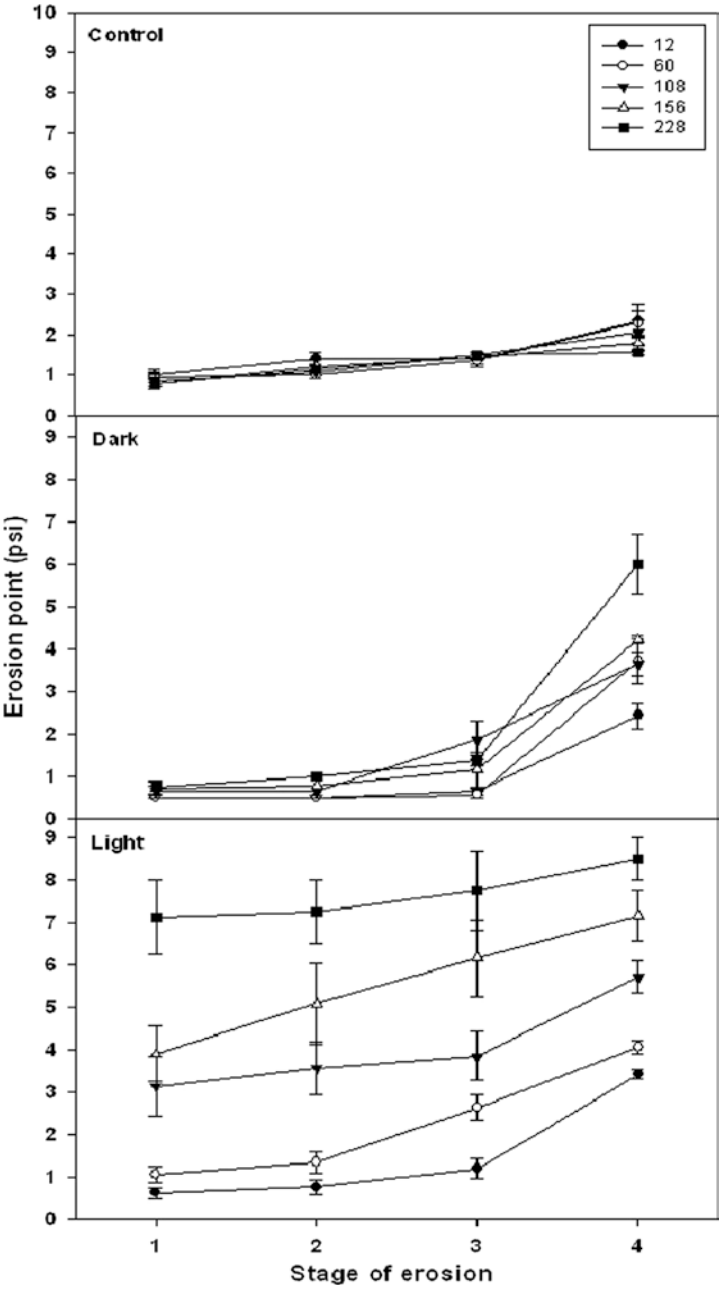

Figure 5. Stability of reconstituted stromatolite kept in light and dark conditions was measured after 12, 60, 108, 156, and 228 h using the cohesive strength meter. The control plot was kept in light conditions but contained stromatolite material free of any microbial assemblage. The erosion point describes the mean pressure required to cause a specific level of erosion (particle resuspension causing a reduction in transmission within the CSM chamber). Four stages of erosion were observed: (1) slight erosion, 10% reduction in transmission, (2) moderate erosion, 20% reduction in transmission, (3) significant erosion, 50% reduction in transmission, and (4) severe erosion, 75% reduction in transmission.

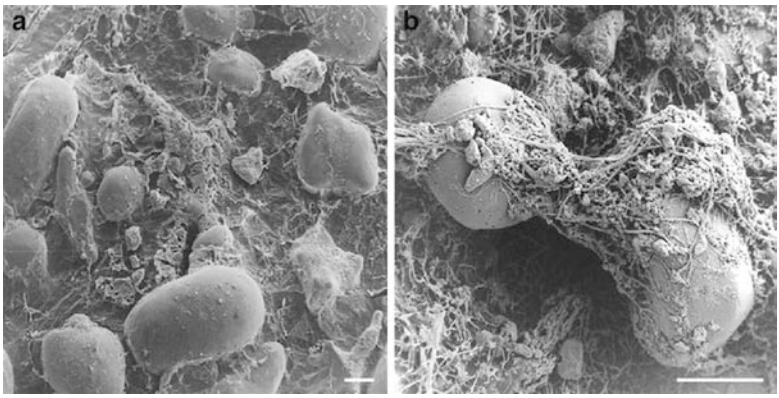

Figure 6. Low-temperature scanning electron microscopy images: (**a**) Absence of microphytobenthic assemblages within samples subjected to dark conditions (Bar marker = 100 μm). (**b**) Ooids within samples subjected to normal light conditions were observed to be trapped within cyanobacterial filaments and the extracellular polymeric substances produced by the cyanobacteria and diatomaceous assemblages present (Bar marker = 100 μm).

The formation of modern stromatolites is highly dependent on sediment accretion rates and their associated microbial assemblages that trap and bind sediment particles that fall on the surface of the structures. The ability to rapidly stabilize the surface material promotes the growth of stromatolites despite ambient hydrodynamic forces acting on them.

The requirement for a light period in the biostabilization process suggests that initial stabilization of surface layers is carried out by autotrophic organisms or their products, such as cyanobacteria and diatoms and their related EPS (Reid et al., 2000). This is supported by previous studies (Reid et al., 2000; Kawaguchi and Decho, 2002; Decho et al., 2005), in which the initial stage of stromatolite formation occurred because of the influence of the cyanobacterium *Schizothrix* sp. through polymer production and filamentous binding. Personal observations also provided evidence of various stalked and chain-forming diatoms present within the stromatolites surface communities. Although the study does not replicate the formation processes that occur naturally, an indication of the ability of the structures to recover following a disturbance event, and the capacity of the microbial assemblages present within the systems to biostabilize the material found naturally was demonstrated. The reactivation of photosynthetic capabilities of the microbial mats was demonstrated by further studies carried out at Highborne as part of the RIBS program (Perkins et al., 2007).

2.2. HOW MUCH STROMATOLITE MATERIAL IS NEEDED TO STABILIZE

Working with dispersed stromatolite material provides the opportunity to conduct experiments to determine how much relative biomass was required to establish

stability of stromatolite material. The reconstitution experiments were repeated using varying concentrations of stromatolitic material (including the microbial mats) mixed in different proportions with beach ooids. A log series of dilution was used (100%, 10%, 1%, 0.1%) to determine the effects of microbial concentration on the regeneration capacity of the system. Samples formed from 100% stromatolite and microbial material exhibited rapid sediment stabilization. Material below 100% exhibited no obvious stabilization (Fig. 7).

The lack of stabilization below 100% stromatolite material suggests that the microbial assemblages present within the stromatolites are responsible for the stabilization of the material, but that a threshold biomass must be reached before this stabilization becomes significant. Therefore, the growth of microbial assemblages to a certain threshold is required for effective and rapid stabilization. The presence of EPS, produced by microphytobenthos, has been shown to promote the physical stabilization of microbial cells, which in turn provides a matrix in which the ooids become attached (Kawaguchi and Decho, 2002; Decho et al., 2005).

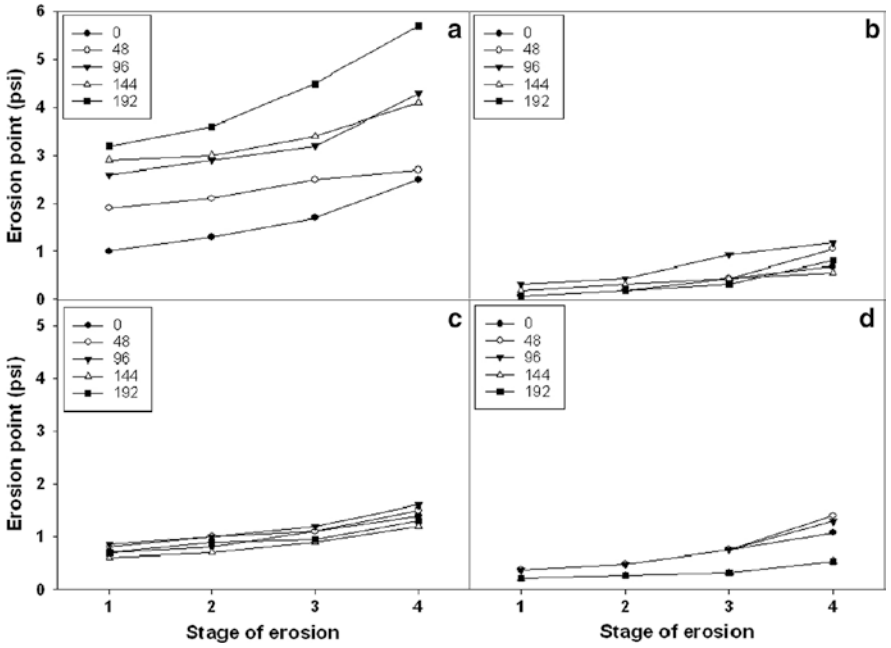

Figure 7. Stability of reconstituted stromatolite, with varying concentrations of microbial assemblage (**a** = 100%, **b** = 10%, **c** = 1%, **d** = 0.1%) was measured after 0, 48, 96, 144, and 192 h using the cohesive strength meter. The erosion point describes the mean pressure required to cause a specific level of erosion (particle resuspension causing a reduction in transmission within the CSM chamber). Four stages of erosion were observed: (1) slight erosion, 10% reduction in transmission, (2) moderate erosion, 20% reduction in transmission, (3) significant erosion, 50% reduction in transmission, (4) severe erosion, 75% reduction in transmission.

3. Discussion

The importance of ancient stromatolites as a means of interpreting the past is often postulated (Krumbein et al., 2003). The extent and accuracy of these interpretations depends not only on the understanding of the form and ecology of stromatolites but also on our ability to interpret their biomechanical properties. The arguments for and against the relative importance of physical and biological processes in stromatolite formation are now largely set aside since it is clear that these factors interact in determining the nature and response of the assemblages. The initial processes of biostabilization seem to precede the deposition of mineral material and the capture and retention of sediment is important. Some aquatic habitats may be more quiescent than others but certainly in the case of the Bahamian systems, shear stress at the surface of the bed is a significant and routine stressor (Eckman et al., 2008). The evolution of individual forms and perhaps more importantly cooperative assemblages that act to capture and retain sediment may be seen as the first "ecosystem engineering" (Jones et al., 1994) and as such represent an important milestone in the development of mutually dependent relationships and ecosystem responses. The studies outlined here have shown that the microbial mats that construct stromatolites at Highborne Cay, Bahamas, are capable of rapid ecosystem engineering, that they perform better under conditions of light (Paterson et al., 2008), emphasizing the importance of photosynthesis and its by-products, and also that a certain biomass of microbial material is required before an effective ecosystem response can be observed. There is a great deal more to be learned about the biomechanics of stromatolites and it is arguable that these modern analogs cannot be assumed to be truly representative of the capabilities of ancient systems but they do provide a window that may help to interpret the form and function of ancient systems even if this process must be treated with some caution. Scientists are beginning to examine stromatolite systems in greater detail, to establish models (Havemann and Foster, 2008), and still use ancient stromatolites to interpret the geological and environmental setting dating back billions of years (van Kranendonk et al., 2008).

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5. References

Admiraal, W. (1984) The ecology of estuarine sediment-inhabiting diatoms. Prog. Phycol. Res. **3**: 269–322.

Airoldi, L. and Cinelli, F. (1996) Early patterns of recovery of filamentous algal turf on a rocky subtidal shore (Meditteranean Sea). Ital. Soc. Ecol. **17**: 341–344.

Aspden, R.J., Vardy, S. and Paterson, D.M. (2004) Salt marsh microbial ecology: microbes, benthic mats and sediment movement, In: S. Fagherazzi, M. Marani and L.K. Blum (eds.) *The Ecogeomorphology of Tidal Marshes.* American Geophysical Union, Washington, DC, pp. 115–136.

Awramik, S.M. and Riding, R. (1988) Role of algal eukaryotes in subtidal columnar stromatolite formation. Proc. Natl. Acad. Sci. USA **85**: 1327–1329.

Chapin, F.S., Schulze, E.D. and Mooney, H.A. (1997) Biodiversity and ecosystem processes. Trends Ecol. Evol. **7**: 107–108.

Decho, A.W., Visscher, P.T. and Reid, R.P. (2005) Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Palaeogeogr. Palaeoclimatol. Palaeoecol. **219**: 71–86.

Eckman, J.E., Andres, M.S., Marinelli, R.L., Bowlin, E., Reid, R.P., Aspden, R.J. and Paterson, D.M. (2008) Wave and sediment dynamics along a shallow subtidal sandy beach inhabited by modern stromatolites. Geobiology **6**: 22–32.

Gersonde, R. and Harwood, D.M. (1990) Lower cretaceous diatoms from ODP Leg 113 Site 693 (Weddell Sea) I. Vegetative Cells, In: P.F. Barker and J.P. Kennett (eds.) *Proceedings of the Ocean Drilling Programme Results*. Ocean Drilling Programme, College Station, TX, pp. 365–402.

Golubic, S. and Browne, K.M. (1996) *Schizothrix gebelinii* sp. nova builds subtidal stromatolites, Lee Stocking Island. Algol. Studies **83**: 273–290.

Harwood, D.M. (1988) Upper Cretaceous and lower Palaeocene diatom and silicoflagellate biostratography of Seymour Island, Eastern Antartic Peninsula. Geol. Soc. Am. **169**: 55–129.

Havemann, S.A. and Foster, J.S. (2008) Comparative characterization of the microbial diversities of an artificial microbialite model and natural stromatolites. Appl. Environ. Microbiol. **74**: 7410–7421.

Jones, C.G., Lawton, J.H. and Shachak, M. (1994) Organisms as ecosystem engineers. Oikos **69**: 373–386.

Jørgensen, B.B., Revsbech, N.P. and Cohen, Y. (1983) Photosynthesis and structure of benthic microbial mats – microelectrode and SEM studies of 4 cyanobacterial communities. Limnol. Oceanogr. **28**: 1075–1093.

Kawaguchi, T. and Decho, A.W. (2002) Isolation and biochemical characterization of extracellular polymeric secretions (EPS) from modern soft marine stromatolites (Bahamas) and its inhibitory effect on $CaCO_3$ precipitation. Prep. Biochem. Biotechnol. **32**: 51–63.

Kendrick, G.A. (1991) Recruitment of coralline crusts and filamentous turf algae in the Galapagos archipelago: effect of simulated scour, erosion and accretion. J. Exp. Mar. Biol. Ecol. **147**: 47–63.

Kruger, M., Blumenberg, M., Kasten, S., Wieland, A., Kanel, L., Klock, J.H., Michaelis, W. and Seifert, R. (2008) A novel, multi-layered methanotrophic microbial mat system growing on the sediment of the Black Sea. Environ. Microbiol. **10**: 1934–1947.

Krumbein, W.E. (1994) The year of the slime, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilisation of Sediments*. BIS-Verlag, Oldenburg, pp. 1–7.

Krumbein, W.E., Paterson, D.M. and Zavarzin, G.A. (eds.) (2003) *Fossil and Recent Biofilms. A Natural History of Life on Earth*. Kluwer, Dordrecht.

Larson, F., Lubarsky, H., Gerbersdorf, S. and Paterson, D.M. (2009) Surface adhesion measurements in aquatic biofilms using magnetic particle induction: MagPI. Limnol. Oceanogr. Meth. **7**: 490–497.

Macintyre, I.G., Reid, P. and Steneck, R.S. (1996) Growth history of stromatolites in a Holocene fringing reef, Stocking Island, Bahamas. J. Sediment. Res. **66**: 231–242.

Mann, D.G. (1999) The species concept in diatoms. Phycologia **38**: 437–495.

Medlin, L.K., Williams, D.M. and Sims, P.A. (1993) The evolution of diatoms (Bacillariophyta). I. Origin of the group and assessment of the morphology of its major divisions. Eur. J. Phycol. **28**: 261–275.

Paterson, D.M. and Black, K. (2000) Siliciclastic intertidal microbial sediments, In: R. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Heidelberg, pp. 76–83.

Paterson, D.M., Wiltshire, K.H., Miles, A., Blackburn, J., Davidson, I., Yates, M.G., McGrorty, S. and Eastwood, J.A. (1998) Microbiological mediation of spectral reflectance from intertidal cohesive sediments. Limnol. Oceanogr. **43**: 1207–1221.

Paterson, D.M., Aspden, R.J., Visscher, P.T., Consalvey, M., Andres, M., Decho, A.W., Stolz, J. and Reid, P.R. (2008) Light-dependant biostabilisation of sediments by stromatolite assemblages. PLoS One **3**: e3176.

Paterson, D.M., Aspden, R.J. and Black, K.S. (2009) Intertidal flats: ecosystem functioning of soft sediment systems, In: E. Wolanski, M.M. Brinson, D.R. Cahoon and G.M.E. Perillo (eds.) *Coastal Wetlands: An Integrated Ecosystem Approach.* Elsevier, Amsterdam, pp. 317–343.

Perkins, R.G., Kromkamp, J.C. and Reid, R.P. (2007) Importance of light and oxygen for photochemical reactivation in photosynthetic stromatolites communities after natural sand burial. Mar. Ecol. Prog. Ser. **349**: 23–32.

Prufert-Bebout, L.E. and Garcia-Pichel, F. (1994) Field and cultivated *Microcoleus chthonoplastes*: The search for clues to its prevalence in marine microbial mats, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 111–116.

Reid, R.P., MacIntyre, I.G., Browne, K.M., Steneck, R.S. and Miller, T. (1995) Modern marine stromatolites in the Exuma Cays, Bahamas: uncommonly common. Facies **33**: 1–18.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., MacIntyre, I.G., Paerl, H.W., Pinckney, L., Prufert-Bebout, L., Steppe, T.F. and DesMarais, D.J. (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature **406**: 989–992.

Scoffin, T.P. (1970) The trapping and binding of subtidal carbonate sediments by marine vegetation in Bimini Lagoon, Bahamas. J. Sediment. Petrol. **40**: 249–273.

Stewart, J.G. (1983) Fluctuations in the quantity of sediments trapped among algal thalli on intertidal rock platforms in southern California. J. Exp. Mar. Ecol. **73**: 205–211.

Stoltz, J.F., Feinstein, T.N., Salsi, J., Visscher, P.T. and Reid, R.P. (2001) TEM analysis of microbial mediated sedimentation and lithification in modern marine stromatolites. Am. Mineral. **86**: 826–833.

Underwood, G.J.C. and Paterson, D.M. (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. Adv. Bot. Res. **40**: 183–240.

van Kranendonk, M.J., Philippot, P., Lepot, K., Bodorkos, S. and Pirajno, F. (2008) Geological setting of Earth's oldest fossils in the ca. 3.5 Ga Dresser Formation, Pilbara Craton, Western Australia. Precambrian Res. **167**: 93–124.

Walter, M.R. (ed.) (1976) *Stromatolites: Developments in Sedimentology 20*. Elsevier, Amsterdam.

Yallop, M.L., DeWinder, B., Paterson, D.M. and Stal, L.J. (1994) Comparative structure, primary production and biogenic stabilization of cohesive and noncohesive marine-sediments inhabited by microphytobenthos. Estuar. Coast. Shelf Sci. **39**: 565–582.

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ENTOPHYSALIS MATS AS ENVIRONMENTAL REGULATORS

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1. Introduction

Microbial mats are considered as the oldest benthic ecosystems constructed predominantly by the efficient primary production of cyanobacterial oxygenic photosynthesis. Cyanobacteria acted as the main architects of sedimentary structures that characterized intertidal coastal flats as well as illuminated parts of the ocean floor for most of the Earth's history (Schopf and Klein, 1992; Golubic and Seong-Joo, 1999; Golubic et al., 2000). A wide variety of other microorganisms have since found a life support and created their ecological niches within the framework of microbial mat structures. Such stratified ecosystems persisted prior to the evolution of metazoans and introduction of grazing and bioturbation, processes whose dramatic global effect was termed "agricultural revolution" (Seilacher and Pflüger, 1994). Stratified microbial mats persist in modern environments under conditions of elevated salinity or rapid cementation (Abed et al., 2008; Gischler et al., 2008). Their preserved organosedimentary products are termed microbialites (Burne and Moore, 1987). Microbial diversity associated with microbialite structures is increasingly being studied by using molecular methods (Reid et al., 2003; Abed et al., 2003; Burns et al., 2004; Papineau et al., 2005), digital fluorescence imaging (e.g., Kawaguchi and Decho, 2002; Paterson et al., 2008; Foster et al., 2009), and biochemical analysis of specific products (e.g., Palmisano et al., 1989; Gautret et al., 2004, 2006; Bühring et al., 2009).

Stromatolites are defined as finely laminated structures, which require continuity of a rhythmic process involving sequential preservation of microbial mats (Kalkowsky, 1908). However, some laminated rocks did not involve microbial mats in their formation (Grotzinger and Rothman, 1996) and other microbialites are not internally laminated but have clotted and cavernous internal textures. These microbialites are also called thrombolites. They are formed by different processes in a discontinuous fashion, each leaving a different sedimentary signature. Alternatively, the original texture has been changed by postdepositional taphonomy (Planavsky and Ginsburg, 2009).

In this chapter, we discuss the role of *Entophysalis*, one of the oldest recognized coccoid stromatolite-forming cyanobacterium, in the formation and

regulation of modern microbial mats and ancient stromatolites. During our studies of the hypersaline microbial mats of Abu Dhabi, this cyanobacterium was frequently encountered (Abed et al., 2008). Although *Entophysalis* was observed in many microbial mats as well as in stromatolitic records, studies on its distribution, morphology, and role in mat formation are very scarce.

2. *Entophysalis* in the Microbial Mats of Abu Dhabi (UAE)

Cyanobacterial morphotypes in the microbial mats of Abu Dhabi are numerous and morphologically complex as discernible by high magnification transmitted light microscopy. They occur in various microbial mats distributed in zones across intertidal ranges (Golubic, 2000). *Entophysalis major* (Fig. 1a) was observed in almost all mats regardless of their tidal position but with a clear preference to the lower intertidal ranges where it usually dominates (for detail see Abed et al., this volume). Most other mats in middle and upper intertidal ranges are dominated by *Lyngbya aestuarii* (Fig. 1b) and *Microcoleus chthonoplastes* (Fig. 1e). The other major morphotype is *Schizothrix splendida*, which forms pinnacle mat (see Abed et al., this volume, Fig. 1j). Among other filamentous cyanobacteria that were observed were: the free gliding *Oscillatoria* spp., which include helically twisted forms, characterized by constriction at the cross-walls between cells (Fig. 1c) and solitary cells of a large *Synechocystis* sp. (Fig. 1d); Rod-shaped coccoid cyanobacteria of the morphogenus *Aphanothece* (*Halothece*), which produced large slimy masses in hypersaline tidal pools in the uppermost tidal ranges (Fig. 1f). Other distinct types of unicellular cyanobacteria include a large *Chroococcus* with asymmetrical production of EPS, which forms stalks, with embedded UV-protecting pigment scytonemin (Fig. 1g, h) and *Aphanocapsa* sp. (Fig. 1i). *Johannesbaptistia pellucida* (Fig. 1j) and a smaller taxon with similar cell organization (Fig. 1k) have cell division in one dimension. *Johannesbaptistia* has been cultured and sequenced (Richert et al., 2006). Coccoid cyanobacteria with cell division in two dimensions are represented by *Gomphosphaeria* and *Merismopedia* (Fig. 1l, m). Among filamentous forms, the most common and diverse are those with narrow trichomes classified as *Leptolyngbya* spp. (Fig. 1n, o).

3. Morphotypic Properties of *Entophysalis*

Entophysalis is a coccoid cyanobacterium belonging to the order Chroococcales (Fig. 1a). It divides by binary fission and produces layered exopolymer (EPS) envelopes intermittent to cell division. The envelopes have a firm gelatinous consistency; they are highly hydrated, supported by ultrafine fibers as perceived under transmission electron microscopy. The fibers are oriented tangentially, arranged in

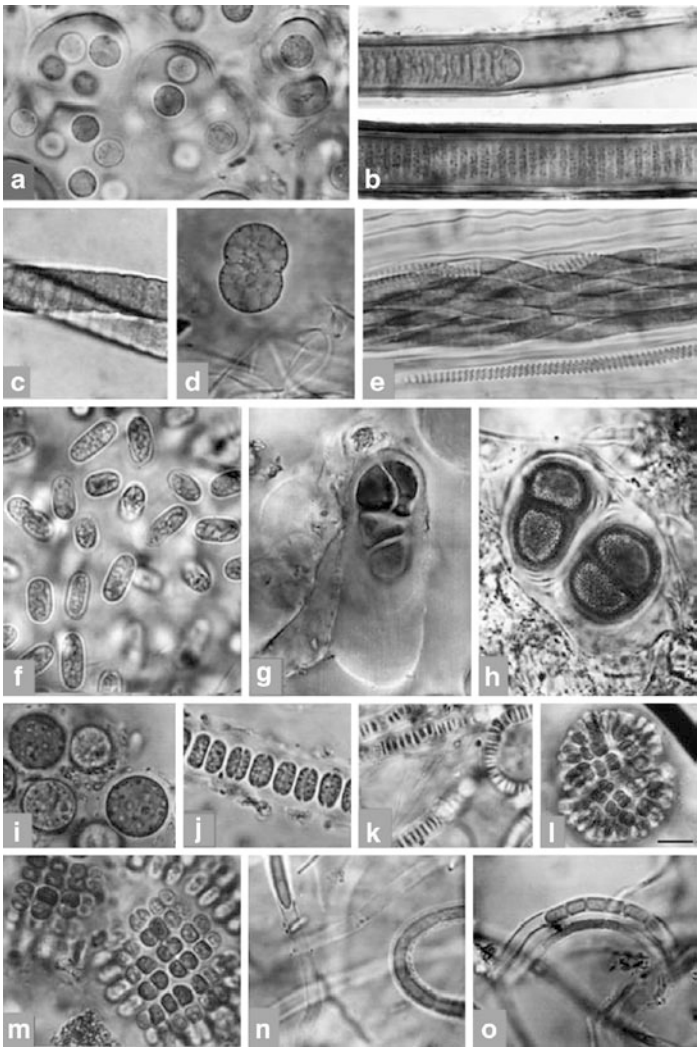

Figure 1. Cyanobacteria in Abu Dhabi microbial mats. The scale in (**a**) is 10-μm long valid for all pictures. (**a**) *Entophysalis major* Ercegović (**b**) two sheathed filaments of *Lyngbya aestuarii,* the upper with distinct apical cell; (**c**) *Oscillatoria* sp.: intertwined helically twisted trichomes; (**d**) *Aphanocapsa* sp.: cell early in division process; (**e**) *Microcoleus chthonoplastes*: intertwined bundle of trichomes within a common sheath, with trichomes of *Spirulina subtilissima* penetrating between sheath layers; (**f**) *Aphanothece* (*Halothece*); (**g**) *Chroococcus* sp. with unidirectional production of EPS; (**h**) *Chroococcus* sp.: four cell stage following two divisions; (**i**) *Aphanocapsa* sp.: the cells assume spherical shape immediately after division; (**j**) *Johannesbaptistia pellucida*; (**k**) *Johannesbaptistia* sp.; (**l**) *Gomphosphaeria* sp.; (**m**) *Merismopedia* sp.; (**n**) *Leptolyngbya* sp. with conical end cell; (**o**) *Leptolyngbya* sp. with firm sheath.

concentric zones within hydrated gelatinous envelopes, which are able to expand and contract by water imbibitions and loss. It is assumed that the EPS envelopes, in addition to providing adherence of cells to the substrate, have a protective role to the cells, which includes retarding of water loss, thus assuring the time needed for the transition from active cellular functions to a dormant state. The organism does not form any distinctive resting stages. The hydrated envelopes expand during cell division, able to include the progeny of a number of cell generations within billowing clonal colonies overgrowing the substrate. New envelope layers are excreted by each cell generation pushing the older layers outward. *Entophysalis* differs from similarly organized coccoid cyanobacteria such as *Gloeocapsa* and *Gloeothece* by its tendency for asymmetric, polarized growth that includes adherence to the substrate. The protective function of gelatinous envelopes includes incorporation of the UV-screening pigment scytonemin (Garcia-Pichel and Castenholz, 1991), which is deposited in response to solar irradiation in the upper, impacted side of the colony.

Two species of *Entophysalis* commonly occur on marine coasts: *E. granulosa* Kützing forms small colonies in intertidal ranges of the world oceans, whereas the larger *E. major* Ercegovićforms extensive coherent mats on arid tropical coasts. So far, there are no cultures of *E. major* in spite of numerous attempts. There are also no 16S rRNA sequences available. These may have been retrieved from mats but were assigned as unknown, because of the lack of axenic cultures for reference.

4. Macro- and Microscale Distribution of *Entophysalis*

Entophysalis dominates in the lower intertidal ranges of the coast of the Arabian Gulf at Abu Dhabi, and is the main constituent of the intertidal stromatolites in the Hamelin Pool, Shark Bay, Western Australia (Golubic, 1983, 1985). It occupies similar environments in the Bahamas and the Guerrero Negro, Baja California, Mexico. In Abu Dhabi, *Entophysalis* mats occupy the lower ranges of the intertidal landscape, which are characterized by megaripples, surrounded by ponds and channels, a landscape inherited from the energy impact of currents and waves preceding the regression of the Arabian Gulf. The landscape resulting from its settlement is shown in Fig. 2.

Entophysalis mat development appears optimized by the high frequency of flooding and efficient drainage. The mat occupies tidal channels where it favors swift currents. It often produces significant biomass building dams across tidal channels. In the process, *Entophysalis* modifies the flow of water, which is then enhanced over the dams while pools form behind them (Fig. 3a, left). The growth accompanied by high production of EPS makes *Entophysalis* an environmentally invasive species, which is able to transform the tidal channel into a series of alternating lotic and lentic sections.

The conditions on the floor of the ponds accumulating behind dams no longer support *Entophysalis*; instead the ponds are invaded by the filamentous

Figure 2. Aerial view of the intertidal landscape on the Arabian Gulf coast at Abu Dhabi with tidal channels and pools covered by microbial mats. The topography is a result of increased sediment accumulation in waterlogged ponds relative to drained elevations, both triggered by the dams built by *Entophysalis*.

cyanobacterium *M. chthonoplastes* (Fig. 3b, left). A stratified mat develops composed of a set of microbial communities across a steep vertical redox gradient. Aerobic metabolism is restricted to a thin top layer, whereas the predominantly anaerobic metabolism reigns in deeper layers of that mat. These mats have higher sediment accumulation rates, which fill the ponds and expose the mat to desiccation and cracking into polygons (Fig. 3c, left). In the lowermost intertidal ranges, the colonization by *Entophysalis* starts on the crest of the megaripples (Fig. 3a, right), but higher in the intertidal zone it moves into depressions between the crests and into the channels. The conditions on the elevations between channels are becoming too dry during low tides, thus also unfavorable to *Entophysalis*, which is gradually replaced by the pinnacle mat composed of the filamentous cyanobacterium *S. splendida.* At the middle level of the intertidal range of the Abu Dhabi coast, the landscape is shared by three different mat types: the crests of former megaripples and banks of the creeks grow pinnacle mats shaped by *S. splendida*, the floor of the ponds are covered by flat mat formed by *M. chthonoplastes*, whereas the pustular mat of *E. major* forms a narrow ring around the ponds balancing adequate water supply and air exposure (Fig. 3c, right).

Well-drained pustular and pinnacle mats are both decomposed aerobically. The pinnacle mat is underlain only by a thin layer of purple bacteria and a transient anoxic zone over oxidized sediments. The mats are inundated by high tides and drained during low tides, so that the mat is completely decomposed and recycled. The sediment remaining beneath the mat is mostly carbonate, sprinkled by rusty specs of ferric iron. In contrast, the sediment beneath the flat *Microcoleus*

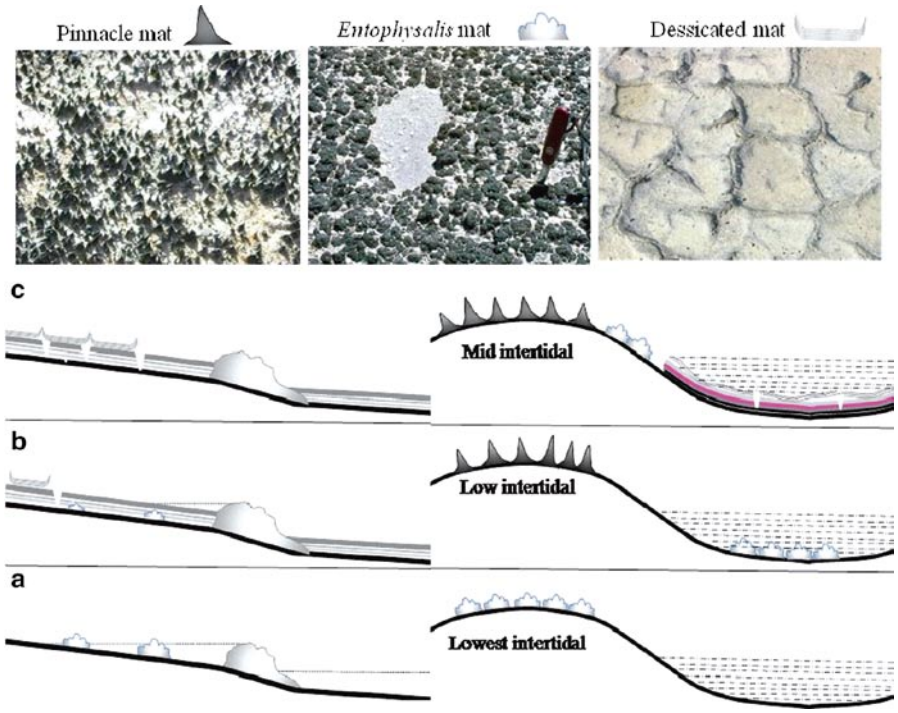

Figure 3. Colonization sequence (**a**–**c**) and distribution of three main microbial mat types in the intertidal zone of the prograding coast at Abu Dhabi, starting from the lowermost intertidal ranges. The distribution of mats and formation of ponds by *Entophysalis* growth is shown in longitudinal section of a tidal creek (*left*), and in a cross-section (*right*) (Representative pictures of the mats are above the diagrams. Swiss army knife is for scale).

mat is stained black by FeS and remains anoxic throughout the tidal cycle. Anoxygenic phototrophs (purple and green bacteria) form a horizon at the oxic−anoxic interface of these mats. Decomposition of organic product in the flat mat is incomplete, so that substantial quantities of it remain incorporated in the sediment (Abed et al., this volume, Fig. 1e).

The consequences of differential sedimentation rates between well-drained topographic heights and ponded lows can be observed while moving further landward across the intertidal zone. The landscape flattens as the channels filled by sediment no longer provide efficient drainage. The water spills over larger surfaces and is retained longer over the low-tide period. Waterlogged condition favors the development of the flat *Microcoleus* mat, which expands over larger areas (Fig. 2). When exposed to severe water loss owing to evaporation, the mat contracts around randomly distributed points, the distance between them depending on the average amount of water retained between the tides. Between the points of contraction,

the mat cracks to produce a polygonal pattern. Contraction affects the surface layers disproportionally so that each polygon curves with upward-turned margins.

Mat cracking changes the conditions for the growth of microorganisms at a scale of 1 to 10 cm. The process transforms a uniform surface of an intact flat mat into ecologically different microhabitats of the polygon. The water in the next tidal cycle is retained longer in the depression in the center of each polygon, but drains away from its margins and into the cracks. The biological consequence is a rearrangement of competitive mat-building organisms according to the change of local conditions as follows: *Microcoleus* remains dominant in the depressed center of each polygon; its margins become overgrown by the pinnacle mat with *Schizothrix*, while *Entophysalis* is attracted to the cracks (Fig. 4).

Further changes in growth and arrangements of mat-forming organisms in the upper intertidal ranges take place without the presence of *Entophysalis*. The landscape, flattened by differential sediment accumulation, has poor drainage, so that tidal flooding leaves mats over soft, swampy ground. High temperature and evaporation promote decomposition of accumulated organic matter, accompanied by gas release, and precipitation of gypsum and halite. The convoluted mats

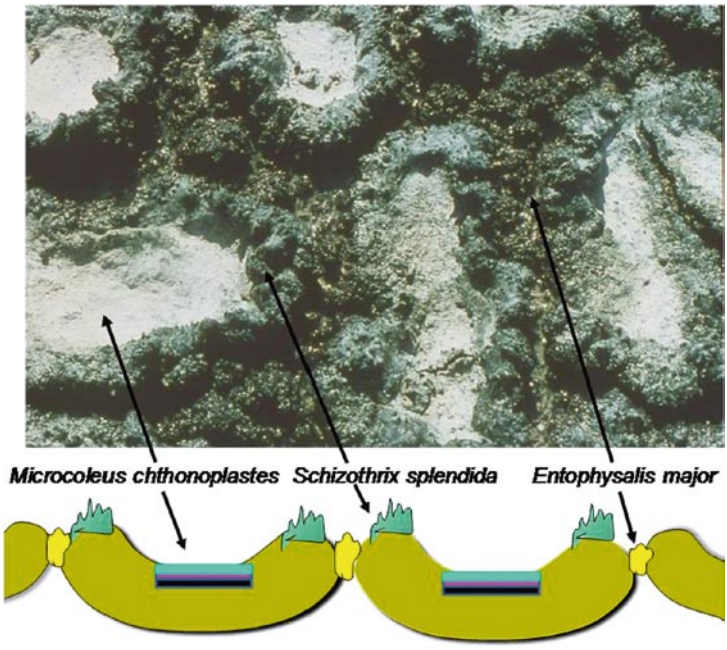

Figure 4. Rearranged growth of microbial mats following desiccation cracking and polygon formation in intertidal mats: stratified *Microcoleus*-dominated mat occupies the water-retaining palm of the polygon, *Schizthrix* colonizes the upturned polygon marges, forming pinnacles, whereas *Entophysalis* colonizes passages of tidal waters draining through the cracks.

in these ranges are shared by *Microcoleus* and *Schizothrix*. The mats are gradually thinning toward the top of the intertidal range; they fold and ultimately dry out and crumble. At the supratidal level, the landscape is a perfectly even sabkha plain, controlled by capillary connection with the groundwater. With the rising and falling in groundwater level, the sabkha plain is maintained by aeolien input and erosion, respectively (Kinsman and Park, 1976).

5. *Entophysalis* and Stromatolites

Entophysalis mats that coat biologically active surfaces of the intertidal stromatolites in Hamelin Pool of Shark Bay, Western Australia (Fig. 5) were made famous since their discovery to science by Phil Playford and Brian Logan (Logan, 1961). Many of these stromatolites are shaped and oriented consistent with the wave action driven by prevalent winds. However, the optimum environment for the development of *Entophysalis* mats in Hamelin Pool appears to be in protected bays, where these mats cover large surfaces over sand. The major role of

Figure 5. *Entophysalis* mat formations in the intertidal zone of Shark Bay, Australia. (**a**) Contiguous platform formed by *Entophysalis* mat in the background of the picture is undercut by wave erosion with a drooping margin. The remaining fragments of the platform are protected by a calcareous crust (*arrow*) with slopes healed by *Entophysalis* overgrowth, forming a domal mound; (**b**) lithified crust with recognizable outlines of *Entophysalis* colonies. Dark color stems from secondary invasion by carbonate penetrating cyanobacteria; (**c**) lithified stromatolite mound with eroded base surrounded by an addie.

Entophysalis mats in these habitats is to stabilize loose sandy sediment by forming extensive coherent platforms (Fig. 5a). Sediment particles are passively trapped between the irregularities of the mat's surface, a process different and significantly slower than that observed in sediment "trapping and binding" (Awramik et al., 1976) by gliding filamentous cyanobacteria (e.g., Reid et al., 2000; MacIntyre et al., 1996). No mineral precipitation has been observed in actively growing *Entophysalis* mats although microbialites, which obviously originated from the same mats, were often found lithified (Fig. 5b). Soft, actively growing *Entophysalis* mats occur often next to hard lithified colonies of the same size and shape, without detectable transitions between them. The transition occurs rapidly within weeks during austral summer, when interstitial water in the mat reaches critical levels of carbonate supersaturation (Golubic, 1983). The process of lithification by massive precipitation of calcium carbonate occurs episodically and is destructive to the mat (Golubic, 1985).

The formation of domed stromatolites (microbialites) is a result of interaction of several processes. In Hamelin Pool, the *Entophysalis* mats extend from protected bays to wave-exposed promontories, where they are involved in the formation of dome-shaped microbialite structures. Sediment stabilization and platform formation are frequently interrupted by erosional events, which may undercut the platform (Fig. 5a, background) or carve channels into it. The mat responds by overgrowing the damaged areas. The scours subdivide the platform and improve local drainage of the remaining fragments. During periods of intense heat and evaporation, carbonate precipitates and forms a crust on the surface of the fragmented platform (Fig. 5a, arrow), which constitutes additional protection from erosion. The continuing erosion tends to remove the surrounding nonlithified parts of the platform leaving mesa-like residual mounds. Erosional events alternate with periods of *Entophysalis* growth, which heals and stabilizes the slopes of the mounds (Fig. 5a, foreground). Resumption of *Entophysalis* growth leads in some areas to healing of the eroded channels and restoration of the platform, which is an exceedingly slow process. For example, tracks left by camel-pulled carts over the mats are still noticeable almost a century later. In other areas, the recurring lithification periods contribute to hardening of the entire structure, followed by internal recrystallization and cementation. The surfaces of lithified microbialites do not support *Entophysalis,* but are occupied by carbonate-penetrating (euendolithic) cyanobacteria (Golubic, 1985). Continued erosion, enhanced by addies around stromatolite mounds, may erode stromatolite base and cause them to collapse (Fig. 5c).

6. Fossil *Entophysalis*

Well-preserved colonies of an organism that shares many morphotypic properties with *Entophysalis* produced stromatolites in the Belcher Island Formation, Hudson Bay, Canada (Golubic and Hofmann, 1976) and was described as *Eoentophysalis*

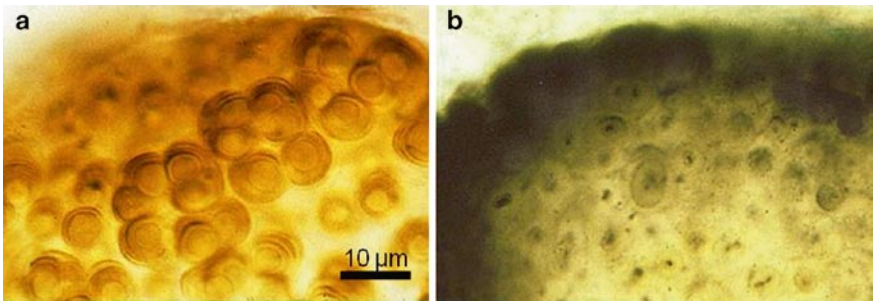

Figure 6. Modern and ancient coccoid stromatolite-building cyanobacteria. (**a**) *Entophysalis major* with cells in different stages of division, surrounded by multiple pigmented EPS envelopes; (**b**) Mesoproterozoic *Eoentophysalis belcherensis* showing the same cell division pattern, growth orientation, and response to solar irradiation as its modern couterpart (Note that the cells collapsed to single and paired granules, whereas the multiple envelopes preserved the original rounded outlines).

belcherensis (Hofmann, 1976). Both organisms show the same type of cell division pattern, both produce multiple envelopes with pigmentation on their upper surfaces (Fig. 6a, b). *E. belcherensis* formed stromatolites about 2,000 My ago, whereas its modern counterpart, *E. major*, forms them today (Fig. 6a). Diagenetic changes in cellular structures start early following death of the organism and can be studied in degraded modern microbial mats.

An important property of the EPS envelopes and sheaths is in the preservation potential of these structures so that their shape and arrangements can be observed in fossils, particularly in those that remained preserved embedded in silica. Unlike plasma membrane-associated cell walls, the EPS envelopes are not part of the osmotic system. Accordingly, they retain their shape as they lose water, contracting like elastic balloons, whereas the cells shrivel and collapse under the tension of the cross-linked peptidoglycan fabric of their walls. Following death, the cells cave in to form polyhedral bodies, shrink to small starlet-shaped bodies, and ultimately remain preserved as small irregular granules (Fig. 6b), whereas the envelopes retain the approximate rounded outlines of the cells that have produced them (Golubic, 1980). Elongated and dumbbell-shaped granules result from cells that died and collapsed in the course of cell division, whereas the recently divided cells produce paired granules within a common envelope. Multiple envelopes that enclose the cells retain their oval shape and reveal the hierarchic arrangement of capsules leaving a trace of the cell division history (Fig. 6b).

The assemblage of microbial fossils of the Belcher Island stromatolites is the oldest that shows close similarity to modern marine cyanobacteria in sedimentary structures comparable with those known in modern settings such as those in Shark Bay, Australia, and Abu Dhabi, UAE. It is quite different from the almost contemporaneous assemblage of microfossils known from the Gunflint Banded

Iron Formation of Canada, indicating that the differences must have been in the ecology of the original habitats (Hofmann, 1976). *E. belcherensis* has been recognized in a number of Proterozoic microbial assemblages preserved by silicification (Seong-Joo et al., 1999).

7. Concluding Remarks

Microbial mats are distributed according to environmental requirements of the dominant microorganism, which also determines the shape and internal structure of the mat. In the case under study, *Entophysalis* mats have introduced a cascade of environmental and microbial changes: they have constructed dams across tidal channels, which produced a series of waterlogged ponds, and in the process, triggered differential sediment accumulation, which ultimately resulted in the formation of a sabkha plain, with its own world of microbial and environmental processes and controls.

8. References

Abed, R.M.M., Golubic, S., Garcia-Pichel, F., Camoin, G.F. and Sprachta, S. (2003) Characterization of microbialite-forming cyanobacteria in a tropical lagoon: Tikehau Atoll, Tuamotu, French Polynesia. J. Phycol. **39**: 1–13.

Abed, R.M.M., Kohls, K., Schoon, R., Scherf, A.-K., Schacht, M., Palinska, K.A., Rullkötter, J. and Golubic, S. (2008) Lipid biomarkers, pigments and cyanobacterial diversity of microbial mats across intertidal flats of the arid coast of the Arabian Gulf (Abu Dhabi, UAE). FEMS Microbiol. Ecol. **65**: 449–462.

Awramik, S.M., Margulis, L. and Barghoorn, E.S. (1976) Evolutionary processes in the formation of stromatolites, In: M.R. Walter (ed.) *Stromatolites. Developments in Sedimentology 20*. Elsevier, Amsterdam, pp. 149–162.

Bühring, S.I., Smittenberg, R.H., Sachse, D., Lipp, J.S., Golubic, S., Sachs, J.P., Hinrichs, K.-U. and Summons, R.E. (2009) A hypersaline microbial mat from the Pacific Atoll Kirimati: insight into composition and carbon fixation using biomarker analyses and a ^{13}C-labeling approach. Geobiology **7**: 308–323.

Burne, R.V. and Moore, L.S. (1987) Microbialites: organosedimentary deposits of benthic microbial communities. Palaios **2**: 241–254.

Burns, B.P., Goh, F., Allen, M. and Neilan, B.A. (2004) Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia. Environ. Microbiol. **6**: 1096–1101.

Foster, J.S., Green, S.J., Ahrendt, S.R., Golubic, S., Reid, R.P., Hetherington, K.L. and Bebout, L. (2009) Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. ISME J. **3**: 573–587.

Garcia-Pichel, F. and Castenholz, R.W. (1991) Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J. Phycol. **27**: 395–409.

Gautret, P., Camoin, G., Golubic, S. and Sprachta, S. (2004) Biochemical control of calcium carbonate precipitation in modern lagoonal microbialites, Tikehau Atoll, French Polynesia. J. Sediment. Res. **74**: 462–478.

Gautret, P., de Wit, R., Camoin, G. and Golubic, S. (2006) Are environmental conditions recorded by the organic matrices associated with precipitated calcium carbonate in cyanobacterial microbialites? Geobiology **4**: 93–107.

Gischler, E., Gibson, M.A. and Oschmann, W. (2008) Giant Holocene freshwater microbialites, Laguna Bacalar, Quintana Roo, Mexico. Sedimentology **55**: 1293–1309.

Golubic, S. (1980) Early photosynthetic microorganisms and environmental evolution, In: R. Holmquist (ed.) *Life Science and Space Research (COSPAR),* Vol. 18. Pergamon, Oxford, pp. 101–107.

Golubic, S. (1983) Stromatolites, fossil and recent: a case history, In: P. Westbroek and E.W. Jong (eds.) *Biomineralization and Biological Metal Accumulation*. D. Reidel Publishing, Dordrecht, pp. 313–326.

Golubic, S. (1985) Microbial mats and modern stromatolites in Shark Bay, Western Australia, In: D.E. Caldwell, J.A. Brierley and C.L. Brierley (eds.) *Planetary Ecology*. Van Nostrand Reinhold, New York, pp. 3–16.

Golubic, S. (2000) Microbial landscapes: Abu Dhabi and Shark Bay, In: L. Margulis, C. Matthews and A. Haselton (eds.) *Environmental Evolution: Effects of the Origin and Evolution of Life on Planet Earth*. MIT Press, Boston, MA, pp. 117–138.

Golubic, S. and Hofmann, H.J. (1976) Comparison of modern and mid-Precambrian Entophysalidaceae (Cyanophyta) in stromatolitic algal mats: cell division and degradation. J. Paleontol. **50**: 1074–1082.

Golubic, S. and Seong-Joo, L. (1999) Early cyanobacterial fossil record: preservation, palaeoenvironments and identification. Eur. J. Phycol. **34**: 339–348.

Golubic, S., Seong-Joo, L. and Browne, K.M. (2000) Cyanobacteria: architects of sedimentary structures, In: R.E. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Berlin, pp. 57–67.

Grotzinger, J.P. and Rothman, D.H. (1996) An abiotic model for stromatolite morphogenesis. Nature **383**: 423–425.

Hofmann, H.J. (1976) Precambrian microflora, Belcher Islands, Canada: significance and systematics. J. Paleontol. **50**: 1040–1073.

Kalkowsky, E. (1908) Oolith und stromatolith im norddeutschen Buntsandstein. Zeitschr. Deut. Geol. Ges. **60**: 68–125.

Kawaguchi, T. and Decho, A. (2002) *In situ* imaging using two-photon and confocal laser scanning microscopy of bacteria and extracellular polymeric secretions (EPS) within marine stromatolites. Mar. Biotechnol. **4**: 127–131.

Kinsman, D.J.J. and Park, R.K. (1976) Algal belt and coastal sabkha evolution, Trucial Coast, Persian Gulf, In: M.R. Walter (ed.) *Stromatolites: Developments in Sedimentology*. Elsevier, Amsterdam, pp. 421–433.

Logan, B.W. (1961) Cryptozoon and associate stromatolites from the Recent, Shark Bay, Western Australia. J. Geol. **69**: 517–588.

MacIntyre, I.G., Reid, R.P. and Steneck, R.S. (1996) Growth history of stromatolites in a Holocene fringing reef, Stocking Island, Bahamas. J. Sediment. Res. **66**: 231–242.

Palmisano, A.C., Summons, R.E., Cronin, S.E. and Des Marais, D.J. (1989) Lipophilic pigments from cyanobacterial (blue-green-algal) and diatom mats in Hamelin Pool, Shark Bay, Western Australia. J. Phycol. **25**: 655–661.

Papineau, D., Walker, J.J., Mojzsis, S.J. and Pace, N.R. (2005) Composition and structure of microbial communities in stromatolites of Hamelin pool in Shark Bay, Western Australia. Appl. Environ. Microbiol. **71**: 4822–4832.

Paterson, D.M., Aspden, R.J., Visscher, P.T., Consalvey, M., Andres, M.S., Decho, A.W., Stolz, J. and Reid, P. (2008) Light-dependent biostabilisation of sediments by stromatolite assemblages. PLoS ONE **3**: e3176.

Planavsky, N. and Ginsburg, R.N. (2009) Taphonomy of modern marine Bahamian microbialites. Palaios **24**: 5–17.

Reid, R.P., James, N.P., Macintyre, I.G., Dupraz, C.P. and Burne, R.V. (2003) Shark Bay stromatolites: microfabrics and reinterpretation of origins. Facies **49**: 299–324.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, G., Paerl, H.W., Pinckney, J.L., Prufert-Bebout, L., Steppe, T.F. and Des Marais, D.J. (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature **406**: 989–992.

Richert, L., Golubic, S., De Le Gue, R., Herve, A. and Payri, C. (2006) Cyanobacterial populations that build 'kopara' microbial mats in Rangiroa, Tuamotu Archipelago, French Polynesia. Eur. J. Phycol. **41**: 259–279.

Schopf, J.W. and Klein, C. (eds.) (1992) *The Proterozoic Biosphere, A Multidisciplinary Study*. Cambridge University Press, Cambridge, 1348 pp.

Seilacher, A. and Pflüger, F. (1994) From biomats to benthic agriculture: a biohistoric revolution, In: W.E. Krumbein, D.M. Peterson and L.J. Stal (eds.) *Biostabilization of Sediments*. Bibliotheks- und Informationssystem der Carl von Ossietzky Universität, Oldenburg, pp. 97–105.

Seong-Joo, L., Golubic, S. and Zhang, Y. (1999) Paleoenvironmental distribution of silicified microfossil assemblages from Gaoyuzhuang Formation, North China. Acta Micropalaeontologica Sinica **16**: 247–268.

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DIVERSITY AND ROLE OF CYANOBACTERIA AND AEROBIC HETEROTROPHIC MICROORGANISMS IN CARBON CYCLING IN ARID CYANOBACTERIAL MATS

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1. Introduction

The south-east coast of the Arabian Gulf has been explored intensively since the 1960s, and is considered a model area for the study of marine carbonate sedimentation under extreme arid conditions (Kinsman, 1964; Evans, 1966; Purser, 1973). The coast consists of a set of barrier islands separating the shallow open waters (<20 m) from a series of protected lagoons, each rimmed by a broad intertidal flat drained by ponded meandering tidal creeks and covered by microbial mats. The geological base of Miocene and Pleistocene deposits protrudes with a few outcrops above the Holocene deposits; the latter include an extended evaporitic sabkha plain developed behind a broad intertidal belt of microbial mats. Different mats are zonally distributed within the belt as described by Kendall and Skipwith (1968) for the lagoons in the west, and by Kinsman and Park (1976) for lagoons in the east of the old Abu Dhabi town. An aerial view of algal belt in Fig. 1a, taken in the 1960s, represents a historic record in view of the enormous development that has taken place along this coast in recent years.

The coast at Abu Dhabi is gradually prograding seaward in the course of several thousand years (Kinsman and Park, 1976) following relatively fast early Holocene transgression (Sarnthein, 1972). The coastal progression initially above 1 m year^{-1} has more recently added ca. 0.7 m of land annually, whereas the average vertical sediment accretion has remained less than 3 mm year^{-1} (Lokier and Steuber, 2008). The sediment accretion selectively favors topographic depressions: most of the sediment is deposited within the intertidal zone where it is mediated by microbial mats. Microbial activities, mainly cyanobacterial primary production

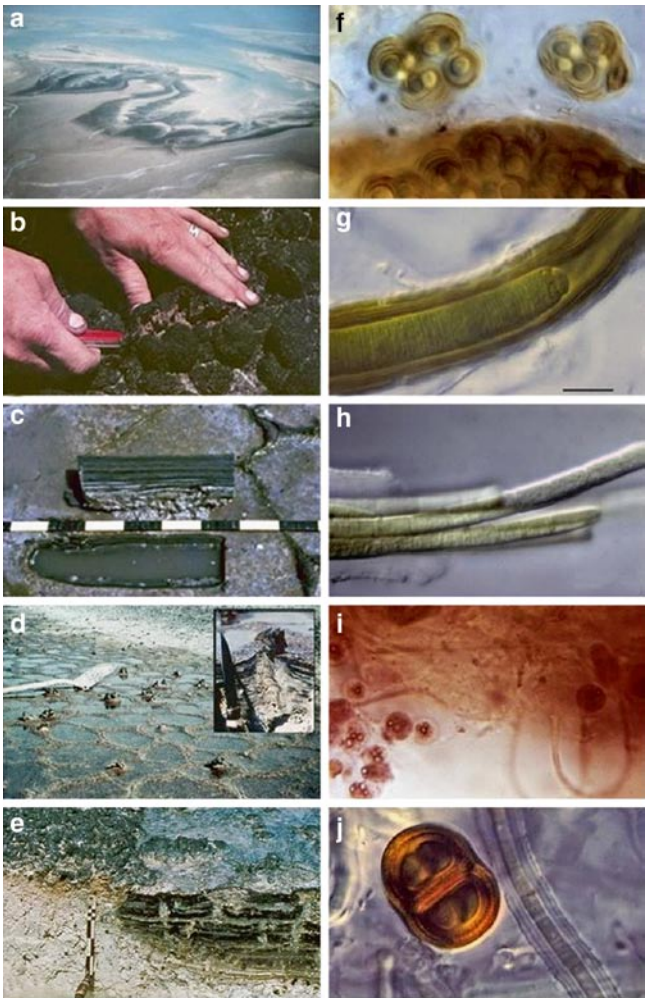

Figure 1. Photographs showing (**a**) aerial view of microbial belt and sabkha plain (*below*) east of Abu Dhabi (taken in 1967); (**b**) pustular mat showing domed structure with degradational holes beneath; (**c**) inundated flat mat cracked into polygons, section shows alternating organic and carbonate layers. Scale sections are 5 cm each; (**d**) flat mat cracked into polygons with pinnacles growing on elevated parts; (**e**) contact between well drained pinnacle mat (*left*) with complete aerobic decomposition below, and inundated flat mat (*right*) with anaerobic decomposition leaving an organic residue of polygons preserved. The scale sections are 5 cm (Field photographs are courtesy of David J.J. Kinsman and Robert K. Park); (**f**) *Entophysalis major*, a coccoid cyanobacterium producing pustular mats; (**g**) *Lyngbya aestuarii* from the surface layer of the Abu Dhabi flat mat. The scale is 10 μm for all photomicrographs; (**h**) *Microcoleus chthonoplastes* forms blue-green layer of flat mats; (**i**) the purple layer of flat mats with *Chromatium vinosum* and *Chloroflexus* spp.; (**j**) *Chroococcus* sp. and *Schizothrix splendida* from the Abu Dhabi pinnacle mat.

as well as aerobic and anaerobic decomposition of organic matter are responsible for the distribution and rates of incorporation of sediments and, ultimately, for the formation of the coastal sabkha plain (Golubic, 1991, 2000). As a part of this process, the coast is transformed from a high energy sedimentary system of channels and megaripples shaped by waves and currents to an almost perfectly flat supratidal sabkha plain with its own hydrological regime, geochemistry, evaporate mineralogy, and regulation mechanisms (Barth and Böer, 2002).

The tidal regime and sedimentation patterns within the microbial belt are strongly modified by the growth of microbial communities dominated by cyanobacteria as the principal primary producers. Their environmental preferences and growth significantly affect water drainage and retention, thus producing and modifying the environments for microbial decomposition of organic matter, which takes place in an array of oxic and anoxic microenvironments affecting nutrient cycling.

The above observations resulting from an interdisciplinary study conducted in the late 1960s analyzed the distribution of cyanobacteria and associated sedimentary structures in terms of the dynamics of growth and decomposition of microbial mats (Kinsman and Park, 1976; Park, 1977; Golubic, 1976, 1991, 2000). Microbial mats of Abu Dhabi were revisited in 2004, and subjected to a study of microbial diversity and activities using biochemical, molecular, and microsensor approaches (Abed et al., 2006, 2007, 2008). Here, we focus on the role of the most active processes driving the carbon cycle located at the uppermost layers of ponded mats.

1.1. EXTREME ARID ENVIRONMENTAL CONDITIONS AND MULTIPLE STRESS

The harsh climatic conditions of the Abu Dhabi coast were summarized by Kinsman and Park (1976) as follows: The coast is exposed to predominant winds from the NW; tidal range of ca. 200 cm diminishes in protected lagoons to ca. 120 cm; air temperature changes seasonally from 15°C to 47°C, and temperature of lagoonal waters from 15°C to 40°C; salinities of lagoonal waters range from 4.2% to 7%; the climate is extremely arid with sporadic rainfall ranging from 400 to 600 mm, in contrast to the evaporation rates of approximately 1,500 mm per annum. This account does not take into consideration the wind-dependent irregularity of tidal flooding, which exposes isolated ponds to complete desiccation and solar heating between tides. Due to the tidal regime, salinity fluctuates locally from 5% to 22% at a diurnal cycle depending on the mat's tidal position, whereas the water temperature increases up to 55°C in hot summers. The combined stress by the simultaneous effect of multiple extreme factors (hypersalinity, very high temperatures, UV and light intensity, and desiccation) constitutes strong selective pressure (Abed et al., 2008). Specialized cyanobacterial mats often develop under these extreme conditions, which control the abundance and activity of grazing organisms (Javor and Castenholz, 1984; Cohen, 1989; Farmer, 1992).

1.2. MICROBIAL MAT TYPES

Zonal distribution of various microbial mats across the intertidal ranges of Abu Dhabi coast has been described on the basis of their macroscopic appearance (color and texture), associated sedimentary features, and mineral precipitates (Kendall and Skipwith, 1968, 1969; Abed et al., 2008). The lowest intertidal ranges are characterized by the Pustular Mat ("cynder" mat of Kendall and Skipwith 1968; "mamillate" mat of Golubic, 1991), which drains effectively during low tide, providing oxidized conditions in the underlying sediments. The mat is completely decomposed, leaving little organic residue in the sediment. Growth and decomposition of the mat produce shallow 5–10 cm diameter vaults detached from the underlying sediment (Fig. 1b). The Flat Mats are physico-chemically and biologically stratified and inundated (Fig. 1c) and occur at different levels within the intertidal zone. In the upper intertidal ranges, the ponds periodically dry out between tidal cycles. The mats shrink due to water loss and crack to form polygons. The polygon edges bend upward, leaving a central depression. At the elevated edges of polygons a different microbial mat forms upright pointed pinnacles (Fig. 1d). The main habitat of the Pinnacle Mat is the crests and slopes of previously subtidal sandbars and the banks of tidal creeks. These habitats are drained between tides, assuring adequate oxygenation to the sediment underneath. The black FeS layer found in other mats is generally missing.

In the upper ranges of the intertidal zone the mats have a tough leathery texture. They are commonly detached from the underlying sediment by gas blisters, and continue to grow as Convoluted Mat ("Blister" mat of Kinsman and Park, 1976). This mat is associated with gypsum precipitation phase in the evaporitic sequence. In the uppermost ranges, the mat becomes thin (Fig. 1e, left) and finally dries and disintegrates with fragments scattered by wind. The ponded depressions at the upper tidal ranges are very hypersaline and lined with a slimy gelatinous mat. Beyond that level extends the surface of the sabkha plain proper with the dry and flaky texture of porous and fractured saline soils. The dominant cyanobacteria building the mats are illustrated in Fig. 1f–j and discussed below.

2. Autotrophy and Heterotrophy: General Overview of Carbon Cycling in Microbial Mats

Cyanobacterial mats are composed of physiologically different groups of autotrophic and heterotrophic microorganisms. Autotrophy, defined as the ability to convert CO_2 to organic carbon, is mainly performed by cyanobacteria and diatoms as oxygenic phototrophs in the uppermost parts of mats (Stal, 1995). Chemoautotrophy is performed by colorless sulfur bacteria, nitrifiers, methanogens, and some sulfate-reducing bacteria (Madigan et al., 2006). As a result of autotrophic activity, organics are directly excreted into the mat and utilized by heterotrophic bacteria (Bateson and Ward, 1988). Organics become available to the heterotrophs after

cell death and lysis of both autotrophs and heterotrophs (Fig. 2). Excretion products of autotrophs include low molecular weight compounds and extrapolymeric substances (EPS). Mannitol and arabinose were identified as excretion products using paper chromatography (Hellebust, 1965). Studies on hot spring mats demonstrated that hyperoxic and alkaline conditions resulting from photosynthetic activity promoted excretion of glycolate as the main compound among dissolved photosynthates (Bateson and Ward, 1988). Dissolved organic compounds produced in mats are apparently different during day and night (Nold and Ward, 1996; Jonkers and Abed, 2003). In the dark and under anoxic conditions of the mat, oxygenic phototrophs are able to ferment intracellular storage compounds such as glycogen. Fermentation products such as acetate, propionate, lactate, or ethanol (Anderson et al., 1987; Jørgensen et al., 1992; Stal, 1995; Nold and Ward, 1996; Stal and Moezelaar, 1997) are excreted and may provide additional soluble carbon substrates for heterotrophs (Fig. 2). EPS excretion by cyanobacteria was found to be stimulated under conditions of nutrients limitation, high salinity, and desiccation (Myklestad et al., 1989; Staats et al., 2000). EPS is predominantly composed of polysaccharides but also contain proteins, lipids, and nucleic acids (Decho, 1990; Flemming and Wingender, 2001), thus serving as a diverse source of organics for heterotrophs.

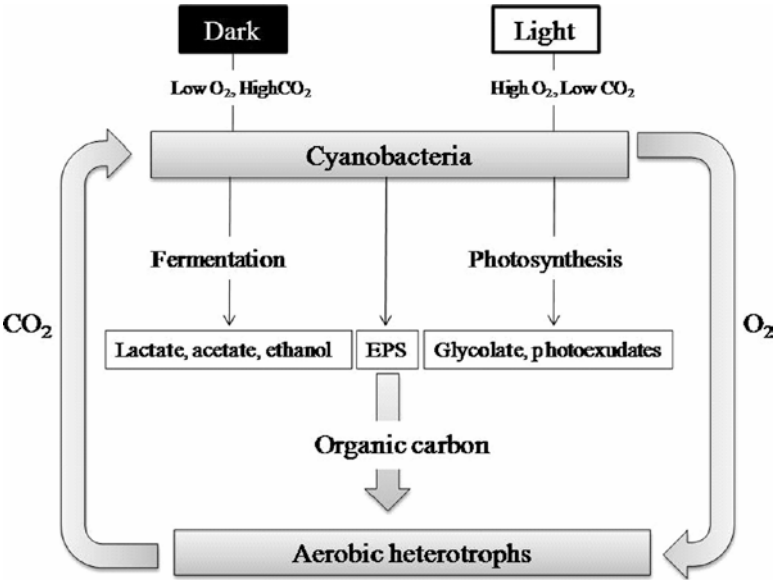

Figure 2. A scheme proposed to account for carbon cycling in the uppermost (2–3 mm) layer of a cyanobacterial mat. Cyanobacteria perform photosynthesis during daytime and fermentation during night producing organic compounds that are respired by aerobic heterotrophic microorganisms. The carbon dioxide produced by respiration can be consumed by cyanobacteria for photosynthesis (Bateson and Ward, 1988).

The organics produced by mat autotrophs nourish other functional groups that comprise aerobic heterotrophs, anaerobic primary degraders (e.g., polymer degraders), sulfate-reducing bacteria, and phototrophic (anoxygenic) as well as chemotrophic (aerobic) sulfide oxidizers. Under oxic conditions, aerobic heterotrophs oxidize organic compounds directly to CO_2. Aerobic utilization of glycolate and acetate was recently demonstrated in different depths of mats (Grötzschel et al., 2002) as well as in isolates of the *Roseobacter* group enriched from the photic zone of a mat (Jonkers and Abed, 2003). In the later study, it was demonstrated that aerobic heterotrophs of the genera *Roseobacter* and *Rhodobacter* grew preferably on simple photosynthetic exudates, whereas *Marinobacter* and *Halomonas* grew better on more complex organics such as yeast extract. Under anoxic conditions, fermentation and anaerobic respiration processes contribute increasingly to the consumption of organic substrates. Fermentative bacteria utilize sugars, amino acids, and complex substrates to produce alcohols and low molecular weight fatty acids (e.g., lactate, acetate, propionate) (Madigan et al., 2006). Sulfate reduction is believed to be the most dominant anaerobic respiration process in hypersaline microbial mats, where sulfate-reducing bacteria oxidize organics using sulfate as an electron acceptor. While previous studies postulated that the release of photosynthates may stimulate daytime sulfate-reduction (Fründ and Cohen, 1992; Canfield and Des Marais, 1993), others demonstrated the possibility of sulfate reduction taking place in fully oxic layer of microbial mats (Fründ and Cohen, 1992; Teske et al., 1998). Denitrification is believed to take place at the uppermost part of the anoxic layer because of its dependence on the continuous supply of organics and NO_3^- (Joye and Paerl, 1993; Paerl et al., 2000). Acetogenesis and methanogenesis have also been reported in microbial mats (Anderson et al., 1987). Acetogenic bacteria, for example, members of the *Clostridiales* were found to grow on complex organics like sugars and amino acids, whereas methanogenic *Archaea* convert fermentation products like H_2, CO_2, and other one-carbon compounds, and acetate to methane.

3. Carbon Flow in the Uppermost Mat Layer

With respect to carbon cycling, the biologically most active layers are the upper few millimeters, dominated by cyanobacteria and aerobic heterotrophs (Fig. 2). During daytime, this part is supersaturated with photosynthetically produced oxygen, while the CO_2 concentration remains low (Canfield and Des Marais, 1994; Wieland and Kühl, 2000). In contrast, during the night, anoxic conditions tend to spread upwards in mats due to continued oxygen consumption and sulfide production. In cyanobacterial mats, gross photosynthesis (i.e., O_2 production by photosynthesis) can reach up to ten times higher values than net photosynthesis (i.e., O_2 production by gross photosynthesis minus O_2 consumption by respiration)

(Wieland and Kühl, 2000). Gross photosynthesis can be measured accurately by O_2 microsensors using the light–dark shift technique (Revsbech and Jørgensen, 1983; Wieland and Kühl, 2000). Respiration in the light is higher than in the dark, indicating the use of soluble photosynthates by aerobic heterotrophs (Bateson and Ward, 1988; Glud et al., 1992; Paerl et al., 1993). Furthermore, aerobic heterotrophs utilize the complex, mostly polymeric carbon compounds of dead cells. As a result of all these aerobic activities, most of the oxygen produced by gross photosynthesis is immediately respired, making aerobic degradation quantitatively as important as photosynthesis for the mat carbon budget. Indeed, most of the CO_2 for photosynthesis is believed to be supplied by respiration within the mat, and net accretion of biomass from external CO_2 is very low, leading to a growth of only a few millimeters per year (Reid et al., 2000).

Even though the importance of aerobic heterotrophs for the carbon cycle of mats is intuitively obvious, this component has been treated in previous research projects merely as a "bulk community." Most progress in research of mats has been based on the macroscopically and microscopically most obvious organisms, viz. the cyanobacteria and bacteria of the sulfur cycle. The study of individual anaerobes has led to the recognition of gliding and oxygen-tolerant sulfate-reducing bacteria as inhabitants of mats (Teske et al., 1998; Fukui et al., 1999; Minz et al., 1999). In comparison, insights into the diversity and carbon utilization characteristics of aerobic heterotrophs are few (Bateson and Ward, 1988; Jonkers and Abed, 2003).

4. Oxygenic Phototrophs: The Driving Force for Carbon Cycling

Cyanobacteria are the major primary producers in microbial mats, and their activity supports the carbon needs of the overall microbial community. Abu Dhabi microbial mats harbor diverse cyanobacteria, and the composition depends on the mat's tidal position and environmental conditions. *Microcoleus chthonoplastes* and *Lyngbya aestuarii* are the dominant species in many mats. Chlorophyll *a* analysis showed that the abundance of oxygenic phototrophs decreased going from the lower to the upper tidal zones (Abed et al., 2008). Many of the cyanobacteria that were present contained the extracellular amber-colored sun-screening pigment scytonemin, whose concentration was dependent on the increase of solar irradiation, salinity, and desiccation (Abed et al., 2008). The pustular mat was mainly composed of the coccoid cyanobacterium *Entophysalis major* (Fig. 1f). This cyanobacterium grows in three dimensions producing ample EPS, which makes the mat translucent, and is protected from UV by the pigment scytonemin (Garcia-Pichel et al., 1993; Proteau et al., 1993).

The surface of the inundated flat mats is coated by a layer of the filamentous cyanobacterium *L. aestuarii.* This organism comprises trichomes build of short cells arranged like stacked coins and is characterized by a calyptra-covered terminal cell. Trichomes are surrounded by multilayered sheaths, heavily pigmented by

scytonemin (Fig. 1g). A minor component in the surface layer of the mat includes large coccoid cyanobacterium *Chroococcus* sp. Beneath this layer, bundles of *M. chthonoplastes* (Fig. 1h) dominate. This organism is surrounded by a thick EPS sheath, but does not show the ability to produce scytonemin. It is characterized by bundled trichomes and a vigorous gliding movement within compounded sheaths. Minor components of this cyanobacterial layer are comprised of sheathed thin filamentous cyanobacteria, usually classified as *Leptolyngbya* sp. The cyanobacterial layer becomes pale-green to salmon-pink in its lower parts, occupied increasingly by the green non-sulfur *Chloroflexus* bacteria (Fig. 1i).

Like in the flat mats, the surface layers of the pinnacle mats are protected by scytonemin-containing cyanobacteria: *L. aestuarii* and *Chroococcus* sp., with an occasional colony of *E. major.* The latter is less competitive in mid-tidal ranges due to increased air-exposure during low tide. The main constituent of the cyanobacterial layer in the pinnacle mat is the mulltitrichomous cyanobacterium *Schizothrix splendida* (Fig. 1j) which, like *M. chthonoplastes*, does not produce scytonemin. This organism has rounded-conical terminal cells and one to several trichomes per filament. The trichomes are slightly narrower than those of *M. chthonoplastes*. The sheath is hyaline, internally distinctly layered, and externally undulated (Golubic, 1973).

The convoluted mats consist of upward concave, water-holding depressions with *M. chthonoplastes*, and upward convex vaults built by *S. splendida*, but without pinnacle formation. The gelatinous mats are mainly populated by extremely halophilic unicellular cyanobacteria of the *Halothece* group (Garcia-Pichel et al., 1998). The surrounded sediments are dry and salt-saturated harboring coccoid cyanobacteria of the *Chroococcidiopsis* morphotype (Wierzchos et al., 2006).

5. Aerobic Heterotrophic Prokaryotes: Diversity and Fundamental Role in Microbial Mats

5.1. DIVERSITY AND ABUNDANCE OF AEROBIC HETEROTROPHIC MICROORGANISMS

Quantification of total bacteria, including cyanobacteria, in the hyperoxic stratum (top 2–3 mm) using real-time-PCR of a single Abu Dhabi mat showed bacterial cell numbers of ca. 10^8 cells g^{-1} (Kohls and Abed, unpublished data, 2008). Interestingly, it was also shown that this layer contained around 10^7 archaeal cells per gram. This indicated that aerobic *Bacteria* as well as *Archaea* were abundant in the uppermost aerobic layer of mats and may have played an essential role in carbon metabolism.

16S rRNA gene cloning of the oxic layers of the different mats along an intertidal transect of Abu Dhabi revealed a high diversity of bacteria, including cyanobacteria, which varied from one mat to the other. The major detected

non-cyanobacterial groups were *Bacteriodetes*, *Chloroflexus*-related bacteria and *Proteobacteria*. Other clones were distributed among the groups *Actinobacteria*, *Deinococci*, *Chlorobiales*, *Gemmatimonadetes*, *Planctomycetes*, and *Spirochaetes* (Abed et al., 2007). The detection of groups like *Bacteriodetes*, the *Chloroflexus* group, and *Proteobacteria* in all the mats points out to their importance in hypersaline microbial mats. *Bacteriodetes* are chemoorganotrophic, possessing the capability to degrade polymeric substrates such as polysaccharides (Kirchman, 2002; Madigan et al., 2006). The mats with the highest salinity (≈20%), possessed a large number of sequences related to the species *Salinibacter ruber*, which is known as an extremely halophilic bacterium (Antón et al., 2002). The genus *Salinibacter* contains bacteria that resemble archaeal halophiles of the family *Halobacteriaceae* in their extreme tolerance to high salinities (Corcelli et al., 2004). They do not accumulate organic osmotic solutes as all other known halophilic and halotolerant aerobic bacteria (Oren et al., 2002) and their enzymes are adapted to function in the presence of high salinities (Oren and Mana, 2002).

Chloroflexus-like bacteria possess the capability of anoxygenic photoautotrophy, photoheterotrophy, and aerobic chemoorganotrophy (Madigan et al., 2006). The close coexistence of cyanobacteria and *Chloroflexus* group (Fig. 3a, b) suggests a role of this group in carbon cycling within mats, a role that has been already demonstrated in previous studies (D'Amelio et al., 1987; Canfield and Des Marais, 1993; Ley et al., 2006). *Chloroflexus*-like bacteria dominate in the uppermost mat layer, whereas cyanobacteria were located underneath (Fig. 3a). This spatial distribution was revealed using Catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH). In the oxic layer, where no H_2S for anoxygenic photosynthesis is available, *Chloroflexus*-related bacteria may switch to a

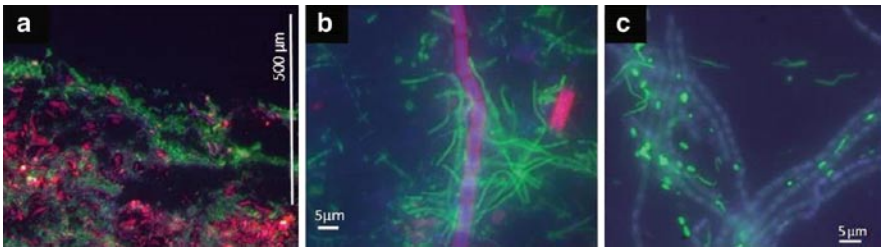

Figure 3. Catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) of 5 µm thick cross-section of a microbial mat using the probes (CFX1223 and GSNB-941) specific for *Chloroflexus*-related bacteria: (**a**) green color shows probe signal; pink color shows cyanobacterial autofluorescence; blue color shows remaining DAPI signals of the remaining cells; (**b**) a closer view of the same cross-section; (**c**) CARD-FISH hybridization of unialgal cyanobacterial culture with associated heterotrophic bacteria, probe- specific for bacteria from the *Bacteriodetes* group (CF319a).

heterotrophic mode, consuming photosynthetic products using oxygen (Madigan et al., 2006; Hanada and Pierson, 2007).

Archaeal 16S rRNA clones from the oxic layer of the mats exhibited a unique diversity. Most sequences were similar to the extremely halophilic archaeal groups *Halobacterium*, *Halohabdus*, and *Halorubrum*. In addition, high abundance of sequences related to the unknown *Euryarchaeota* and *Crenarchaeota* from the marine benthic group B (MBGB) was detected (unpublished data). *Crenarchaeota* are widely distributed among aquatic environments, but their metabolic functions are still unknown. The bacterial and archaeal diversities and abundances in Abu Dhabi mats seem to be very similar to those in Guerrero Negro mats, Baja California, Mexico (Ley et al., 2006).

5.2. INTERACTION BETWEEN CYANOBACTERIA AND AEROBIC HETEROTROPHIC BACTERIA IN CARBON CYCLING

The close association between cyanobacteria and aerobic heterotrophic bacteria in the upper oxic layer of microbial mats and in cyanobacterial cultures (Fig. 3c) suggests a close functional interaction. This interaction may be mutualistic, promoting the survival and persistence of both groups, but can also be competitive for scarce and dilute nutrients and other resources (Paerl, 1996). A number of studies have shown that cyanobacterial exudates can be easily assimilated and recycled by associated heterotrophic bacteria (Bauld and Brock, 1974; Bateson and Ward, 1988; Epping et al., 1999). High concentrations of O_2 released by cyanobacteria during oxygenic photosynthesis can be toxic to a variety of metabolic and biosynthetic pathways. The respiring bacteria counteract the chemical changes in O_2, CO_2, and pH induced by photosynthesis (Wieland and Kühl, 2006).

The interaction between cyanobacteria and aerobic heterotrophs is specific and depends on the type of available cyanobacterial exudates and the cyanobacterial species (Kohls et al., unpublished data, 2008). Using 67 strains isolated from the top layer of a mat from the Arabian Gulf, it was shown that bacteria belonging to the genera *Marinobacter*, *Halomonas*, *Roseobacter*, *Rhodobacter*, *Bacillus*, and *Micrococcus* were specialized in degrading acetate and glycolate. On the other hand, *Bacteroidetes* and some *Archaea* like *Halobacterium*, *Halorubrum*, and *Haloferax* were only enriched on *Spirulina*-extract, indicating their specialization in the degradation of complex polymeric substrates and dead cyanobacterial cells. Comparison of mono-cyanobacterial cultures isolated from different mats showed that associated heterotrophs differed from one cyanobacterial culture to another, although some bacteria were common. The associated bacteria of these cultures belonged to the groups of *Alpha-* and *Gammaproteobacteria*, *Chloroflexus*-related bacteria, and most of them belonged to the *Bacteroidetes* group (unpublished data). Cultivation of an axenic culture of *M. chthonoplastes* PCC 7420 (Pasteur Culture Collection of Cyanobacteria, Paris) with and without the addition of a filtrate containing aerobic heterotropbic bacteria and released

substances (obtained from a closely related unialgal culture), showed that the growth of cyanobacteria was stimulated upon addition of the aerobic heterotrophs. The heterotrophs apparently used the carbon compounds as well as photosynthetically produced oxygen to degrade cyanobacterial exudates aerobically. Furthermore, aerobic heterotrophs might have provided cyanobacteria with necessary vitamins and other growth factors. In contrast to this beneficial relationship, *Flexibacter*-related species were shown to lyse cyanobacterial filaments and grow directly on their fragments (Kohls et al., unpublished data, 2008).

6. Effect of Salinity on Carbon Cycling in Microbial Mats

6.1. SALINITY DETERMINES THE DIVERSITY OF PHOTOTROPHS AND HETEROTROPHS

Salinity plays a crucial role in carbon cycling in microbial mats by affecting the diversity and activities of the involved microorganisms. Many studies on solar salterns and evaporation ponds along salinity gradients demonstrated a decrease in bacterial diversity when salinity increased (Gerdes et al., 1985; Benlloch et al., 2002; Casamayor et al., 2002; Jungblut et al., 2005). Studies on Abu Dhabi intertidal flats, where mats are subjected to different daily and seasonal regimes of salinity, depending on their tidal position (Fig. 4, lower scheme), showed that this is not always the case. The total bacterial diversity in the upper tidal mats where salinity fluctuates between 6% and 20% was higher than in middle and lower tidal mats at salinities ranging between 6% and 10% (Abed et al., 2007). This might be due to the existence of halophilic bacteria adapted to a broad range of salt concentrations and/or the presence of different bacterial groups with different salinity optima. Salinity fluctuation might have promoted the coexistence of a salinity-resistant fraction of the original community and the newly introduced halophilic bacteria, resulting in an increase of biodiversity (Buckling et al., 2000; Johst and Huth, 2005). Cyanobacteria exhibited their highest abundance and diversity in the lower and middle tidal mats, but decreased significantly in the uppermost tidal mat, probably in favor of halotolerant and halophilic species. In contrast, the aerobic heterotrophic CFB group was more abundant and diverse in the upper tidal mat than in the middle and lower tidal mats (Abed et al., 2007, 2008). This could be attributed to the abundance of EPS in the upper tidal mat (Kirchman, 2002), the production of which is stimulated under high salt stress (Liu and Buskey, 2000; Abdullahi et al., 2006).

6.2. TIDAL POSITION AND CARBON CYCLING

Rates of photosynthesis and aerobic respiration are controlled by salinity. Previous studies using cultures of *Microcoleus*, *Anabaena*, and *Spirulina* spp. or intact mat pieces showed a significant reduction in rates of photosynthesis and respiration

by osmotic stress (Vonshak et al., 1988; Fernandes et al., 1993; Karsten, 1996, Garcia-Pichel et al., 1999, Wieland and Kühl, 2006). When microbial mats located at different tidal transects in Abu Dhabi were compared, the rates of gross photosynthesis and aerobic respiration showed a decreasing trend from lower to upper tidal range with maximum rates detected in the low tidal mat (Fig. 4) (Abed et al., 2007). The two processes remained coupled and any decrease in one process resulted in a decrease in the other. Gross photosynthesis was inhibited more than aerobic respiration (44% and 34%, respectively) from low tidal to middle tidal mat, but when moving to the upper tidal mat, aerobic respiration was inhibited more strongly relative to gross photosynthesis (84% and 65%, respectively). The inhibition of gross photosynthesis along the increasing salinity gradient correlated well with reduction in cyanobacterial abundance and diversity (Abed et al., 2007). However, the diversity and abundance of aerobic heterotrophs showed an opposite trend to the rates of aerobic respiration. This might indicate that all respiring bacte-

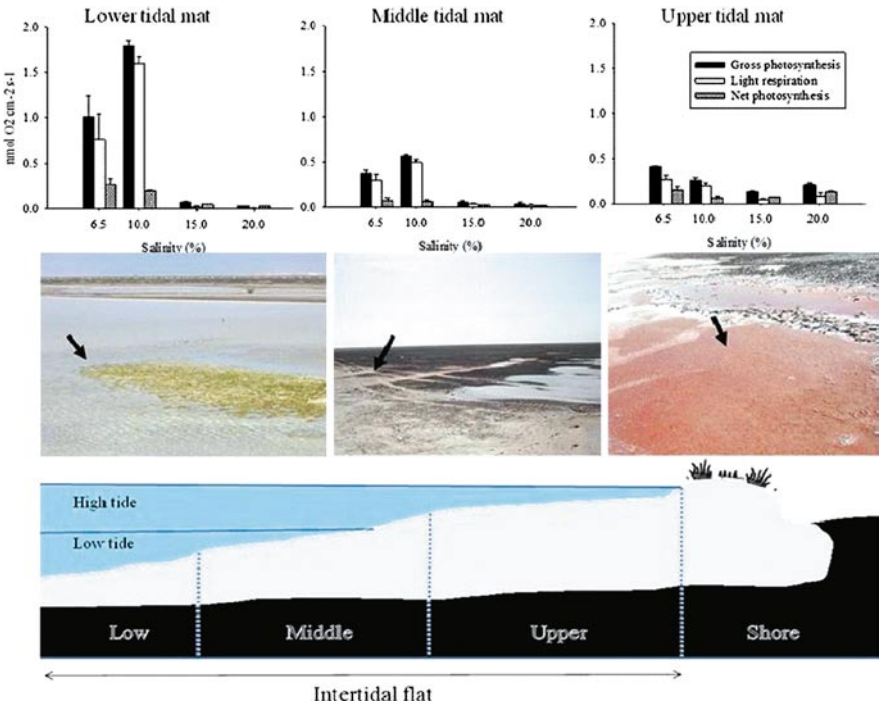

Figure 4. Salinity dependence of areal rates of gross photosynthesis, net photosynthesis and light respiration in three microbial mats from lower, middle and upper tidal ranges (Abed et al., 2007). (The photographs show the locations of the studied mats, their relative position with respect to tides indicated on the scheme below. Note that the mats are exposed to different regimes of salinities depending on their tidal position.)

rial populations survived at 20% salinity, but only the halophilic component of the community was active, the rest becoming active only when salinity decreased during the tidal cycle (Abed et al., 2007).

6.3. WHY HIGH SALINITIES INHIBIT PHOTOSYNTHESIS AND AEROBIC RESPIRATION?

All mats from Abu Dhabi intertidal flat, irrespective of their tidal location, showed a decrease in gross and net photosynthesis and light respiration with increasing salinity above 10%. High salinities exert an osmotic stress on photosynthetic organisms, leading to limited metabolic activity and inhibition of photosynthesis (Garcia-Pichel et al., 1999). Cyanobacteria reallocate a large fraction of cell resources for the production of inorganic and organic osmoregulators in order to maintain an osmotic equilibrium with the surrounding brine (Blumwald and Tel-Or, 1983; Mackay et al., 1983; Reed et al., 1984; Csonka, 1989; Fernandes et al., 1993; Galinski, 1995). Inhibition of photosynthesis by high salinity could also be due to the limited availability of CO_2, given that the solubility and diffusion coefficient of gases decrease with increasing salinity. Garcia-Pichel et al. (1999) explained the inhibition of photosynthesis by salinity by postulating that when salinity increases, oxygen solubility in the overlying seawater decreases, and the oxygen produced by photosynthesis cannot be dissolved completely in the seawater, but remains trapped within mats. This results in the creation of localized elevated oxygen tension within mats, which may have a negative effect on photosynthesis. High oxygen tension result in photooxidation in mats and oxygen acts as a competitive inhibitor of RubisCO carboxylase activity. The inhibition of light respiration might be a direct consequence of photosynthesis inhibition, since the two processes are coupled (Canfield and Des Marais, 1993; Kühl et al., 1996). Alternatively, it may be due to the decrease in oxygen solubility and diffusion coefficient at elevated salinities (the brine at 20% salinity has almost half the capacity to hold oxygen than the brine at 6.5% salinity).

7. Temperature Regulation of Carbon Cycling

Temperature controls bacterial diversity and rates of bacterial metabolic processes. Abu Dhabi mats are exposed to a daily temperature fluctuation due to tidal regime and temperatures exceeding 50°C in summer. Using microsensors, we studied the effect of different temperatures on rates of photosynthesis and aerobic respiration as well as the interaction between carbon and sulfur cycling in an upper tidal mat from Abu Dhabi (Abed et al., 2006). Rates of gross and net photosynthesis increased with increasing temperature with maximum detectable rates at 45°C, above which both processes were inhibited. The thickness of the photosynthetic

zone decreased with increasing temperature. While gross and net photosynthesis showed increasing rates with temperatures below 45°C, aerobic respiration in the light remained constant. These trends were statistically evaluated and were shown to be significant. Increasing temperature apparently resulted in the uncoupling of gross photosynthesis and light respiration (Abed et al., 2006). This is unlike the case of elevated salinity, where rates of the two processes changed but remained coupled (Abed et al., 2007). Sulfide production remarkably increased in the light and dark to produce amounts that could not be removed by sulfide oxidation at 50–60°C. The sulfide produced had a clear and dramatic effect on other mat processes (see below). The upward diffusion of sulfide triggered migration of *M. chthonoplastes* to the mat surface (Abed et al., 2006), and probably also caused the organism to switch from oxygenic to anoxygenic photosynthesis at higher temperatures, by using sulfide as alternate electron donor (Jørgensen et al., 1983; Cohen et al., 1986; Stal, 1991).

Temperature has a direct effect on the enzymes involved in CO_2 fixation (Li et al., 1984; Sukenik et al., 1987; Raven and Geider, 1988; Davison, 1991) and on the diffusion of CO_2/HCO_3^- and nutrients across the plasmalemma and/or chloroplast membrane(s) (Davison, 1991). This explains the increase in rates of gross photosynthesis between 25°C and 45°C. The inhibition of gross photosynthesis at 50°C was most likely due to the inhibition of photosystem II (PSII) by sulfide (and not by temperature), which reached maximum concentrations at this temperature. By removing this sulfidic layer, the mat was still able to photosynthesize at 60°C. Sulfide was shown to inhibit photosystem II (PSII), which is responsible for the generation of electrons. It was also shown to block the electron transport chain by reacting with cytochromes, heme-proteins, and metal proteins (Cohen et al., 1986). Temperature might have inhibited aerobic respiration by directly influencing the membrane composition and enzyme properties of respiring bacteria (Thamdrup et al., 1998). Otherwise, the dramatic increase in the activity of sulfate-reducing bacteria might have lead to scavenging of photosynthetic and other excretion products, which are normally respired by the aerobic bacteria (Bateson and Ward, 1988; Glud et al., 1992; Paerl et al., 1993). Previous studies postulated that the release of photosynthates may stimulate daytime sulfate reduction (Fründ and Cohen, 1992; Canfield and Des Marais, 1993). Therefore, the decrease in light respiration and its uncoupling with gross photosynthesis could be explained by the inability of aerobic bacteria to compete for organics with sulfate reducing bacteria in the light (Abed et al., 2006).

8. Concluding Remarks

Microbial mats are complex ecosystems that grow by vigorous oxygenic photosynthesis at the sediment–water interface, producing high levels of oxygen and organics. Similarly vigorous are the degradation processes of the produced organic matter carried out by aerobic heterotrophic bacteria with the consequence

of oxygen depletion and introduction of anoxic conditions at night and in the sediment under the mat. The diversity of aerobic heterotrophs differ from one mat to another and even from one cyanobacterial strain to another, depending on the environmental conditions and the available organics. Salinity and temperature control the rates of photosynthesis and respiration, thus every mat exhibits variable rates depending on its position along the tidal flat. The balance between organics production and decomposition determines the accretion rate of mats, which is normally very low.

9. References

Abdullahi, A.S., Underwood, G.J.C. and Gretz, M.R. (2006) Extracellular matrix assembly in diatoms (*Bacillariophyceae*). V. Environmental effects on polysaccharide synthesis in the model diatom, *Phaeodactylum tricornutum*. J. Phycol. **42**: 363–378.

Abed, R.M.M., Polerecky, L., Al Najjar, M. and de Beer, D. (2006) Effect of temperature on photosynthesis, oxygen consumption and sulfide production in an extremely hypersaline cyanobacterial mat. Aquat. Microb. Ecol. **44**: 21–30.

Abed, R.M.M., Kohls, K. and de Beer, D. (2007) Effect of salinity changes on the bacterial diversity, photosynthesis and oxygen consumption of cyanobacterial mats from an intertidal flat of the Arabian Gulf. Environ. Microbiol. **9**: 1384–1392.

Abed, R.M.M., Kohls, K., Schoon, R., Scherf, A.K., Schacht, M., Palinska, A.K., Al-Hassani, H., Hamza, W., Rullkötter, J. and Golubic, S. (2008) Lipid biomarkers, pigments and cyanobacterial diversity of microbial mats across intertidal flats of the arid coast of the Arabian Gulf (Abu Dhabi, UAE). FEMS Microbiol. Ecol. **65**: 449–462.

Anderson, K.L., Tayne, T.A. and Ward, D.M. (1987) Formation and fate of fermentation products in hot spring cyanobacterial mats. Appl. Environ. Microbiol. **53**: 2343–2352.

Antón, J., Oren, A., Benlloch, S., Rodríguez-Valera, F., Amann, R. and Rosselló-Mora, R. (2002) *Salinibacter ruber* gen. nov., sp nov., a novel, extremely halophilic member of the bacteria from saltern crystallizer ponds. Int. J. Syst. Evol. Microbiol. **52**: 485–491.

Barth, H.-J. and Böer, B. (eds.) (2002) *Sabkha Ecosystems. Vol. 1. The Arabian Peninsula and Adjacent Countries.* Kluwer, Dordrecht.

Bateson, M.M. and Ward, D.M. (1988) Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. Appl. Environ. Microbiol. **54**: 1738–1743.

Bauld, J. and Brock, T.D. (1974) Algal excretion and bacterial assimilation in hot spring algal mats. J. Phycol. **10**: 101–106.

Benlloch, S., López-López, A., Casamayor, E.O., Øvreås, L., Goddard, V. and Daae, F.L. (2002) Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environ. Microbiol. **4**: 349–360.

Blumwald, E. and Tel-Or, E. (1983) Salt adaptation of the cyanobacterium *Synechococcus* 6311 growing in continuous culture (turbidostat). Plant Physiol. **74**: 183–185.

Buckling, A., Kassen, R., Bell, G. and Rainey, P.B. (2000) Disturbance and diversity in experimental microcosms. Nature **408**: 961–964.

Canfield, D.E. and Des Marais, D.J. (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim. Cosmochim. Acta **57**: 3971–3984.

Canfield, D.E. and Des Marais, D.J. (1994) Cycling of carbon, sulfur, oxygen and nutrients in a microbial mat. Science **251**: 1471–1473.

Casamayor, E.O., Massana, R., Benlloch, S., Øvreås, L., Diez, B. and López-López, A. (2002) Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ. Microbiol. **4:** 338–348.

Cohen, Y. (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions, In: Y. Cohen and E. Roseberg (eds.) *Microbial Mats. Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, pp. 22–36.

Cohen, Y., Jørgensen, B.B., Revsbech, N.P. and Poplawski, R. (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl. Environ. Microbiol. **51**: 398–407.

Corcelli, A., Lattanzio, V.M.T., Mascolo, G., Babudri, F., Oren, A. and Kates, M. (2004) Novel sulfonolipid in the extremely halophilic bacterium *Salinibacter ruber*. Appl. Environ. Microbiol. **70**: 6678–6685.

Csonka, L.N. (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. **53**: 121–147.

D'Amelio, E.D., Cohen, Y. and Des Marais, D.J. (1987) Association of a new type of gliding, filamentous, purple phototrophic bacterium inside bundles of *Microcoleus chthonoplastes* in hypersaline cyanobacterial mats. Arch. Microbiol. **147**: 213–220.

Davison, I.R. (1991) Environmental effects on algal photosynthesis: temperature. J. Phycol. **27**: 2–8.

Decho, A. (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Annu. Rev. **28**: 73–153.

Epping, E.H.G., Khalili, A. and Thar, R. (1999) Dynamics of photosynthesis and respiration in an intertidal biofilm. Limnol. Oceanogr. **44**: 1936–1948.

Evans, G. (1966) The recent sedimentary facies of the Persian Gulf Region. Philos. Tr. R. Soc. S-A. **259**: 291–298.

Farmer, J.D. (1992) Grazing and bioturbation in modern microbial mats, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere – A Multidisciplinary Study*. Cambridge University Press, Cambridge, pp. 247–251.

Fernandes, T.A., Lyer, V. and Apte, S.K. (1993) Differential responses of nitrogen fixing cyanobacteria to salinity and osmotic stresses. Appl. Environ. Microbiol. **59**: 899–904.

Flemming, H.C. and Wingender, J. (2001) Relevance of microbial extrapolymeric substances (EPSs) – Part I: structural and ecological aspects. Water Sci. Technol. **43**: 1–8.

Fründ, C. and Cohen, Y. (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl. Environ. Microbiol. **58**: 70–77.

Fukui, M., Teske, A., Aßmus, B., Muyzer, G. and Widdel, F. (1999) Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus *Desulfonema*). Arch. Microbiol. **172**: 193–203.

Galinski, E.A. (1995) Osmoadaptation in bacteria. Adv. Microb. Physiol. **37**: 274–328.

Garcia-Pichel, F., Wingard, C.E. and Castenholz, R.W. (1993) Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. Appl. Environ. Microbiol. **59**: 170–176.

Garcia-Pichel, F., Nübel, U. and Muyzer, G. (1998) The phylogeny of unicellular, extremely halotolerant cyanobacteria. Arch. Microbiol. **169**: 469–482.

Garcia-Pichel, F., Kühl, M., Nübel, U. and Muyzer, G. (1999) Salinity dependent limitation of photosynthesis and oxygen exchange in microbial mats. J. Phycol. **35**: 184–195.

Gerdes, G., Krumbein, W.E. and Holtkamp, E. (1985) Salinity and water activity related zonation of microbial communities and potential stromatolites of the Gavish Shabka, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems. The Gavish Shabka*. Springer, New York, pp. 238–266.

Glud, R.N., Ramsing, B. and Revsbech, N.P. (1992) Photosynthesis and photosynthesis coupled respiration in natural biofilms quantified with oxygen microsensors. J. Phycol. **28**: 51–60.

Golubic, S. (1973) Three new species of *Schizothrix* Kützing (Cyanophyta) from marine algal mats. Schweiz. Z. Hydrol. **35**: 152–156.

Golubic, S. (1976) Organisms that build stromatolites, In: M.R. Walter (ed.) *Stromatolites: Developments in Sedimentology*. Elsevier, Amsterdam, pp. 113–126.

Golubic, S. (1991) Microbial mats of Abu Dhabi, In: L. Margulis and L. Olendzenski (eds.) *Environmental Evolution, Effects of the Origin and Evolution of Life on Planet Earth*. MIT Press, Cambridge, MA, pp. 131–147.

Golubic, S. (2000) Microbial landscapes: Abu Dhabi and Shark Bay, In: L. Margulis, C. Matthews and A. Haselton (eds.) *Environmental Evolution: Effects of the Origin and Evolution of Life on Planet Earth*. MIT Press, Cambridge, MA, pp. 117–138.

Grötzschel, S., Abed, R.M.M. and de Beer, D. (2002) Metabolic shifts in hypersaline microbial mats upon addition of organic substrates. Environ. Microbiol. **4**: 683–695.

Hanada, S. and Pierson, B.K. (2007) The family *Chloroflexaceae*, In: S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt (eds.) *The Prokaryotes. A Handbook on the Biology of Bacteria*, Vol. 7. Springer, New York, pp. 815–842.

Hellebust, J. (1965) Excretion of some organic compounds by marine phytoplankton. Limnol. Oceanogr. **10**: 192–206.

Javor, B.J. and Castenholz, R.W. (1984) Invertebrate grazers of a microbial mat, Laguna Gurrero Negro, Mexico, In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites*. Alan R. Liss, New York, pp. 85–94.

Johst, K. and Huth, A. (2005) Testing the intermediate disturbance hypothesis: when will there be two peaks of diversity? Biodivers. Res. **11**: 111–120.

Jonkers, H.M. and Abed, R.M.M. (2003) Identification of aerobic heterotrophic bacteria from the photic zone of a hypersaline microbial mat. Aquat. Microb. Ecol. **30**: 127–133.

Jørgensen, B.B., Cohen, Y. and Revsbech, N.P. (1983) Transition from anoxygenic to oxygenic photosynthesis in a *Microcoleus chthonoplastes* cyanobacterial mat. Appl. Environ. Microbiol. **51**: 408–417.

Jørgensen, B.B., Nelson, D.C. and Ward, D.M. (1992) Chemotrophy and decomposition in modern microbial mats, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge University Press, Cambridge, pp. 287–293.

Joye, S.B. and Paerl, H.W. (1993) Contemporaneous nitrogen fixation and dentrification in intertidal microbial mats: rapid response to runoff events. Mar. Ecol. Prog. Ser. **94**: 267–274.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ. Microbiol. **7**: 519–529.

Karsten, U. (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (Cyanobacteria) from benthic microbial mats: response to salinity change. J. Phycol. **32**: 501–506.

Kendall, C.S.St. and Skipwith, P.A.d,E. (1968) Recent algal mats of a Persian Gulf lagoon. J. Sediment. Petrol. **38**: 1040–1058.

Kendall, C.S.St. and Skipwith, P.A.d'E. (1969) Holocene shallow-water carbonate and evaporite sediments of the Khor al Bazam, Abu Dhabi, the southwest Persian Gulf. Bull. Am. Assoc. Petr. Geol. B **53**: 841–869.

Kinsman, D.J.J. (1964) Recent carbonate sedimentation near Abu Dhabi, Trucial Coast, Persian Gulf. Thesis, University of London, 302 pp.

Kinsman, D.J.J. and Park, R.K. (1976) Algal belt and coastal sabkha evolution, Trucial Coast, Persian Gulf, In: M.R. Walter (ed.) *Stromatolites: Developments in Sedimentology*. Elsevier, Amsterdam, pp. 421–433.

Kirchman, D.L. (2002) The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol. **39**: 91–100.

Kühl, M., Glud, R.N., Plough, H. and Ramsing, N.B. (1996) Microenvironmental control of photosynthesis and photosynthesis coupled respiration in an epilithic cyanobacterial biofilm. J. Phycol. **32**: 799–812.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

Li, W.K.W., Smith, J.C. and Platt, T. (1984) Temperature response of photosynthetic capacity and carboxylase activity in Arctic marine phytoplankton. Mar. Ecol. Prog. Ser. **17**: 237–243.

Liu, H.B. and Buskey, E.J. (2000) Hypersalinity enhances the production of extracellular polymeric substances (EPS) in the Texas brown tide alga *Aureoumbra lagunensis* (*Pelagophyceae*). J. Phycol. **36**: 71–77.

Lokier, S. and Steuber, T. (2008) Quantification of carbonate-ramp sedimentation and progradation rates for the late Holocene Abu Dhabi Shoreline. J. Sediment. Res. **78**: 423–431.

Mackay, M.A., Norton, R.S. and Borowitzka, L.J. (1983) Marine blue green algae have a unique osmoregulatory system. Mar. Biol. **73**: 301–307.

Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2006) *Brock Biology of Microorganisms*, 12th ed. Pearson Benjamin Cummings, San Francisco, CA.

Minz, D., Flax, J.L., Green, S.J., Muyzer, G., Cohen, Y., Wagner, M., Rittmann, B.E. and Stahl, D.A. (1999) Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl. Environ. Microbiol. **65**: 4666–4671.

Myklestad, S., Holm-Hansen, O., Varum, K.M. and Volcani, B.E. (1989) Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis*. J. Plankton Res. **11**: 763–773.

Nold, S.C. and Ward, D.M. (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. Appl. Environ. Microbiol. **62**: 4598–4607.

Oren, A. and Mana, L. (2002) Amino acid composition of bulk protein and salt relationships of selected enzymes of *Salinibacter ruber*, an extremely halophilic bacterium. Extremophiles **6**: 217–223.

Oren, A., Heldal, H., Norland, S. and Galinski, E.A. (2002) Intracellular ion and organic solute concentrations of the extremely halophilic bacterium *Salinibacter ruber*. Extremophiles **6**: 491–498.

Paerl, H.W. (1996) Microscale physiological and ecological studies of aquatic cyanobacteria: macroscale implications. Microsc. Res. Tech. **33**: 47–72.

Paerl, H.W., Bebout, B.M., Joye, S.B. and Des Marais, D.J. (1993) Microscale characterization of dissolved organic matter production and uptake in marine microbial mat communities. Limnol. Oceanogr. **38**: 1150–1161.

Paerl, H.W., Pinckney, J.L. and Steppe, T.F. (2000) Cyanobacterial–bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ. Microbiol. **2**: 11–26.

Park, R.K. (1977) The preservation potential of some recent stromatolites. Sedimentology **24**: 485–506.

Proteau, P.J., Gerwick, W.H., Garcia-Pichel, F. and Castenholz, R. (1993) The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia **49**: 825–829.

Purser, B.H. (ed.) (1973) *Persian Gulf*. Springer, New York, 471 pp.

Raven, J.A. and Geider, R.J. (1988) Temperature and algal growth. New Phytol. **110**: 441–461.

Reed, R.H., Richardson, D.L., Warr, S.R.C. and Stewart, W.D.P. (1984) Carbohydrate accumulation and osmotic stress in cyanobacteria. J. Gen. Microbiol. **130**: 1–4.

Reid, R.P., Vischer, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, I.G., Paerl, H.W., Pinckney, J.L., Prufert-Bebout, L., Steppe, T.F. and Des Maraia, D.J. (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature **406**: 989–992.

Revsbech, N.P. and Jørgensen, B.B. (1983) Photosynthesis of benthic microflora measured with high spatial resolution by the oxygen microprofile method: capabilities and limitations of the method. Limnol. Oceanogr. **28**: 749–756.

Sarnthein, M. (1972) Sediments and history of the postglacial transgressionin the Persian Gulf and northwest Gulf of Oman. Mar. Geol. **12**: 245–266.

Staats, N., Stal, L.J. and Mur, L.R. (2000) Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions. J. Exp. Mar. Biol. Ecol. **249**: 13–27.

Stal, L.J. (1991) The metabolic versatility of the mat-building cyanobacteria *Microcoleus chthonoplastes* and *Oscillatoria limosa* and its ecological significance. Arch. Hydrobiol. Suppl. **92**: 453–467.

Stal, L.J. (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol. **131**: 1–32.

Stal, L.J. and Moezelaar, R. (1997) Fermentation in cyanobacteria. FEMS Microbiol. Rev. **21**: 179–211.

Sukenik, A., Bennett, J. and Falkowski, P. (1987) Light-saturated photosynthesis limitation by electron transport or carbon fixation. Biochim. Biophys. Acta **891**: 205–215.

Teske, A., Ramsing, N.B., Habicht, K., Fukui, M., Kuver, J., Jørgensen, B.B. and Cohen, Y. (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. **64**: 2943–2951.

Thamdrup, B., Hansen, J.W. and Jørgensen, B.B. (1998) Temperature dependance of aerobic respiration in a coastal sediment. FEMS Microbiol. Ecol. **25**: 189–200.

Vonshak, A., Guy, R. and Guy, M. (1988) The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. Arch. Microbiol. **150:** 417–420.

Wieland, A. and Kühl, M. (2000) Short-term temperature effects on oxygen and sulfide cycling in a hypersaline cyanobacterial mat (Solar Lake, Egypt). Mar. Ecol. Prog. Ser. **196**: 87–102.

Wieland, A. and Kühl, M. (2006) Regulation of photosynthesis and oxygen consumption in a hypersaline cyanobacterial mat (Camargue, France) by irradiance, temperature and salinity. FEMS Microbiol. Ecol. **55**: 195–210.

Wierzchos, J., Ascaso, C. and McKay, C.P. (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. Astrobiology **6**: 1–8.

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OOID ACCRETING DIATOM COMMUNITIES FROM THE MODERN MARINE STROMATOLITES AT HIGHBORNE CAY, BAHAMAS

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1. Introduction

Diatoms occur as common components of living microbial mats and stromatolites (Stolz, 1990; Franks and Stolz, 2009; Reid et al., 2000). In some cases, their populations may be considerable and form distinct layers (Stolz, 2001). Many species are capable of exuding extracellular polymeric substances (EPS), and benthic diatoms in particular can contribute to sediment stabilization (Winsborough, 2000; Underwood and Paterson, 2003). Diatoms have been observed in the stromatolites at Shark Bay, Australia (Awramik and Riding, 1988) and at several different stromatolitic locations in the Bahamas (Riding et al., 1991; Riding, 1994). Diatoms first appear in the rock record in the Early Jurassic (Winsborough, 2000), thus their occurrence in modern flat laminated mats and stromatolites raises questions regarding the relatedness of these structures to fossil mats and stromatolites (Awramik and Riding, 1988; Riding, 1994). Thus, understanding their role in the biogenesis of microbialites has become a paramount issue in interpreting the fossil record.

The modern marine stromatolites at Highborne Cay, Bahamas have been under investigation for over a decade (Reid et al., 1995, 2000; Paterson et al., 2008), most recently under the auspices of the Research Initiative on Bahamian Stromatolites (RIBS). An intensive and extensive monitoring program over a 3-year period (2005–2007) has provided insight into the general ecology and sedimentary processes involved in the biogenesis of these stromatolites. What has

emerged is a picture of a very dynamic system that exhibits heterogeneity on both spatial and temporal scales (Perkins et al., 2007; Eckman et al., 2008, Paterson et al., 2008). The now classic model for stromatolite biogenesis in Highborne Cay involves three different sediment laminations and their associated microbial communities: (1) accreted ooids – Type 1 mat, (2) micrite crust – Type 2 mat, and (3) fused grain layer – Type 3 mat (Reid et al., 2000). The surface layers of unconsolidated carbonate ooids are formed by the trapping and binding by EPS-secreting cyanobacteria, primarily *Schizothrix gebeleinii* (Reid et al., 2000; Stolz et al., 2001). The thin micritic crusts are produced as the result of the activity of a surface biofilm community dominated by sulfate-reducing bacteria (Visscher et al., 1998, 2000). Lastly, the fused grain layers are the result of the boring cyanobacteria (e.g., *Solentia* sp.) (MacIntyre et al., 2000; Reid et al., 2000). It is the alternation in a quasi-successional procession of the different surface mat communities and their associated sediment layers (Type 1 – Type 2 – Type 3) that results in the growth of the stromatolite (Reid et al., 2000). More recently, this rather simplistic model has been augmented by further studies of the microbial diversity (Baumgartner et al., 2006; Franks, 2007; Desnues et al., 2008; Havemann and Foster, 2008; Foster et al., 2008), and additional ooid-accreting surfaces have been recognized. Two of these ooid-accreting communities are dominated by diatoms that have been designated "yellow fur" and "pustular blanket" based on their gross morphology and color. Yellow fur is dominated by stalked diatoms, whereas pustular blanket is populated by tube-forming diatoms. We have used a combination of light microscopy, confocal scanning laser microscopy (CSLM) enhanced by immunohistochemistry, and transmission electron microscopy (TEM) in concert with field observations and conclusions from previous related studies to assess the potential contribution of diatoms to stromatolite biogenesis.

2. Stalked Diatoms

Stalked diatoms can be found throughout the year at Highborne Cay as a minor component of the different surface communities. During the summer and early fall, however, their populations may grow and develop into a conspicuous thick layer (0.5–1cm) on the surface of the stromatolites (Fig. 1a). The community is comprised primarily of the stalked diatoms *Licmophora remulus*, *L. paradoxa*, and *Striatella unipunctata* (Fig. 1b). Although the cells of the two species of *Licmophora* (baseball bat-shaped) differ from that of the *Striatella* (pillow-shaped), they all produce a similar type of stalk. We have found that these stalks bind a lectin–FITC conjugate (Concanavalin A) as demonstrated for the stalked marine diatom *Achnanthes longipes* (Wustman et al., 1997). Conjugate binding is also not sensitive to acid treatment, such that the carbonate ooids can be removed from the mat material (Fig. 1b). Figure 1b shows an example of a yellow fur that has colonized the surface of a Type 1 mat. The individual cells of the diatoms appear to float in the overlying surface waters, tethered to the surface by their stalks.

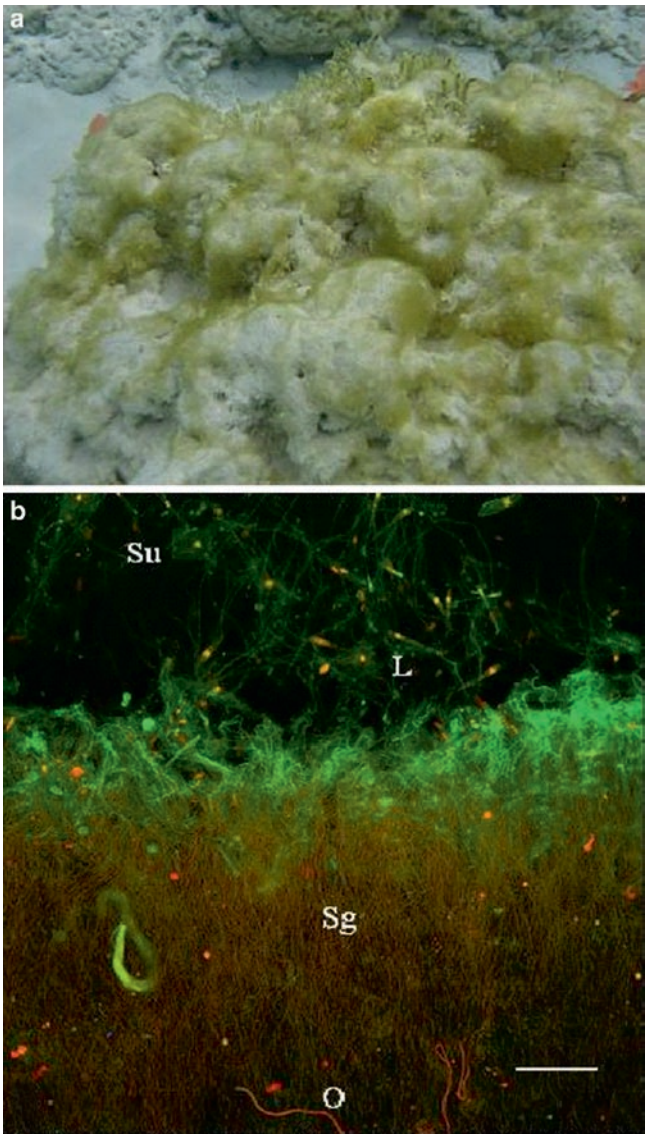

Figure 1. The "yellow fur" surface community. (**a**) A stromatolitic bioherm with an extensive surface mat of stalked diatoms which impart the yellow color. (**b**) The surface community in cross section. The sample has been acid treated (HCl) to remove the ooids, then stained with lectin–FITC conjugate to reveal the layer of stalks (green). Cells of *S. unipunctata* (Su) and *Licmophora* spp. (L) can be seen at the top of the micrograph. Filaments of *Schizothrix gebeleinii* (Sg) form a distinct layer below the stalks. A few filaments of *Oscillatoria* sp. (O) are also present. CLSM, bar 200 μm.

The surface is a dense weave of stalks almost a quarter centimeter in thickness. Immediately below this, the stalks interweave with filaments of *S. gebeleinii* for about another quarter centimeter depth (Fig. 1b). The filaments of *S. gebeleinii* appear to be oriented perpendicular to the surface possible indicating an upward migration towards the surface. The layer of *S. gebeleinii* persists for another centimeter down section, with little evidence of the diatom stalks. Individual filaments of *Oscillatoria* sp. were found further down in the mat (Fig. 1b).

The cells of *Licmophora* readily attach to surfaces and individual filaments of cyanobacteria and red algae (Fig. 2a). Initially, the diatom attaches via a holdfast region, but as the stalk grows, the cell extends away from the surface to which it is attached (Franks, 2007). Manual manipulations of the yellow fur material indicate that ooids adhere to both holdfast and stalk (Fig. 2a inset). Samples of stromatolites collected in July 2007 with well-developed yellow fur had several millimeters of trapped but unconsolidated ooids at the surface (Fig. 2b). Although there was some course-grained material (e.g., coral skeletal fragments), the bulk of the trapped sediment were fine- to medium-sized ooids (Fig. 2). This is in contrast to the reports that eualgal/cyanobacterial stromatolites are coarse-grained (Awramik and Riding, 1988; Riding, 1994). A conspicuous feature was the presence of ooid-free gelatinous pockets both near the surface and well into the subsurface (Fig. 2b, c). Lectin–FITC conjugate staining revealed that the voids were actually bundles of stalks (Fig. 2b, c). No diatom cells or their frustules were evident, indicating that they had either detached and remained at the surface, or had degraded upon burial. In some cases, the bundles of stalks became selectively colonized by filaments of *Oscillatoria* sp. (Fig. 2b inset). This raises the intriguing possibility that the stalks are providing a conduit for light into the subsurface of the stromatolite. Alternatively, the degrading stalks could be a source of nutrients. More importantly, the persistence of the stalks in the subsurface for extended periods of time (e.g., months) suggests that they could contribute to the overall integrity of the layer.

3. Tube-Forming Diatoms

Pustular blanket, the colorless cohesive surface layer, is usually seen in the fall and winter. It begins as small individual pustules (Fig. 3a), eventually coalescing into a blanket covering the surface. The identity of the organism responsible for these unique accretionary structures was not initially obvious, as dissection of the pustules often indicated only the presence of empty sheaths. However, further observation of freshly obtained material revealed the presence of a tube-forming diatom with individual cells resembling *Navicula* (Fig. 3b). The diatom is light sensitive and highly motile. Often aligned end to end inside the sheath (Fig. 3b), when disturbed (e.g., under the intense light of the microscope) the chain constricts and the cells overlay each other (Fig. 3c). The cells rapidly evacuate the sheath, leaving behind an empty tube. Ooids readily adhere to the sheath material, giving the appearance of a string of pearls. There is also evidence for carbonate

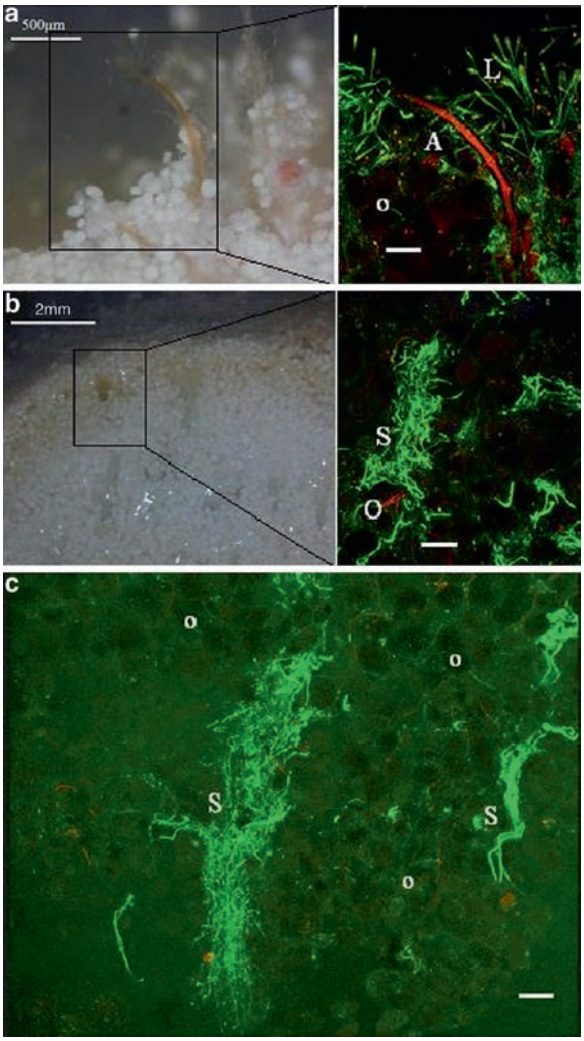

Figure 2. Stalks and ooid accretion. (**a**) *Licmophora* spp. stalks and cells (L) adhering to a red algal filament (A) and ooids (o) (inset, CLSM), (**b**) gelatinous voids in the subsurface are actually clusters of diatom stalks (S), which often get colonized by *Oscillatoria* sp. (O) (*inset*, CLSM), (**c**) clusters of diatom stalks surrounded by ooids (o) further down section (CLSM). Stalks were stained with FITC–lectin conjugate. All bars 100 µm.

precipitation associated with the sheath (Fig. 3c). The lectin–FITC conjugate did not readily bind to the sheath indicating that the composition of the EPS is different than that of the stalks of *Licmophora* and *Striatella*.

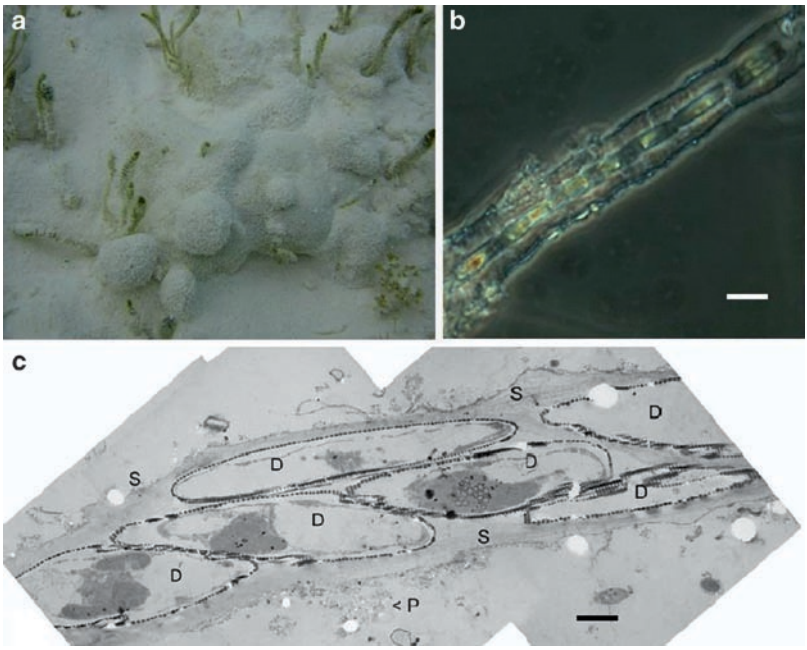

Figure 3. Pustular blanket and tube-forming diatom. (**a**) Distinctive surface accretions of "pustular blanket," (**b**) chain of *Navicula*-like diatoms inside a common sheath (tube). Phase contrast light microscopy, bar 10 μm, (**c**) cross section through chain of tubular diatoms (D) in a common sheath (S). Note precipitation (P) associated with the sheath. TEM, bar 1 μm.

4. Diatoms and Stromatolite Biogenesis

Previous reports have shown that stromatolites at Highborne Cay, Bahamas, can be produced solely by bacterial processes (Reid et al., 2000). In particular, micrite crusts are formed by bacterial biofilms (Type 2 mats) and the fused grain layers by endolithic cyanobacterial communities (Type 3 mats; Visscher et al., 1998, 2000; Macintyre et al., 2000). In addition, layers of unconsolidated sand grains are formed as a result of the trapping and binding of cyanobacteria (Type 1 mats). Nevertheless, the presence of diatoms often as conspicuous surface communities (e.g., yellow fur) raises the question of what if any role do the diatom communities described above play in stromatolite accretion?

Several field observations suggest that physical conditions at the Highborne Cay reef complex restrict the growth of diatom communities. The reef complex (including the stromatolites) is located in a high-energy environment (the surf zone) and is subject to strong winds, periods of intense wave action, and burial events (Eckman et al., 2008; Paterson et al., 2008). Both yellow fur and pustular

blanket are readily eroded by wave action. In addition, highly mobile sand bars move across the reef crest and reef flat frequently, burying the stromatolites under tens of centimeters of sediment (Andres and Reid, 2006). The duration of the burial events varies with the relative location in the reef complex (Gaspar, 2007). These burial events are key to the survival and proliferation of the stromatolites as burial inhibits the growth of macroalgae (e.g., *Batophora*), and boring macrofauna (Andres and Reid, 2006). Burial events also impact the diatom populations, which are extremely sensitive to perturbations. In one set of experiments, the photosynthetic activity of the yellow fur community was unrecoverable after 7 days of burial (Perkins et al., 2007). In contrast, the cyanobacteria are more resilient and survive prolonged burial (Kromkamp et al., 2007). The photosynthetic activity in cyanobacterial-dominated mats (e.g., Type 1 mat) buried for 5 days was reactivated within 1–2 h after exposure (Kromkamp et al., 2007), and even those buried for over 2 weeks quickly rebounded (Perkins et al., 2007).

The occurrence of the surface diatom communities as "transient blooms" at specific times of the year, their susceptibility to erosion by wind and wave action, and the fact that diatoms do not survive burial, suggest that the contribution of diatoms to the growth of stromatolites in the Highborne Cay reef system may be minimal. Nevertheless, stalked diatom mats have been observed attached to a variety of stromatolite surfaces including Mat Types 1, 2, and 3. These stalked mats trap ooids, resulting in rapid and significant accretion. The tube-forming diatoms are also capable of trapping sediment and form extensive surfaces of pustular blanket. Thus both communities could form layers of accreted ooids. Although no evidence of diatom frustules are found at depth (Stolz et al., 2001), silica is undersaturated in seawater and the frustules should readily dissolve after the death of the diatom. Thus, recognition of diatom-accreted sediment in the stromatolite subsurface is difficult. Additional work involving pigment accretion studies and detailed grain size analyses is being conducted in an effort to determine the amount of diatom-trapped sediment that is incorporated into the stromatolite subsurface.

5. Acknowledgments

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6. References

Andres, M.S. and Reid, R.P. (2006) Growth morphologies of modern marine stromatolites: a case study from Highborne Cay, Bahamas. Sed. Geol. **185**: 319–328.

Awramik, S.M. and Riding, R. (1988) Role of algal eukaryotes in subtidal columnar stromatolite formation. Proc. Natl. Acad. Sci. USA **85**: 1327–1329.

Baumgartner, L.K., Reid, R.P., Dupraz, C., Decho, A.W., Buckley, D.H., Spear, J.R., Przekop, K.M. and Visscher, P.T. (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment. Geol. **185**: 131–145.

Desnues, C.G., Rodriguez-Brito, B., Rayhawk,· S., Kelley,· S., Tran, T., Haynes, M., Liu, H., Hall, D., Angly, F.E., Edwards, R.A., Thurber, R.V., Reid, R.P., Siefert, J., Souza, V., Valentine, D., Swan, B., Breitbart, M. and Rohwer, F. (2008) Biodiversity and biogeography of phages in modern stromatolites and thrombolites. Nature **452**: 340–345.

Eckman, J.E., Andres, M.S., Marinelli, R.L., Bowlin, E., Reid, R.P., Aspden, R.J. and Paterson, D.M. (2008) Wave and sediment dynanmics along a shallow sub-tidal sandy beach inhabited by modern marine stromatolites. Geobiology **6**: 21–32.

Foster, J.S., Green, S.J., Ahrendt, S.R., Golubic, S., Reid, R.P., Hetherington, K.L. and Bebout, L. (2010) Cyanobacterial diversity in marine stromatolites: evidence for redundant ecotypes. ISME J. (in press).

Franks, J. (2007) Confocal and TEM analysis of microbial communities in modern marine stromatolites at Highborne Cay Bahamas. M.S. thesis, Duquesne University, 79 pp.

Franks, J. and Stolz, J.F. (2009) Flat laminated microbial mat communities. Earth Sci. Rev. **96**: 163–172.

Gaspar, A.G. (2007) Stromatolites: unique seascapes of the Bahamas – multiscale mapping and management guidelines for a living example of an ancient ecosystem. M.S. thesis, University of Miami, Coral Gables, 178 pp.

Havemann, S.A. and Foster, J.S. (2008) Comparative characterization of the microbial diversities of an artificial microbialite model and a natural stromatolite. Appl. Environ. Microbiol. **74**: 7410–7421.

Kromkamp, J.C., Perkins, R., Dijkman, N., Consalvey, M., Andres, M. and Reid, R.P. (2007) Can cyanobacteria survive burial? Aquat. Microb. Ecol. **48**: 123–130.

Macintyre, I.G., Prufert-Bebout, L. and Reid, R.P. (2000) The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. Sedimentology **47**: 915–921.

Paterson, D.M., Aspden, R.J., Visscher, P.T., Consalvey, M., Andres, M.S., Decho, A.W., Stolz, J. and Reid, R.P. (2008) Light dependent biostabilisation of sediments by stromatolite assemblages. PLoS ONE **3**: e3176.

Perkins, R.G., Kromkamp, J.C. and Reid, R.P. (2007) Importance of light and oxygen for photochemical reactivation in photosynthetic stromatolite communities after natural sand burial. Mar. Ecol. Prog. Ser. **349**: 23–32.

Reid, R.P., Macintyre, I.G., Steineck, R.S., Browne, K.M. and Miller, T.E. (1995) Stromatolites in the Exuma Cays: uncommonly common. Facies **33**: 1–18.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B., Dupraz, C., MacIntyre, I., Paerl, H.W., Pinckney, J., Prufert-Bebout, L., Steppe, T. and Des Marais, D. (2000) The role of microbes in accretion, lamination, and early lithification in modern marine stromatolites. Nature **406**: 989–992.

Riding, R.R. (1994) Stromatolite survival and change: the significance of Shark Bay and Lee Stocking Island subtidal columns, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments*. Bibiotheks und Informationssystem der Unversitat Oldenburg, Oldenburg, pp. 183–202.

Riding, R.R., Awramik, S.M., Winsborough, B.M., Griffin, K.M. and Dill, R.F. (1991) Bahaman giant stromatolites: microbial composition of surface mats. Geol. Mag. **128**: 227–234.

Stolz, J.F. (1990) Distribution of phototrophic microbes in the flat laminated microbial mat at Laguna Figueroa, Baja California, Mexico. BioSystems **23**: 345–357.

Stolz, J.F. (2001) Structure in marine biofilms, In: W.E. Krumbein, D.M. Paterson and G.A. Zavarzin (eds.) *Fossil and Recent Biofilms: A Natural History of Life on Earth*. Kluwer, Dordrecht, pp. 227–240.

Stolz, J.F., Feinstein, T.N., Salsi, J., Visscher, P.T. and Reid, R.P (2001) TEM analysis of microbial mediated sedimentation and lithification in a modern marine stromatolite. Am. Mineral. **86**: 826–833.

Underwood, G.J.C. and Paterson, D.M. (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. Adv. Bot. Res. **40**: 184–240.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., Macintyre, I.G. and Thompson, J. Jr. (1998) Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am. Mineral. **83**: 1482–1491.

Visscher, P.T., Reid, R.P. and Bebout, B.M. (2000) Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology **28**: 919–922.

Winsborough, B.M. (2000) Diatoms and benthic microbial carbonates, In: R.R. Riding and S.M. Awramik (eds.) *Microbial Sediments*. Springer, Berlin, pp. 76–83.

Wustman, B.A., Gretz, M.R. and Hoagland, K.D. (1997) Extracellular matrix assembly in diatoms (Bacillariophyceae) I. A model of adhesives based on chemical characterization and localization of polysaccharides from the marine diatom *Achnanthes longipes* and other diatoms. Plant Physiol. **113**: 1059–1069.

Biodata of **Graham J.C. Underwood**, author of ***"Exopolymers (Extracellular Polymeric Substances) in Diatom: Dominated Marine Sediment Biofilms"***

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EXOPOLYMERS (EXTRACELLULAR POLYMERIC SUBSTANCES) IN DIATOM-DOMINATED MARINE SEDIMENT BIOFILMS

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1. Introduction

Microphytobenthic biofilm microbial mats, consisting of assemblages dominated by cyanobacteria are well described 'classical' microbial communities. Such cyanobacterial mats are characterized by long-term persistence, a layered physical structure and complex biogeochemical interactions. Often, these cyanobacterial mats are the dominant community in harsh environments (hot springs, polar lakes, alkaline lakes and salinas).

Another type of microbial biofilm community, termed microphytobenthos (MPB) or benthic microalgae (BMA), occurs widely on soft sediment substrata in freshwater, estuarine and marine habitats. In contrast to 'classical' microbial mats, microphytobenthic biofilms form rapidly under favourable conditions and rapidly reform after physical disturbance. They are therefore transient in comparison to the long-term persistence of cyanobacterial mats. However, microphytobenthic mats are extremely widely distributed, occurring wherever light penetrates to the sediment surface, and are abundant on intertidal mud and sandflats and in shallow subtidal regions (Cahoon, 1999; Underwood and Kromkamp, 1999; Stal, 2003; Underwood and Paterson, 2003). Under favourable conditions, many hundreds of square meters of intertidal soft sediment can be covered with thick biofilms of MPB, which form a discreet structure in the surface matrix of the sediment (Fig. 1a, b). Microphytobenthic gross primary production can be high, even exceeding that of phytoplankton in the overlying water column, despite the activity of MPB being compressed into a biofilm only a few millimeters thick (Underwood and Kromkamp, 1999). These high levels of activity mean that MPB play a significant ecological role in estuarine food webs, with biofilms grazed by a wide variety of deposit-feeding invertebrates, fish, and after resuspension, by filter-feeding animals (Heip et al., 1995). Microphytobenthic biofilms are also sites of high rates of biogeochemical cycling (Pinckney and Zingmark, 1991; Underwood et al., 2005), mediating nitrogen cycling processes (Risgaard-Petersen, 2003; Cook et al., 2007) and contributing to the physical stability of the underlying sediment (Orvain et al., 2003; Underwood and Paterson, 2003), thus influencing geomorphological processes in coastal zones.

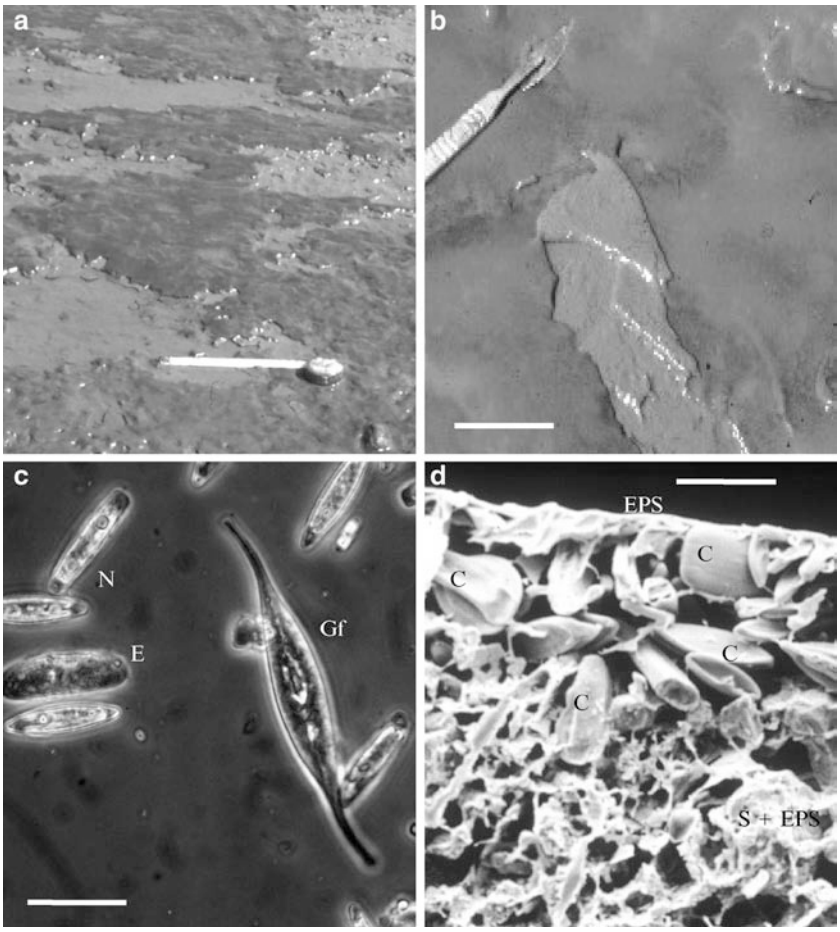

Figure 1. (**a**) Intertidal mudflat showing extensive patches of microphytobenthic biofilms, note the elevation above the sediment bed; scale bar = 15 cm. (**b**) Microphytobenthic biofilm peeled back by wave action, demonstrating the physical integrity of an established biofilm; scale bar = 2 cm. (**c**) Typical microphytobenthic diatoms, Gf = *Gyrosigma fasciola*, N = *Navicula* sp. and E = euglenophyte cells; scale bar = 20 µm. (**d**) Low temperature scanning electron micrograph of vertical fracture through MPB biofilm, showing smooth EPS surface layer, cells, (**c**) on top of a matrix of sediment and EPS; scale bar = 20 µm.

Microphytobenthic biofilms are dominated by motile diatoms. Other microalgae groups are found, including some green algae, dinoflagellates, euglenophytes, as well as cyanobacteria, but diatoms dominate the biomass in the majority of habitats. Resuspension and deposition of sediments caused by the constant tidal and wave movement in marine environments selects for motile taxa (typically biraphid pennate diatoms) able to cope with burial and with the ability to move back to the illuminated sediment surface (Admiraal, 1984).

There is potentially an extremely high level of species richness in marine benthic diatom biofilms, with an excess of 1,500 species described from different geographical regions (Witkowski et al., 2000). Yet within a particular environment, it is more usual to find only a few (20+) taxa that are numerically dominant within assemblages (Colijn and Dijkema, 1981; Underwood, 1994; Thornton et al., 2002; Forster et al., 2006) (Fig. 1c). The species composition of biofilms influences the function of the system. There are differences in migratory and photosynthetic behavior between species (Underwood et al., 2005), taxa have different optima in terms of nutrients, pH, salinity and potentially toxic chemical species, such as ammonia or H_2S (Admiraal, 1984; Underwood et al., 1998; Underwood and Provot, 2000), and species richness influences biofilm primary productivity (Forster et al., 2006). Different benthic diatoms also differ in the amount and nature of the EPS that they produce (see below).

The dense populations of benthic diatoms (cell densities exceed 10,000 cells cm^{-2}, Underwood et al., 1998) form biofilms due to the production of large quantities of extracellular polymeric substances (EPS, or exopolymers) within the sediment matrix and also on the surface of sediments. Many microorganisms produce extracellular polymeric substances (EPS), consisting of polysaccharides, glycoproteins and other constituents, that form a mucilaginous matrix surrounding the microbial cell (Decho, 1990). These EPS play an important role in the interaction of microorganisms and their environment, being involved in cell signalling, pathogen–host interactions, disease and creating localised microenvironments (Bhaskar and Bhosle, 2005; Bhinu, 2005). In microphytobenthic biofilms, diatoms and cyanobacteria are major producers of EPS (Stal, 2003; Underwood and Paterson, 2003), and the resultant biofilms can be up to 3–4 mm thick (Fig. 1d) and form sediment-stabilising 'skins' on the surface of intertidal sediments (Fig. 1b, d).

2. Measuring EPS in Natural MPB Biofilms

A major issue regarding accurately determining the concentrations of EPS in marine biofilms relates to the extraction methods utilized. Most work has taken the techniques devised for working with cell cultures and applied these to sediments. Because EPS exists in the environment in a range of forms along a continuum from dissolved molecules, though highly hydrated molecules, loose and firm gels and finally as discrete structures (tubes, pads, envelopes surrounding cells), it is possible to apply a sequence of extractions using increasingly stringent solutions to remove these EPS (Fig. 2). Generally, this starts with an aqueous extraction (at ambient temperature) to remove what is termed the colloidal carbohydrate. This fraction includes a range of molecular sizes, from monosaccharides and small oligosaccharides to large polymer chains (>100 kDa in size), consisting of many thousands of monosaccharide units. EPS can be separated from the colloidal fraction (colloidal EPS or c-EPS) by precipitation in 70% ethanol at 4°C for 24 h (Decho, 1990; Underwood et al., 2005).

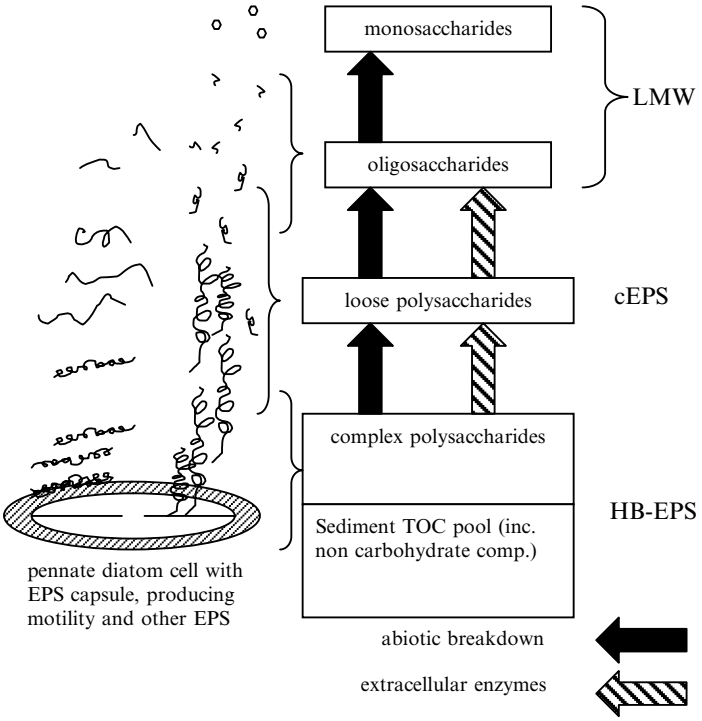

Figure 2. Schematic diagram showing the continuum of EPS types produced by benthic diatoms and their assumed relationship to the extraction regimes used in sediments and culture studies. LMW = low molecular weight fraction, cEPS = colloidal EPS (LMW + cEPS = colloidal carbohydrate), HB-EPS = hot bicarbonate extracted EPS. (Note that the extraction procedures are not discrete but overlap.)

The precipitated polymers can then be dialysed to remove bound salt, and the carbohydrate composition determined (Underwood and Paterson, 2003). Varying the proportions of ethanol precipitates different EPS fractions, with larger EPS precipitating at a lower alcohol content. This approach has shown that the colloidal EPS of the diatom *Cylindrotheca closterium* contains at least two distinct EPS (Underwood et al., 2004). Similar separations can be done using gel permeation chromatography (GPC) (Magaletti et al., 2004), or molecular cut-off filters (de Brouwer et al., 2003). The colloidal and colloidal–EPS fractions of biofilms show rapid changes in concentration over hourly time scales, with production during illuminated periods of exposure (Fig. 3c, e), and reductions in concentrations during tidal cover (Underwood and Paterson, 2003; Stal, 2003; Hanlon et al., 2006). There is a close correlation between biofilm biomass (measured as chlorophyll *a*) and colloidal and c-EPS content (Fig. 3a–f), a pattern that has been found to exist across a wide range of temperate intertidal locations (Underwood and Smith, 1998; Blanchard et al., 2000; Bellinger et al., 2005).

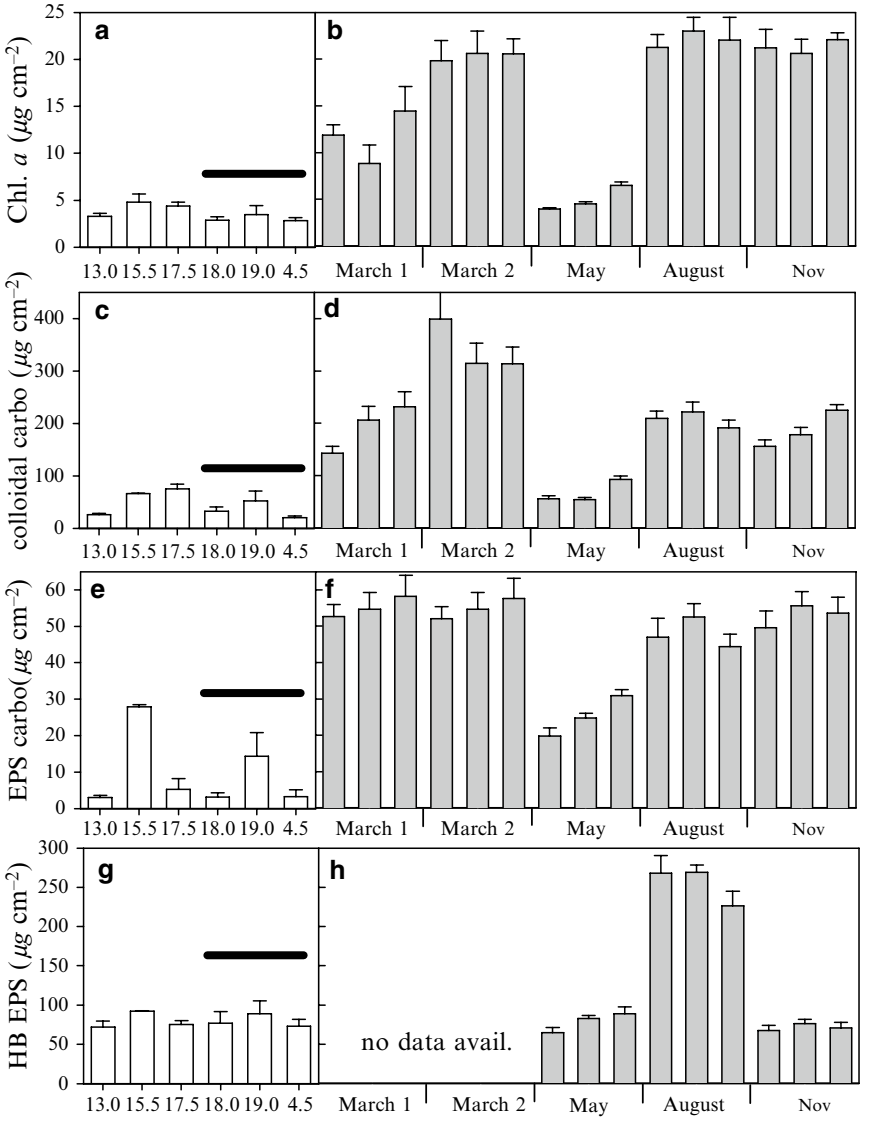

Figure 3. Concentrations of chlorophyll *a* (**a**, **b**), colloidal carbohydrate (**c**, **d**), colloidal EPS (**e**, **f**) and hot bicarbonate-extracted EPS (**g**, **h**) in intertidal biofilms from the midshore of mudflats at Alresford Creek in the Colne estuary, UK, showing (**a**, **c**, **e**, **g**) changes in concentration over an 18 h tidal period (bar indicates period of tidal immersion and darkness) on 27–28 May 2003 (mean values, ± standard error, $n = 20$) and (**b**, **d**, **f**, **h**) during five sampling periods during 2003 (mean values, ± standard errors, $n = 10$, for three 1 m^2 quadrats). Concentrations of carbohydrates are μg glucose equivalents cm^{-2} mm^{-1} biofilm depth.

Hot water extractions can be used to remove a large proportion of the intracellular storage carbohydrates (glucans) of diatoms and possibly other bound extracellular fractions (Chiovitti et al., 2004; de Brouwer and Stal, 2004). Extraction in 0.5 M $NaHCO_3$ at 95°C solubilizes stalks and complex EPS material (hot bicarbonate, HB-EPS, Fig. 2) (Wustman et al., 1997; Chiovitti et al., 2003; Bellinger et al., 2005; Hanlon et al., 2006). HB-EPS extracted from biofilms has a complex sugar profile (Bellinger et al., 2005), and appears more refractory to microbial breakdown, particularly over short time periods, for example, Fig. 2g (Haynes et al., 2007; Hofmann et al., 2009). Other extraction procedures have been used, with EDTA extractions, various modifications of hot water extractions, and different treatment of sediment before extraction (Underwood et al., 1995; Underwood and Paterson, 2003; Stal, 2003). Given the spectrum of EPS material present within biofilms, and the operational nature of all these procedures, care needs to be taken when comparing data from fractions derived using different techniques (Chiovitti et al., 2004; de Brouwer and Stal, 2004).

An additional consideration when measuring EPS in situ, is that EPS will be subject to physical alteration (e.g., photooxidation, hydrolysis) and biological degradation by extracellular enzymes produced by heterotrophic microorganisms within the sediment matrix (van Duyl et al., 1999, 2000; Goto et al., 2001; Haynes et al., 2007; Hofmann et al., 2009. The monosaccharide composition of EPS changes during degradation (Giroldo et al., 2003; Hofmann et al., 2009), but the degradation sequences in intertidal sediments (Fig. 2) are not well described. However, these partially degraded EPS will be part of any fraction extracted from natural biofilms.

Concentrations of EPS and other carbohydrate fractions can be as high as 16,000 μg glucose equivalents (6.4 mg C) g^{-1} dry wt. sediment (Underwood and Paterson, 1993). These values (0.64% organic carbon as carbohydrate compared to typical total organic carbon values of 1.8–3.0% w/w, for intertidal mudflats, Thornton et al., 2002) may be underestimates, as the phenol sulfuric acid assay used to determine complex polysaccharides in sediment extracts is usually calibrated with glucose, but is not 100% efficient at hydrolyzing and measuring more structural polymers. However, it is clear that substantial quantities of EPS are present within mudflat biofilms, and that these concentrations fluctuate over seasonal cycles as biofilms grow and are removed by grazers and physical disturbance (Fig. 3).

In situ visualization of EPS in natural sediments using low temperature microscopy usually shows a honeycomb structure of cells and EPS on top of the sediment surface (Paterson et al., 1998; Underwood and Paterson, 2003). The literature is rich with references discussing the strands and filaments of EPS seen in electron microscope studies, many of which are now recognised to be artefacts (Hoagland et al., 1993; Holland et al., 2004; Perkins et al., 2006). It is most likely that EPS is present in sediments as an unstructured, unformed gel–solution matrix surrounding and binding sediment particles and cells (Decho, 1994; Perkins et al., 2006).

3. EPS Production in Diatom Biofilms

The biological polymers produced by diatoms are carbohydrate-rich, and consist of complex macromolecules that contribute significant biological advantages to the diatoms (Hoagland et al., 1993), other organisms within the biofilm matrix (Decho, 2000; Underwood and Paterson, 2003), and the environment in which these biofilms are found (e.g., through biostabilization) (Paterson, 1989; Lucas et al., 2003; Stal, 2003). Though these EPS molecules are predominantly polysaccharide, other constituents (proteins, uronic acids, sulfate, other side arm groups) are present. The relative importance of these other components varies between species, the conditions the cells are grown under, and influences the potential properties of the EPS molecules.

The production of EPS by intertidal biofilms dominated by pennate diatoms is well described. The amount of photoassimilates excreted as extracellular organic carbon can exceed 70% (Smith and Underwood, 1998; Goto et al., 1999; de Brouwer and Stal, 2001; Cook et al., 2007), of which a large proportion (up to 50%) is polymeric material (Underwood and Paterson, 2003). The chemical composition and quantities of the EPS produced vary with irradiance (with excess production under high light and nutrient limitation, and also production in darkness, Smith and Underwood, 1998; Staats et al., 1999; Underwood et al., 2004; Chiovitti et al., 2004), with nutrient limitation (nitrogen or phosphorus, Magaletti et al., 2004; Abdullahi et al., 2006), cell growth stage (Smith and Underwood, 2000) and the tidally linked endogenous rhythms of vertical migration and photosynthesis that intertidal biofilms possess (Apoya-Horton et al., 2006; Abdullahi et al., 2006; Hanlon et al., 2006). The result is a range of different EPS produced by microalgae present within sediment biofilms (Staats et al., 1999; de Brouwer and Stal, 2001; Bellinger et al., 2005), as well as a relatively chemically uncharacterised, but significant contribution from bacteria (Mueller et al., 2006).

A key point to mention is that there is a great deal of plasticity in the quantities and types of EPS produced by diatoms (Underwood and Paterson, 2003; Chiovitti et al., 2003; Abdullahi et al., 2006), and therefore studies often report seemingly incompatible findings. Even the same species can be made to change the EPS they produce when the culture conditions are changed. Diatoms have been shown to respond to environmental changes by modifying their 'behavior' (movement modalities) with concomitant changes in chemical composition of EPS polysaccharides (Apoya-Horton et al., 2006). This highlights not only the dynamic nature of EPS biopolymers and their role in biofilm ecology, but also the problem in determining underlying fundamental models of EPS production in MPB biofilms.

Diatoms use structured EPS for the production of stalks, pads and tubes. These species of diatoms tend to be found on fixed substrata, such as stones, man-made surfaces (i.e., as biofouling assemblages on ships, etc.), plants and macroalgae (Hoagland et al., 1993; Holland et al., 2004). For an authoritative review of diatom stalks and structures see Hoagland et al. (1993). The mobile nature of

marine sediments means that few stalked species are found in microphytobenthic assemblages, though tube dwelling diatoms do occur. The majority of the EPS in MPB biofilms is present as an amorphous gel matrix surrounding the cells and are attached to the sediment particles. These EPS are produced by diatoms and other biofilm inhabitants for a variety of purposes (see below).

Benthic diatoms produce a number of different types of EPS that differ in their chemistry, properties and bioavailability within natural biofilms. In the majority of diatom species, the cell wall is tightly wrapped in a complex EPS that obscures the fine features of the silica frustule. This is clearly seen in LTSEM images of natural biofilms (e.g., Paterson et al., 1998). This material is often termed bound or attached carbohydrate, or extracellular matrix polymers (ECM) (Abdullahi et al., 2006). Detailed structural work has been done on the cell coverings of *Craspedostauros australis*, *Pinnularia viridis*, and *Nitzschia navis-varingica* (Higgins et al., 2000, 2003a, b; Chiovitti et al., 2003), and *Phaeodactylum tricornutum* (Abdullahi et al., 2006). The cell wall-bound EPS can be removed by hot bicarbonate extraction, and had a high sulfate and protein content, a wide range of constituent monosaccharides and complex polysaccharide linkage patterns (Chiovitti et al., 2003; Abdullahi et al., 2006).

Using atomic force microscopy (AFM), Higgins et al. (2003a) found differences in the structure of the surface EPS of *C. australis* and *P. viridis*. The surface polymers on *C. australis* were polysaccharide chains that uncoiled and then detached. These polymers were not particularly adhesive. This was a different property to the EPS produced by the attached diatom *Toxarium undulatum*, whose EPS pads exhibited "saw tooth" patterns under the AFM, indicative of modular proteins unfolding, providing both elasticity and strength (Dugdale et al., 2006).

Biraphid diatoms also use EPS for motility, motion being generated by the extrusion of multiple polymer chains along the raphe, which attach to the substratum. The locomotive force is generated by movement (via an actin-myosin system) of the retained ends of the EPS molecules (see Lind et al., 1997 and Poulsen et al., 1999, for a detailed description of diatom motility mechanisms). These adhesive EPS strands are cast off into the environment as the cell moves. Trails of diatoms moving across surfaces have been observed for over 40 years (Drum and Hopkins, 1966) and are often curved to reflect the shape of the raphe slit. Higgins et al. (2003b) measured "saw tooth" AFM properties in the adhesive strands produced along the raphe of *P. viridis*. These strands are similar to those seen bundled together to form strands of mucilage in *C. australis* and other diatoms (Lind et al., 1997; Higgins et al., 2000; e.g., Fig. 7f in Underwood et al., 1995). Higgins et al. (2003b) found two types of EPS in the trails of diatoms, a central core of tethers that detach from the raphe and amorphous mucilage surrounding this. There is also evidence that diatom motility is enhanced by the trails of other cells, with smaller *Navicula* cells preferentially moving along the trails of much larger *Pleurosigma* individuals (Wenderoth et al., 2004), which suggests that EPS recognition and EPS–EPS binding is taking place. Moving diatoms leave their

trails behind and this EPS helps to form biofilms (Holland et al., 2004). This is a fundamental difference between diatom biofilms in muddy sediments and those biofilms found on hard surfaces, where the cells are permanently fixed to the substratum by pads, stalks and tubes, for example, yellow fur diatom communities on stromatolites (Franks et al., this volume).

Though benthic motile diatoms do produce substantial amounts of EPS during movement, these motility polymers do not account for all the EPS found within biofilms. Diatoms release EPS into their environment during periods of environmental stress (e.g., high irradiance conditions, see references above), and there is a close relationship between colloidal EPS production and photosynthesis. During cell division and cell death, capsular and tightly bound (HB) EPS will become detached from the silica frustule and become located in the sediment matrix (Fig. 2). There is also evidence that diatoms may excrete specific EPS to alter the local microenvironment. The chemical properties of the polysaccharide-rich EPS suggest many putative roles in biofilm ecology, from protection from desiccation, localization of extracellular enzymes, generation of microenvironment, metal binding, cell–cell interactions, stimulation of microbial activity and also anti-bacterial properties (Decho, 1990, 2000; Wigglesworth-Cooksey and Cooksey, 2005). The difficulty for biofilm researchers is that the chemical characterization of EPS requires large quantities of material, which means any EPS produced *in situ* to meet the requirements of a particular sub-environment within the microstructure of biofilms would be diluted by other material during extraction. One possible way to address this is by using imaging techniques such as AFM or confocal microscopy, coupled with specific lectin stains. Though this has been done in cultures, and in attached biofilms (e.g., Wigglesworth-Cooksey and Cooksey, 2005; Mueller et al., 2006), it has not been possible to work on undisturbed natural sediment biofilms using these approaches. The challenge in developing our understanding of the role of EPS in MPB biofilm functioning is to design experimental approaches that allow us to understand EPS properties and function *in situ* and to show that EPS do fulfil the many roles with which it is credited.

4. References

Abdullahi, A.S., Underwood, G.J.C. and Gretz, M.R. (2006) Extracellular matrix assembly in diatoms (Bacillariophyceae). V. Environmental effects on polysaccharide synthesis in the model diatom, *Phaeodactylum tricornutum*. J. Phycol. **42**: 363–378.

Admiraal, W. (1984) The ecology of estuarine sediment inhabiting diatoms. Progr. Phycol. Res. **3**: 269–322.

Apoya-Horton, M.D., Yin, L., Underwood, G.J.C. and Gretz, M.R. (2006) Movement modalities and responses to environmental changes of the mudflat diatom *Cylindrotheca closterium* (Bacillariophyceae). J. Phycol. **42**: 379–390.

Bellinger, B.J., Abdullahi, A.S., Gretz, M.R. and Underwood, G.J.C. (2005) Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. Aquat. Microb. Ecol. **38**: 169–180.

Bhaskar, P.V. and Bhosle, N.B. (2005) Microbial extracellular polymeric substances in marine biogeochemical processes. Curr. Sci. **88**: 45–53.

Bhinu, V.S. (2005) Insight into biofilm-associated microbial life. J. Mol. Microbiol. Biotechnol. **10**: 15–21.

Blanchard, G.F., Paterson, D.M., Stal, L.J., Richard, P., Galois, R., Huet, V., Kelly, J., Honeywill, C., de Brouwer, J., Dyer, K., Christie, M. and Seguines, M. (2000) The effect of geomorphological structures on potential biostabilisation by microphytobenthos on intertidal mudflats. Cont. Shelf Res. **20**: 1243–1256.

Cahoon, L.B. (1999) The role of benthic microalgae in neritic ecosystems. Oceanogr. Mar. Biol. Ann. Rev. **37**: 47–86.

Chiovitti, A., Higgins, M.J., Harper, R.E., Wetherbee, R. and Bacic, A. (2003) The complex polysaccharides of the raphid diatom *Pinnularia viridis* (Bacillariophyceae). J. Phycol. **39**: 543–554.

Chiovitti, A., Molino, P., Crawford, S.A., Teng, R., Spurck, T. and Wetherbee, R. (2004) The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminaran and not extracellular polysaccharides. Eur. J. Phycol. **39**: 117–128.

Colijn, F. and Dijkema, K.S. (1981) Species composition of benthic diatoms and the distribution of chlorophyll *a* on an intertidal flat in the Dutch Wadden Sea. Mar. Ecol. Prog. Ser. **4**: 9–21.

Cook, P.L.M., Veuger, B., Böer, S. and Middelburg, J.J. (2007) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. Aquat. Microb. Ecol. **49**: 165–180.

de Brouwer, J.F.C. and Stal, L.J. (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Mar. Ecol. Prog. Ser. **218**: 33–44.

de Brouwer, J.F.C. and Stal, L.J. (2004) Does warm-water extraction of benthic diatoms yield extracellular polymeric substances or does it extract intracellular chrysolaminaran? Eur. J. Phycol. **39**: 129–131.

de Brouwer, J.F.C., de Deckere, E.M.G.T. and Stal, L.J. (2003) Distribution of extracellular carbohydrates in three mudflats in west-Europe. Est. Coastal Shelf Sci. **56**: 313–324.

Decho, A.W. (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Ann. Rev. **28**: 73–153.

Decho, A.W. (1994) Molecular-scale events influencing the macroscale cohesiveness of exopolymers, In: W.E. Krumbein, D.M. Paterson and L.J. Stal (eds.) *Biostabilization of Sediments*. Bibliotheks und Informationssystem der Universität Oldenburg (BIS), Verlag, Oldenburg, pp. 135–148.

Decho, A.W. (2000) Microbial biofilms in intertidal systems: an overview. Cont. Shelf Res. **20**: 1257–1274.

Drum, R.W. and Hopkins, J.T. (1966) Diatom locomotion: an explanation. Protoplasma **62**: 1–33.

Dugdale, T.M., Dagastine, R., Chiovitti, A. and Wetherbee, R. (2006) Diatom adhesive mucilage contains distinct supramolecular assemblies of a singular modular protein. Biophys. J. **90**: 2987–2993.

Forster, R.M., Creach, V., Sabbe, K., Vyverman, W. and Stal, L.J. (2006) Biodiversity-ecosystem function relationship in microphytobenthic diatoms of the Westerschelde estuary. Mar. Ecol. Prog. Ser. **311**: 192–201.

Giroldo, D., Viera, A.A.H. and Paulsen, B.S. (2003) Relative increase in deoxy sugars during microbial degradation of an extracellular polysaccharide release by a tropical freshwater *Thalassiosira* sp. (Bacillariophyceae). J. Phycol. **39**: 1109–1115.

Goto, N., Kawamura, T., Mitamura, O. and Terai, H. (1999) Importance of extracellular organic carbon production in the total primary production by tidal-flat diatoms in comparison to phytoplankton. Mar. Ecol. Prog. Ser. **190**: 289–295.

Goto, N., Mitamura, O. and Terai, H. (2001) Biodegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. J. Exp. Mar. Biol. Ecol. **257**: 73–86.

Hanlon, A.R.M., Bellinger, B.J., Haynes, K., Xiao, G., Hofmann, T.A., Gretz, M.R., Ball, A.S., Osborn, A.M. and Underwood, G.J.C. (2006) Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom-dominated, microalgal biofilm over a tidal emersion–immersion period. Limnol. Oceanogr. **51**: 179–93.

Haynes, K., Hofmann, T.A., Smith, C.J., Ball, A.S., Underwood, G.J.C. and Osborn, A.M. (2007) Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. Appl. Environ. Microbiol. **73**: 6112–6124.

Heip, C.H.R., Goosen, N.K., Herman P.M.J., Kromkamp, J., Middelburg, J.J. and Soetaert, K. (1995) Production and consumption of biological particles in temperate tidal estuaries. Oceanogr. Mar. Biol. Ann. Rev. **33**: 1–149.

Higgins, M.J., Crawford, S.A., Mulvaney, P. and Wetherbee, R. (2000) The topography of soft, adhesive diatom "trails" as observed by atomic force microscopy. Biofouling **16**: 133–139.

Higgins, M.J., Sader, J.E., Mulvaney, P. and Wetherbee, R. (2003a) Probing the surface of living diatoms with atomic force microscopy: the nanostructure and nanomechanical properties of the mucilage layer. J. Phycol. **39**: 722–734.

Higgins, M.J., Molino, P., Mulvaney, P. and Wetherbee, R. (2003b) The structure and nanomechanical properties of the adhesive mucilage that mediates diatom–substratum adhesion and motility. J. Phycol. **39**: 1181–1193.

Hoagland, K.D., Rosowski, J.R., Gretz, M.R. and Roemer, S.C. (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. J. Phycol. **29**: 537–566.

Hofmann, T., Hanlon, A.R.M., Taylor, J.D., Ball, A.S., Osborn, A.M. and Underwood, G.J.C. (2009) Dynamics and compositional changes in extracellular carbohydrates in estuarine sediments during degradation. Mar. Ecol. Prog. Ser. **379**: 45–58.

Holland, R., Dugdale, T.M., Wetherbee, R., Brennan, A.B., Finlay, J.A., Callow, J.A. and Callow, M.E. (2004) Adhesion and motility of fouling diatoms on a silicone elastomer. Biofouling **20**: 323–329.

Lind, J.L., Heimann, K., Miller, E.A., van Vliet, C., Hoogenraad, N.J. and Wetherbee, R. (1997) Substratum adhesion and gliding in a diatom are mediated by extracellular proteoglycans. Planta **203**: 213–221.

Lucas, C.H., Widdows, J. and Wall, L. (2003) Relating spatial and temporal variability in sediment chlorophyll *a* and carbohydrate distribution with erodibility of a tidal flat. Estuaries **26**: 885–893.

Magaletti, E., Urbani, R., Sist, P., Ferrari, C.R. and Cicero, A.M. (2004) Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom *Cylindrotheca fusiformis* under N- and P-limitation. Eur. J. Phycol. **39**: 133–142.

Mueller, L.N., de Brouwer, J.F.C., Almeida, J.S., Stal, L.J. and Xavier, J.B. (2006) Analysis of a marine phototrophic biofilm by confocal laser scanning microscopy using the new image quantification software PHLIP. BMC Ecol. **6**, doi:10.1186/1472-6785-6-1

Orvain, F., Galois, R., Barnard, C., Sylvestre, A., Blanchard, G. and Sauriau, P.G. (2003) Carbohydrate production in relation to microphytobenthic biofilm development: an integrated approach in a tidal mesocosm. Microb. Ecol. **45**: 237–251.

Paterson, D.M. (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. Limnol. Oceanogr. **34**: 223–234.

Paterson, D.M., Wiltshire, K.H., Miles, A., Blackburn, J., Davidson, I., Yates, M.G., McGroty, S. and Eastwood, J.A. (1998) Microbiological mediation of spectral reflectance from intertidal cohesive sediments. Limnol. Oceanogr. **43**: 1027–1221.

Perkins, R.G., Davidson, I.R, Paterson, D.M., Sun, H., Watson, J. and Player, M.A. (2006) Low-temperature SEM imaging of polymer structure in engineered and natural sediments and the implications regarding stability. Geoderma **134**: 48–55.

Pinckney, J. and Zingmark, R.G. (1991) Effects of tidal stage and sun angles on intertidal benthic microalgal productivity. Mar. Ecol. Prog. Ser. **76**: 81–89.

Poulsen, N.C., Spector, I., Spurck, T.P., Schultz, T.F. and Wetherbee, R. (1999) Diatom gliding is the result of an actin-myosin motility system. Cell Motil. Cytoskeleton **44**: 23–33.

Risgaard-Petersen, N. (2003) Coupled nitrification–denitrification in autotrophic and heterotrophic estuarine sediments: On the influence of benthic microalgae. Limnol. Oceanogr. **48**: 93–105.

Smith, D.J. and Underwood, G.J.C. (1998) Exopolymer production by intertidal epipelic diatoms. Limnol. Oceanogr. **43**: 1578–1591.

Smith, D.J. and Underwood, G.J.C. (2000) The production of extracellular carbohydrate exopolymers (EPS) by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. J. Phycol. **36**: 321–333.

Staats, N., de Winder, B., Stal, L.J. and Mur, L.R. (1999) Isolation and characterisation of extracellular polysacchardies from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur. J. Phycol. **34**: 161–169.

Stal, L.J. (2003) Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. Geomicrobiol. J. **20**: 463–478.

Thornton, D.C.O, Dong, L.F., Underwood, G.J.C. and Nedwell, D.B. (2002) Factors affecting microphytobenthic biomass, species composition and production in the Colne estuary (UK). Aquat. Microb. Ecol. **27**: 285–300.

Underwood, G.J.C. (1994) Seasonal and spatial variation in epipelic diatom assemblages in the Severn estuary. Diatom Res. **9**: 451–472.

Underwood, G.J.C. and Kromkamp, J. (1999) Primary production by phytoplankton and microphytobenthos in estuaries. Adv. Ecol. Res. **29**: 93–153.

Underwood, G.J.C. and Paterson, D.M. (1993) Seasonal changes in diatom biomass, sediment stability and biogenic stabilization in the Severn Estuary. J. Mar. Biol. Assoc. UK **73**: 871–887.

Underwood, G.J.C. and Paterson, D.M. (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. Adv. Bot. Res. **40**: 184–239.

Underwood, G.J.C. and Provot, L. (2000) Determining the environmental preferences of four estuarine epipelic diatom taxa – growth across a range of salinity, nitrate and ammonium conditions. Eur. J. Phycol. **35**: 173–182.

Underwood, G.J.C. and Smith, D.J. (1998) Predicting epipelic diatom exopolymer concentrations in intertidal sediments from sediment chlorophyll *a*. Microb. Ecol. **35**: 116–125.

Underwood, G.J.C., Paterson, D.M. and Parkes, R.J. (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnol. Oceanogr. **40**: 1243–1253.

Underwood, G.J.C., Phillips, J. and Saunders, K. (1998) Distribution of estuarine benthic diatom species along salinity and nutrient gradients. Eur. J. Phycol. **33**: 173–183.

Underwood, G.J.C., Boulcott, M., Raines, C.A. and Waldron, K. (2004) Environmental effects on exopolymer production by marine benthic diatoms – dynamics, changes in composition and pathways of production. J. Phycol. **40**: 293–304.

Underwood, G.J.C., Perkins, R.G., Consalvey, M., Hanlon, A.R.M., Oxborough, K., Baker, N.R. and Paterson. D.M. (2005) Patterns in microphytobenthic primary productivity: species-specific variation in migratory rhythms and photosynthetic efficiency in mixed-species biofilms. Limnol. Oceanogr. **50**: 755–767.

van Duyl, F.C., de Winder, B. Kop, A.J. and Wollenzien, U. (1999) Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. Mar. Ecol. Prog. Ser. **191**: 19–32.

van Duyl, F.C., de Winder, B., Kop, A.J. and Wollenzien, U. (2000) Consequences of diatom mat erosion for carbohydrate concentrations and hetertrophic bacterial activities in intertidal sediments of the Ems-Dollard estuary. Cont. Shelf Res. **20**: 1335–1349.

Wenderoth, K., Marquardt, J. and Rhiel, E. (2004) The big trail: many migrate at the expense of a few. Diatom Res. **19**: 115–122.

Wigglesworth-Cooksey, B. and Cooksey, K.E. (2005) Use of fluorophore-conjugated lectins to study cell-cell interactions in model marine biofilms. Appl. Environ. Microbiol. **71**: 428–435.

Witkowski, A., Lange-Bertalot, H. and Metzeltin, D. (2000) Diatom flora of marine coasts. Iconographia Diatomologica v. 7. Ganter, Ruggell; Koeltz Scientific Books. Königstein.

Wustman, B.A., Gretz, M.R. and Hoagland, K.D. (1997) Extracellular matrix assembly in diatoms (Bacillariophyceae) I. A model of adhesives based on chemical characterisation and localisation of polysaccharides from the marine diatom *Achnanthes longipes* and other diatoms. Plant Physiol. **113**: 1059–1069.

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MICROBIAL MATS FROM WIND FLATS OF THE SOUTHERN BALTIC SEA

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1. The Baltic Sea – Special Features Enabling the Development of Microbial Mats

The Baltic Sea covers an area of 377,000 km² and is the world's largest brackish-water ecosystem. In geological terms, the Baltic Sea is quite young and its development began with the thawing of the Weichselian ice sheet after the last glaciation 15,000 years BP (before present). Because the connection to the North Sea was mostly temporary, the salinity conditions changed often. For the last 8,000 years BP, the salinity regime remained more or less unchanged and brackish (Björck, 1995). The catchment area is 1.6 million square kilometers. The annual input of riverine and precipitation freshwater is about 660 km³, while further 475 km³ of saline water flows in from the North Sea. The discharge of brackish water into the North Sea over the small straits between Denmark and Sweden averages 950 km³ (Björck, 1995). Apart from these small connections, the Baltic Sea is surrounded by land. This has a great impact on the salinity regime. Through the inflow of saline bottom water from the North Sea, salty and oxygen-rich water is delivered. Thus, the salinity of the freshwater-influenced surface water decreases from 25–15 PSU in the western part, to 8–6 PSU in the central Baltic Sea and down to 2 PSU in the Bottnian Gulf in the north–east (Matthäus, 1996). Therefore, the Baltic Sea can be characterized as a very large estuary. Despite this horizontal salinity gradient, the conditions in the Baltic Sea are very different from those in estuaries because of missing tides. The tidal range is extremely low with 12–15 cm, but wind direction and wind speed may temporarily induce high waves and change sea water levels (Brosin, 1965; Lass and Magaard, 1996). Consequently, salinity levels at any point do not vary much, resulting in a rather stable vertical salinity gradient, but strong horizontal salinity gradients along the shore line structure the benthic fauna and flora profoundly.

The evolution of the nowadays diversified Baltic Sea shoreline was very complex. Interactions between melting of the ice-shield, isostatic processes, erosion, and global changes of the sea-level contributed to the different coastal forms. In addition, particularly the Southern Baltic Sea still exhibits a dynamic geomorphology of the coast. Material is steadily eroding from cliff lines, transported by coastal parallel currents and deposited at other places, i.e., the processes of abrasion

and sedimentation lead to a loss of upland relief and at the same time to the formation of spits and wind flats (Schwarzer, 1996).

Wind flats are typical for the Southern Baltic Sea coast. Although these sediment areas are similar to tidal flats with respect to exposition and inundation, they are exposed and irregularly flooded as a sole function of the prevailing wind direction and speed (Rippe and Dierschke, 1997). Worldwide, there are only few tideless brackish or marine sediment regions with a comparable topology. Thus, wind flats are scarce and ecologically poorly studied. Similar wind-influenced ecosystems are located, for example, in the Black Sea and at the northern beaches of the Caspian Sea (Eisma, 1998).

The "Bock" wind flat represents the biggest of the alluvial formations at the German Baltic Sea coast and is located at the eastern end of the Darss-Zingst peninsula where it separates the Bodden chain from the Baltic Sea (Fig. 1). "Bock" is the youngest formation of this peninsula and extends over 10 km in west–east direction and 2–2.5 km in north–south direction (Kube, 1992). The average water level is 5–25 cm (Reinhard, 1953) and the salinity is 10–12 PSU (Schlungbaum and Baudler, 2001). A combination of the natural sediment transport by currents and anthropogenic activities led to the present status of the "Bock" wind flat. Nowadays, the wind flat "Bock" is part of the National Park "Vorpommersche Boddenlandschaft", which encompasses a total area of 805 km^2 with 687 km^2 water area.

In 2001, the working group "Applied Ecology" of the University of Rostock initiated several ecological and microbiological studies on the "Bock" wind flat and its microbial mats (Witte et al., 2004; Woelfel, 2004; Witte, 2005; Heyl, 2007; Woelfel et al., 2007, Kern, 2008). During the first surveys, a few patches of microbial mats were documented with a maximum extension of 200–500 m^2 in summer 2002 (Witte et al., 2004). Three years later, the mat area had grown to ca. 40,000 m^2, while at the beginning of 2008 the mat covered a slightly smaller area of about 30,000 m^2 (Kern, 2008).

2. Microbial Mats in the "Bock" Wind Flat

The irregular water level fluctuations cause partly extreme environmental conditions or frequent strong changes in other abiotic parameters, such as radiation, temperature, salinity, and nutrient concentrations. Under such unstable environmental conditions, laminated microbial mats are generally favored (Stal, 1995). Therefore, wind flats of the German coast line are typically inhabited by microbial mats on exposed sediments. A survey of the occurrence of microbial mats along the German Baltic Sea coast indicated their presence at ten stations, namely from west to the east Flügge/Fehmarn (1), Wohlenberger Wiek (2), Langenwerder (3), Salzhaff (4) , wind flat "Bock" (5), Hiddensee (6), Polchow (7), Lietzow (8), Thiessow (9) and Eldena (10) (Fig. 2).

Laminated microbial mats develop in many different environments, such as thermal springs, hypersaline ponds and lakes, dry and hot deserts, Antarctic

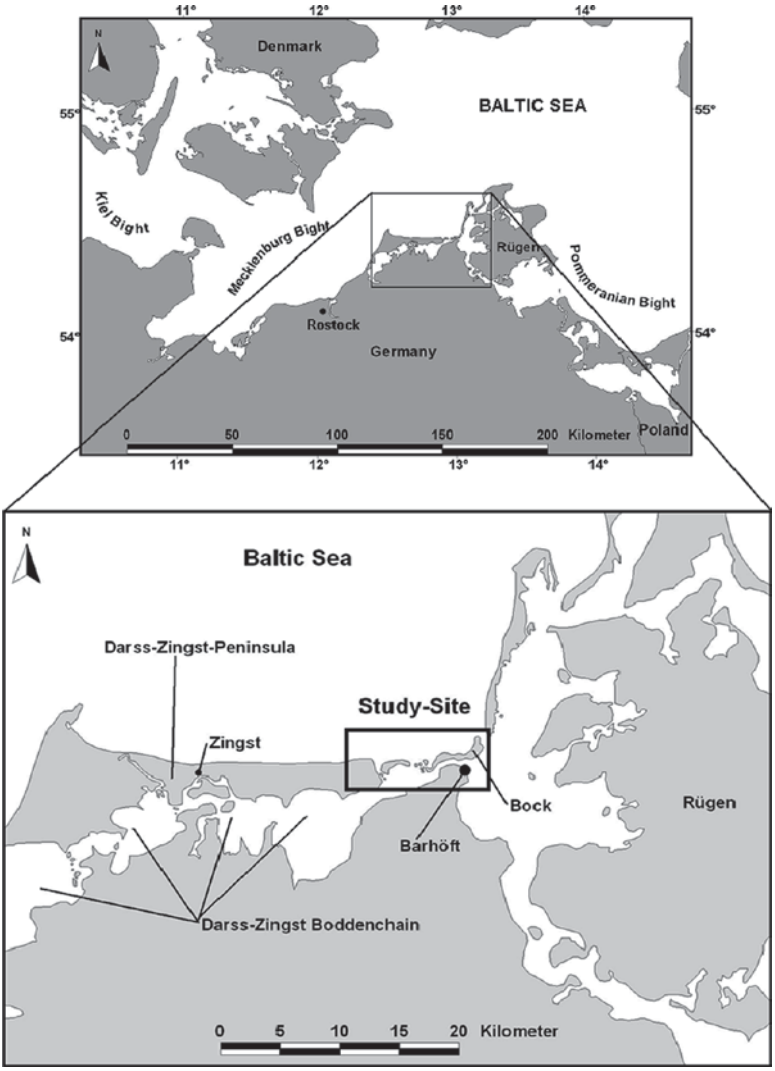

Figure 1. Geographical location of the "Bock" wind flat (study-site) at the eastern end of the Darss-Zingst-Peninsula. (Maps were created by Dr. Florian Peine, University of Rostock, Germany.)

and alkaline lakes, as well as coastal intertidal sediments (Cohen and Rosenberg, 1989; Stal and Caumette, 1994). Most studies on marine microbial mats have focussed on tidal environments (Stal and Krumbein, 1985; van Gemerden, 1993; Stal, 1995, 2003; Karsten et al., 1998. Thus, investigations of microbial mats from the nontidal brackish-water Baltic Sea are very rare (Witte et al., 2004).

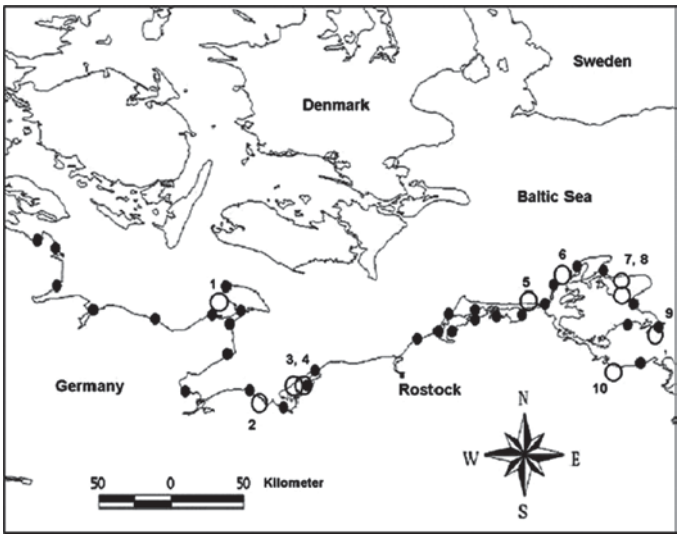

Figure 2. Stations mapped for the establishment of microbial mats along the German Baltic Sea coast (*black circles*). Locations with microbial mats are marked (*open circles*). (Modified after Witte et al., 2004.)

Microbial mats are described as the initial step in colonizing coastal habitats. These communities accumulate organic material and enrich the sediment with nutrients, which may be followed by colonization of higher plants (van Gemerden, 1993). In the "Bock" wind flat, the first occurrence of single individuals of *Puccinella maritima* (Hudson) Parlatore (common salt marsh grass), a typical pioneer plant for coastal regions, was observed in 2002 (Woelfel et al., 2007). In 2006/07, more *P. maritima* were found along the inner part of the wind flat. Since the end of 2007, numerous *P. maritima* formed patches over a wider area (Kern, 2008) where they stabilize the sediment and affect the topography. In conclusion, a microbial mat-induced change in the coastal geomorphology seems likely if this *P. maritima* population permanently establishes in the "Bock" wind flat and further successional species and stages follow.

3. Community Structure of Microbial Mats of the "Bock" Wind Flat

The special features of the Baltic Sea imply a difference of the microbial mats in the "Bock" wind flat compared to those found in typical marine habitats, like tidal flats. Two of the most striking differences between both mat types can be observed even with the naked eye. First of all, the brownish layer of diatoms in tidal flat mats, typically found on top of the cyanobacteria layer, is located deeper (below the layer of cyanobacteria). Secondly, stable layers of purple sulfur bacteria were

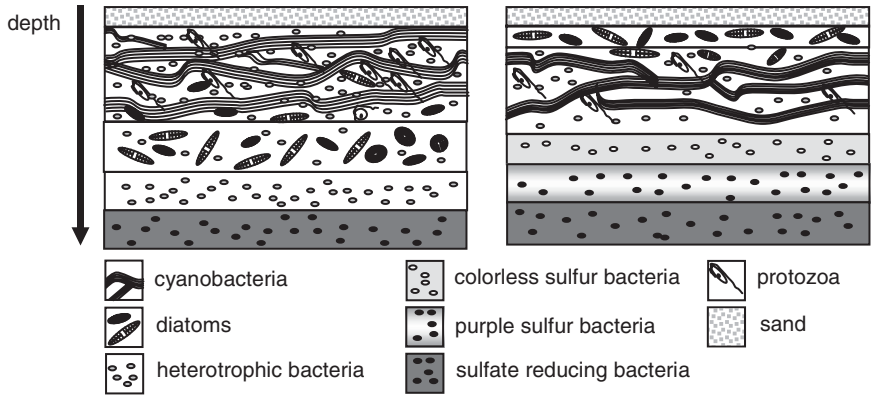

Figure 3. Scheme of two typical laminated microbial mats from the "Bock" wind flat (*left*) and a tidal flat (*right*). (Modified after Woelfel et al., 2007; van Gemerden, 1993.)

rare in the microbial mats of the wind flat. Furthermore, the first quantification of associated heterotrophic bacteria within the phototrophic layer suggested a diverse community with high abundances (Heyl, 2007). Until now it is generally accepted that micrograzers are more or less lacking in microbial mats (Stal, 1995), but various and abundant protozoa, mostly ciliates, were found in the microbial mats of the "Bock" wind flat (Fig. 3) (Kern, 2008). It is likely that this community structure is special for microbial mats at wind flats of the Baltic Sea compared to tidal flats. In addition, apart from the well-studied cyanobacteria and the various groups of sulfur bacteria, diatoms, protozoa, and heterotrophic bacteria should be more extensively studied in terms of their ecological functions in the phototrophic layer of microbial mats.

3.1. CYANOBACTERIA

Almost all microbial mats in intertidal and hypersaline habitats are dominated by the cosmopolitan filamentous cyanobacterium *Microcoleus chthonoplastes* (Garcia-Pichel et al., 1996). The microbial mats in the wind flat are also dominated by this organism (Woelfel et al., 2007). However, Siegesmund et al. (2008) taxonomically revised the genus *Microcoleus* based on phylogenetic analyses on 16S rRNA sequences, internal transcribed spacer (ITS) sequences and secondary structures. *M. chthonoplastes* was assigned to the new genus *Coleofasciculus* as *Colefasciculus chthonoplastes* within the Phormidiaceae. Within this new genus two lineages, one containing strains originating from brackish-marine habitats and the other one with strains from habitats with lower salinity, were recognized. All examined strains from freshwater and terrestrial habitats, such as *Microcoleus*

vaginatus and *M. steenstrupii* remained in their original genus within the Oscillatoriaceae (Siegesmund et al., 2008).

Populations of *C. chthonoplastes* from the North Sea and Baltic Sea were analyzed using multilocus sequence typing (MLST), which analyses the sequences of several housekeeping gene fragments of many strains. The resulting phylogenetic trees for three different genetic loci of metabolic genes (rDNA-ITS, *kaiC*, *petB*/*D*) showed a different clustering of the analyzed cyanobacterial strains for each genetic locus indicating frequent genetic recombination (Lodders et al., 2005). Interestingly, *C. chthonoplastes* strains isolated from the same microbial mat over the period of 1 year are placed in both lineages of the genus *Coleofasciculus*, indicating a high genetic diversity at one place (Siegesmund et al., 2008).

Several *C. chthonoplastes* strains originating from different microbial mats along the salinity gradient of the Southern Baltic Sea were compared according to their potential formation of salinity ecotypes. Physiological investigations proved all strains to be euryhaline. While most isolates showed high growth rates between 15 and 45 PSU with optima at 33 PSU, few strains preferred lower salinities indicating intraspecific differences in physiological response patterns (Karsten, 1996; Witte, 2005). The concentrations of trehalose and glycosyl glycerol as the most important osmoprotective substances correlated to increasing salinities (Karsten, 1996; Witte, 2005).

Another environmental condition with which the phototrophic organisms have to cope is the high irradiance. UV radiation can cause damage of DNA and protein. However, the mat organisms have developed several strategies to compensate for UV stress, for instance by downward migration (avoidance) within the mat (Bebout and Garcia-Pichel, 1995; Quesada and Vincent, 1997), repair of DNA damage, de novo protein synthesis, fast replacement of bleached chlorophyll, and the synthesis of sunscreen compounds (Castenholz and Garcia-Pichel, 2000). Among the *C. chthonoplastes* strains, the physiological and biochemical responses to UV radiation are strain-specific. Some strains are able to acclimate to UV radiation, others bleach. The survival of *C. chthonoplastes* cells is related to the capability of synthesizing and accumulating UV-absorbing mycosporine-like amino acids. Self-shading by degraded cells is another way to protect parts of the population (Pattanaik et al., 2008). Although *C. chthonoplastes* is abundant in many microbial mats all over the world, it seems to be rather UV sensitive (Pattanaik et al., 2008). Perhaps, the joint occurrence with *Lyngbya* cf. *aestuarii* explains the success of *C. chthonoplastes* in spite of its UV sensitivity. The species produces the sunscreen compound scytonemin, which is deposited in the cell walls and extracellular sheaths, thereby providing an efficient UV protection. In a microbial mat from an Australian intertidal mangrove flat, *L.* cf. *aestuarii* formed a protective layer on top of *C. chthonoplastes* especially during summer time (Karsten et al., 1998). Also within the microbial mat of the "Bock" wind flat *C. chthonoplastes* dominated the phototrophic organisms in spring, while during summer the abundance of *L.* cf. *aestuarii* was increased in the top layer (Woelfel et al., 2007).

3.2. DIATOMS

Diatoms, being cosmopolitans in aquatic habitats, are also common in almost all types of microbial mats at tidal and wind flats, e.g. in hypersaline or Antarctic lakes. However, the characteristics of microbial mats and their associated diatoms of a wind flat are slightly different from those described from tidal flats concerning structure, biomass, and species composition. In the study of Woelfel et al. (2007) special attention was paid to the comparison of diatom compositions in a laminated microbial mat as a relatively steady biocoenosis compared to sediments without a mat ("common" microphytobenthos), being probably more disturbed areas at the "Bock" wind flat. Diatoms were found in rather deep layers (down to 1 cm) in the wind flat mat with highest abundances at 3–7 mm depth. In tidal mats, diatoms are typically found directly beneath the sediment surface (0–3 mm) (van Gemerden, 1993; cf. Fig. 3). Coarsely grained sediments in the wind flat area probably enhanced oxygen penetration deeper into the sediment and thus provided microenvironmental conditions preventing the formation of such clearly defined zones.

Investigation of species composition revealed a community typical for sandy shallow-water areas of the Baltic Sea, dominated by the epipelic diatoms *Achnanthes lemmermannii* Hustedt, *Navicula paul-schulzii* Witkowski et Lange-Bertalot sp. nov., *Mastogloia exigua* Lewis, and *Navicula phylleptosoma* Lange-Bertalot. Furthermore, Witkowski (1990, 1991) found a similar species composition of diatoms in microbial mats even at slightly lower salinities at Puck Bay at the Southern Baltic Sea (Poland). In the study by Woelfel et al. (2007), 93 diatom species were identified in total with highest diversity in August 2002 when 62 species were identified in one sample.

While diversity did not change over the course of the seasonal study, the quantitative occurrence varied. In the less settled sediment, lower cell numbers as well as higher occurrences of very small species (<15 μm) were found. The investigated microbial mat was characterized by higher abundances of larger (≥20 μm) and specific taxa. The higher incidence of large diatoms in the mat is possibly related to the extracellular polymeric matrix produced by the cyanobacteria and diatoms, which stabilizes the sediment, thus reducing erosion. Furthermore, they may benefit from the mat EPS in terms of protection against desiccation, UV-irradiance, as well as rapid nutrient recycling performed through the associated heterotrophic bacteria within the mat. However, life deeper inside the mat will have to face stronger competition for space, nutrients, and light.

In conclusion, so far, diatoms of microbial mats are still poorly studied either as associated organisms with cyanobacteria or as percentage biomass of the total phototrophic community. Very little is known about the diversity of diatoms and their possible special ecological function within the microbial mat ecosystem. Furthermore, in contrast to tidal flats, the specific inundation patterns and the related changes of other environmental factors open the opportunity to study responses of species and communities under strongly and irregularly fluctuating conditions.

3.3. HETEROTROPHIC BACTERIA

Although the phototrophic layer of microbial mats is dominated by cyanobacteria and diatoms with respect to their biomass, a high diversity of heterotrophic bacteria is usually observed. Phylogenetic analysis based on the 16S rRNA gene of the upper 3 mm of a hypersaline microbial mat from Mexico indicated that only 6% of the recovered prokaryotic sequences could be assigned to cyanobacteria, whereas over 50% of the sequences represented *Proteobacteria* and *Bacteroidetes*. Most striking was the high abundance of *Chloroflexi* (21% of the sequences), a group of phototrophic facultative anaerobic bacteria (Ley et al., 2006). These data point not only to the close connection and parallel occurrence of photo- and heterotrophic organisms within the phototrophic layer of a microbial mat, but also to a high diversity of microorganisms as well as microclimates.

The community composition of the heterotrophic bacteria within the top layer of microbial mats and mat-free sediments in the "Bock" wind flat is different, compared to those of tidal flats. Card-FISH analyses showed a clear dominance of *Alphaproteobacteria* with 30%. Other examined bacterial domains, like *Betaproteobacteria*, *Gammaproteobacteria*, Gram-positive bacteria with high G + C-content and *Bacteroidetes*, only accounted for 1–14% (Heyl, 2007). In tidal flats of the North Sea, the *Bacteroidetes* group was the dominating bacterial domain with 8–18% in the upper 0.5 cm, whereas *Alphaproteobacteria* only accounted for 1.5–3.5% (Llobet-Brossa et al., 1998). These different dominance patterns indicate different substrate compositions within various mat communities. Many bacteria belonging to the *Bacteroidetes* are consuming refractory high molecular weight molecules, whereas *Alphaproteobacteria* are known to utilize preferably labile low molecular weight molecules (Cottrell and Kirchman, 2000). Therefore, the dominance of *Alphaproteobacteria* in the wind flat may indicate a better availability of readily utilizable substrates. As organic material is mainly produced by the phototrophic organisms and delivered as exudates (Underwood et al., 1995), the community composition of the heterotrophic bacteria is probably determined by the phototrophic organisms, their activity (amount of substrates), and physiological status (quality of exudates) (Jensen, 1983; Cook et al., 2004).

Extracellular enzymes located freely in the surrounding medium or bound extracellularly to the bacterial membrane catalyze the first step of organic matter degradation (Chróst, 1990). These enzymes hydrolyze oligo- or polymers to bioavailable monomers, which can easily pass the cell wall, can be taken up through the membrane, and can be further metabolized within the cell (Wetzel, 1991). Thus, these catalysts exploit also the pool of larger biopolymers. Glycosidases in general are highly specific enzymes (Martinez et al., 1996); many are species specific and/or inducible by the respective substrates. The presence and activity of extracellular glycosidases, therefore, reflects the substrate composition. The common α-glucosidase (EC 3.2.1.20) hydrolyzes 1,4-α-glucosidic bonds (e.g. in starch), which is considered to be a quite labile substrate in contrast to the more refractory cellulose hydrolyzed by β-glucosidase (EC 3.2.1.21) (Bairoch, 2000). The ratio of

the activity of both glucosidases describes, therefore, the amount of labile and refractory carbohydrates (Herndl, 1992). At least in the phototrophic layer of the microbial mat, the cell-specific activity of the α-glucosidase was with 0.2–1.1 amol cell^{-1} h^{-1} (*atto*-mol cell^{-1} h^{-1} = 10^{-18} mol cell^{-1} h^{-1}) as high as that of the β-glucosidase with 0.3–1.0 amol cell^{-1} h^{-1} (Heyl, 2007). However, the source of extracellular enzymes is difficult to determine within microbial mats (Sirová et al., 2006) and it is likely that some hydrolases may originate from protists and algae or have accumulated after cell lyses in the interstitial water. Huge amounts of carbohydrate polymers stem from the sheaths of *C. chthonoplastes* strains and consisted mainly of glucose and galactose (Severin, 2005). Hence, the heterotrophic bacteria present within the microbial mat can rely on sufficient polymeric substrates. The hydrolysis products can also be used by many phototrophs as supportive or "luxury" substrates especially in times of light or nutrient limitation (Kamjunke et al., 2008).

Although various interactions between cyanobacteria and heterotrophic bacteria in aquatic habitats have been described (Cole, 1982), there are most probably many more that are still unexplored. Members of the *Cytophaga*-cluster are known to enzymatically lyse cyanobacteria and Gram-positive bacteria (Marshall, 1989; Madigan et al., 2009). Bacterial respiration can be beneficial because it protects cyanobacteria from photooxidation and promotes N_2 fixation by lowering the oxygen concentrations in the surrounding environment (Marshall, 1989). Furthermore, some associated bacteria produce vitamins and growth factors, which are essential for cyanobacteria and diatoms (Cole, 1982).

3.4. PURPLE SULFUR BACTERIA

The lacking layer of purple sulfur bacteria in the wind flat mat can be explained by the sediment characteristics and its consequences for oxygen penetration into the sediment. The sediment of the "Bock" wind flat is middle-grained with 0.32–0.33 mm average particle size (Woelfel et al., 2007). Tidal flats, which are inhabited by microbial mats, typically feature fine-grained sands with a particle size of 0.063–0.2 mm (Stal, 1994). It is often considered that microbial mats worldwide establish easily on this small grain size (Stal et al., 1985). Therefore, the wind flat microbial mats seem to be unique because of their occurrence on a coarser sediment type. However, it is not yet known if this observation is specific for the Southern Baltic Sea coast. A larger grain size may allow a deeper oxygen penetration into the sediment. Therefore, anaerobic microenvironments with least residual irridation, which are necessary for purple sulfur bacteria, do not appear that often. Only during winter, when primary production and, hence, oxygen production was low, thin layers of purple sulfur bacteria underneath the cyanobacteria established temporarily.

Purple sulfur bacteria use H_2S as an electron donor resulting in oxidation to sulfate. H_2S is the final product of sulfate reduction, which is mediated under anaerobic conditions by sulfate-reducing bacteria in the lowest black layer. It may

be speculated that the sulfur cycle is suppressed in the wind flat mat due to the lack of purple sulfur bacteria as one key group or if other bacterial groups like chemoautotrophic sulfur-oxidizing bacteria replace the ecological function of the purple sulfur bacteria with respect to the oxidation of sulfide. When the sulfur cycle is indeed suppressed, the question arises which bacterial group is responsible for organic matter degradation and recycling of nutrients and whether sulfate as electron acceptor may be replaced by oxygen, nitrate, nitrite, manganese, and/or iron. This would make the microbial mats of the wind flat rather unique because other mat communities are known to develop in microenvironments which are rich in sulfide (Cohen, 1989).

3.5. PROTOZOA AS MICROGRAZERS

So far, not much attention has been paid to the possible impact of grazing pressure on microbial mats by protozoa because it was assumed to be generally low (e.g. Stal, 1995). The first inventory of protozoa within the phototrophic layer of the microbial mat in the "Bock" wind flat indicated relatively high abundances of heterotrophic nanoflagellates (70–380 $\times$ 10^3 individuals cm^{-3}), which were dominated by kineto-plastids and chrysophyceae. Larger heterotrophic flagellates (>20 μm) did not differ in cell density between mat and normal sediments in the wind flat. Surprisingly, pigmented nano- and microflagellates amounted to almost the same abundances as ciliates (0.2–6.5 $\times$ 10^3 individuals cm^{-3}) and were dominated by crypto-, dino-, and euglenophyceae. Ciliates summed up to 1.4–6.8·$\times$ 10^3 individuals cm^{-3}, the numbers being up to 13 times higher within the upper 3 mm of the mat than in the adjacent mat-free sediment. Rhizopods were much less abundant than the other protists, which is not typical for other sediments. Compared to other brackish and marine habitats, protistan abundances in the wind flat are low (Kern, 2008), which may be attributed to the high average grain size of the sediment and their regular erosion or redistribution. In this respect, the cyanobacterial mat stabilizes the protistan habitat allowing an increased abundance.

Heterotrophic nanoflagellates are so small that bacteria or very small phototrophic cells, like rod-shaped cyanobacteria, are the preferred prey organisms. For all larger and pigmented flagellate groups, mixotrophic behavior is described also with bacteria-sized prey. The diet of *Euplotes* sp. and *Protocrusia* sp., which were abundant in the wind flat mat, consists mainly of bacteria. These protozoa are also able to ingest solitary eukaryotic algae, but avoid mat-dominating *C. chthonoplastes* as well as larger (longer) diatoms. In the microbial mat of the "Bock" wind flat, protozoa grazing on bacteria and smaller microalgae always accounted for >75 % of all protozoa (Kern, 2008). Although the grazing pressure on the mat itself, i.e. on the dominating and structuring filamentous cyanobacteria, can still be assumed as negligible, the possible impact on nutrient recycling due to bacteriovory may be high. From these preliminary data, protozoa may by

ecologically important in microbial mats, but their role within the microbial element cycles remains to be investigated.

4. Productivity of Microbial Mats in the "Bock" Wind Flat

The chlorophyll *a* content of the microphytobenthos station was with 10–142 mg m^{-2} (Table 1), similar to other sandy eulittorals at the Baltic Sea (Sundbäck, 1983) or at a sandy tidal flat (Carrot Island, USA) (Pinckney et al., 1995). The biomass of primary producers in microbial mats was always higher than in adjacent microphytobenthic assemblages, e.g. at Mellum Island, North Sea (Stal et al., 1985). In the "Bock" wind flat mat, high biomass of microalgae with up to 1,268 mg chlorophyll *a* m^{-2} were established in the top 3 mm layer of the microbial mat in September 2006, while later investigations in 2007/08 indicated a decrease by about 22–59%. Compared to the seasonal concentration changes in the microbial mat, in the "common" diatom microphytobenthos chlorophyll *a* content did not vary much.

In 2002, the microbial mat within the wind flat exhibited a high biomass from the spring onwards, while at the diatom microphytobenthos station biomass accumulated steadily until late summer (Woelfel et al., 2007). Due to the high biomasses of phototrophic organisms in microbial mats, high primary production rates have to be expected for the whole year. In the intertidal flats of the German Wadden Sea with similar biomass values, gross production rates of 37–109 mg O_2 m^{-2} h^{-1} (14–41 mg C m^{-2} h^{-1}) were measured during a growing season (Billerbeck et al., 2007). The high photosynthetic activities led to an efflux of oxygen during daytime at a sandy site, whereas the oxygen produced at a muddy site was completely consumed within the sediment. Hence, in the sandy sediments of the wind flat, photosynthesis may be equally high due to the deeper light penetration and other physical conditions (gas exchange, mixing, pore water fluxes), while most

Table 1. Maximal and minimal monthly medians of chlorophyll *a* (mg chl *a* m^{-2}) and abundance of bacteria (cells × 109 cm^{-3}) within the upper 1 cm (2002) and upper 3 mm (2006–2008) of the microbial mat and diatom microphytobenthos site within the "Bock" wind flat.

Year	Month	Station	Depth (cm)	Chl *a* (mg m^{-2})	Bacteria (cells 10^9 × cm^{-3})
2002[a]	June–Nov	Microbial mat	1	209–305	n.d.
	Mar–Nov	Diatom phytobenthos		10–142	n.d.
2006[b]	Sept–Dec	Microbial mat	0.3	364–1,268	1.2–12
		Diatom phytobenthos		48–97	0.8–4.0
2007[b,c]	Jan–Mar; Sept–Dec	Microbial mat	0.3	46–722	0.6–6.8
		Diatom phytobenthos		20–46	0.1–0.7
2008[c,d]	Jan–Feb; Aug–Sept	Microbial mat	0.3	27–566	0.6–2.4
		Diatom phytobenthos		37–77	0.2–0.6

[a]Woelfel et al. (2007); [b]Heyl (2007); [c]Kern (2008); [d]Unpublished data; n.d.: no data.

tidal flats may be less productive in terms of oxygen export such as muddy sediments.

There are several other stimulating or inhibitory factors of net primary production, which are partly influenced by the sediment stabilizing effects of microbial mats. During inundation, migration of microalgae into deeper sediment or mat layers may result in a decreased photosynthesis. When water is available, frequent mixing of sediments without a mat can keep a phototrophic community active throughout the whole mixing depth because all cells receive light at least temporarily and grazing might be disturbed (Connor et al., 1982). The high heterotrophic activity embedded in the exopolysaccharide mat matrix can strongly affect net photosynthesis. Thus, net production rates in marine microbial mats can cover a broad span ranging from a low 38 mg O_2 m^{-2} h^{-1} (14 mg C m^{-2} h^{-1}) (Villbrandt et al., 1990) to a high 870 mg O_2 m^{-2} h^{-1} (326 mg C m^{-2} h^{-1}) (Martinez-Alonso et al., 2004).

Another important function of primary producers is the generation of organic matter that is degraded via aerobic or fermentative pathways (mostly) within the microbial mat. These compounds can be used as energy sources by the sulfate-reducing bacteria, which are responsible for producing sulfide or anoxygenic photosynthesis. Anoxygenic photosynthesis represented in 22–46% of the total primary production in Guerrero Negro (Baja California, Mexico) mats (Javor and Castenholz, 1984). In this aspect wind flat microbial mats remain to be studied.

The community of heterotrophic bacteria in the wind flat mat was with up to 12×10^9 cells cm^{-3} in 2006 up to 300 times higher than in tidal flats, where abundances of 0.04–6.4 cells $\times$ 10^9 cm^{-3} are common (Llobet-Brossa et al., 1998; Mußmann et al., 2005; Musat et al., 2006). The community of the heterotrophic bacteria was also affected by the recent decrease of the mat thickness and abundances were reduced by 43–50% in 2007 and another 35% in 2008 (Table 1).

Because microbial mats are able to inhabit extremely nutrient-poor environments, nutrients are very likely limiting the mat's productivity and growth. While on the one hand certain microbial activities compensate for the lack of inorganic nutrients, such as phosphatase production to retrieve phosphorus from organic sources (Rengefors et al., 2001), on the other hand the annual addition of external phosphorus inputs of 10 g P m^{-2} over 3 years led to an extinction of a microbial mat in wetlands of Belize as macrophytes established in this formerly nutrient-poor habitat (Sirová et al., 2006). These observations emphasize the role of nutrient availability for the stability and success of microbial mats in colonizing extreme habitats where competition and grazing are restricted. Measurements of alkaline phosphatase from wind flats revealed apparent K_m-values of 185–246 μmol l^{-1} for the wind flat microbial mat and 100–214 μmol l^{-1} for the diatom-dominated sediment, i.e. no difference between both stations (Table 2). These high values do not indicate any production or adaptation of specific alkaline phosphatases, they rather show that the K_m values are much higher than the phosphate concentrations (as end products) needed for fast phototrophic growth.

The presence of extracellular enzyme activities typically point to nutrient demand within a microbial community (Sinsabaugh et al., 2008). Therefore, the 30–100 times higher V_{max}-values of the alkaline phosphatase within the microbial mat compared to the diatom community indicate higher P requirements for the mat (Table 2). Unlike proteolytic enzymes and esterases, alkaline phosphatases may be different in their activity and species-specificity (Rengefors et al., 2001). Hence, phosphatase activity must be linked directly to the organisms producing them. This will allow a more precise insight and evaluation of the kinetics and activity of hydrolytic enzymes in view of nutrient availability.

Compared to alkaline phosphatase a similar indicator of nitrogen limitation is still missing within the microbial mat. Perhaps, a high proteolytic activity can be the result of the nitrogen demand. The extracellular activity of leucine-aminopeptidase in the wind flat mat ranged from 2 to 20 amol cell^{-1} h^{-1} in the microbial mat, and from 4 to 52 amol cell^{-1} h^{-1} in the diatom community (Heyl, 2007). These higher cell-specific but lower total proteolytic activities (V_{max}) (Table 2) in the diatom microphytobenthos suggest a potential N-limitation and a comparably low amount of nitrogen-rich substrate at this station (Montuelle and Volat, 1998). Amino acids provide nitrogen below the Redfield ratio and can be a preferable nitrogen source (Ludwig et al., 2006). At least some cyanobacteria are able to take up amino acids in high amounts heterotrophically (Zubkov et al., 2004). This would mean that peptides are rapidly recycled to amino acids and are utilized as a primary nitrogen source. The C:N ratio of the organic material from the wind flat was with values of about 8–10 as high as the Redfield ratio of intact growing phytoplankton in summer 2002 (Redfield, 1934; Woelfel, 2004) supporting the hypothesis of sufficient nitrogen supply within the microbial mat. After organic nitrogen input, net productivity did not increase within a hypersaline microbial mat, but gross photosynthesis and oxygen consumption accelerated. This reflects the tight coupling of production, consumption, and recycling within the microbial mats (Ludwig et al., 2006). In contrast to P, N can also be recruited from atmospheric N_2 independent from eutrophication. Within a tidal flat microbial mat setting in a rather undisturbed area, only 2% of the required nitrogen was

Table 2. Extracellular enzyme activities of leucine-aminopeptidase and alkaline phosphatase as parameters of the Michaelis Menten kinetics (V_{max} [µmol l^{-1} h^{-1}] and K_m [µmol l^{-1}]) within the microbial mat and the diatom-dominated microphytobenthos site in the "Bock" wind flat. Measured in sediment slurries with TRIS-HCl (5 mM, pH 8.2, 10 PSU) at room temperature with fluorescently labeled substrates (L-leucine-7-amido-4-methylcoumarin (AMC) hydrochloride; 4-methylumbellyferyl (MUF)-phosphate) at final substrate concentrations of 0, 25, 50, 75, 100, 200, and 400 µmol l^{-1} for leucine–aminopeptidase and of 0, 25, 100, 200, 350, and 500 µmol l^{-1} for alkaline phosphatase.

	Leucine–aminopeptidase		**Alkaline phosphatase**	
Parameter	**Microbial mat**	**Diatom phytobenthos**	**Microbial mat**	**Diatom phytobenthos**
V_{max} (µmol l^{-1} h^{-1})	2.9–6.7	0.6–0.8	2.6–11.7	0.08–0.17
Apparent K_m (µmol l^{-1})	198–329	189–325	185–246	100–214

gained via N_2 fixation for the production of organic material. In contrast, an actively growing microbial mat imported 17% of the needed nitrogen by fixation of atmospheric dinitrogen (Bebout et al., 1992). For an ecosystem, there are two strategies to obtain the needed amount of nitrogen: to utilize and recycle the "same" nitrogen over and over again or to import "new" nitrogen via fixing atmospheric dinitrogen. The high V_{max} values of leucine–aminopeptidase support the first strategy for the wind flat microbial mat (Table 2).

5. Conclusions

Research on the wind flat microbial mats showed a unique form of microbial mats with a missing stable layer of purple sulfur bacteria and the occurrence of diatoms within deeper sediment layers. The absence of a layer of purple sulfur bacteria in the wind flat microbial mats requires further investigation. Particularly, the question whether the sulfur cycle is suppressed or whether other bacterial groups replace purple sulfur bacteria and their function in the sulfur cycle deserves attention. Moreover, high abundance of a special community composition of heterotrophic bacteria was observed for coastal zones within the phototrophic layer of the microbial mats, which remains to be analyzed in more detail particularly with respect to its specificity for the wind flat microbial mats of the Southern Baltic Sea.

A balance of production and degradation of organic matter within the microbial mat has to be calculated in order to understand the regulation by nutrient sources and sinks in this wind flat ecosystem as well as the consequences for the community. The extracellular enzyme activities and kinetics provide valuable data to better understand the potential of the microbial mat community to cope with shortage or excess of nutrients. The heterotrophic bacteria associated with the cyanobacteria and diatoms are of special interest for the recycling of nutrients in the phototrophic layer of the microbial mat.

6. References

Bairoch, A. (2000) The ENZYME database in 2000. Nucleic Acids Res. **28**: 304–305.

Bebout, B.M. and Garcia-Pichel, F. (1995) UV B-induced vertical migrations of cyanobacteria in a microbial mat. Appl. Environ. Microbiol. **61**: 4215–4222.

Bebout, B.M., Paerl, H.W., Bauer, J.E., Canfield, D.E. and Des Marais, D.J. (1992) Nitrogen cycling in microbial mat communities: the quantitative importance of N-fixation and other sources of N for primary productivity, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats – Structure, Development and Environmental Significance.* Springer, Heidelberg, pp. 265–272.

Billerbeck, M., Roy, H., Bosselmann, K. and Huettel, M. (2007) Benthic photosynthesis in submerged Wadden Sea intertidal flats. Estuar. Coastal Shelf Sci. **71**: 704–716.

Björck, S. (1995) A review of the history of the Baltic Sea, 13.0–8.0 ka BP. Quatern. Int. **27**: 19–40.

Brosin, H.J. (1965) Hydrographie und Wasserhaushalt der Boddenkette südlich des Darß und des Zingst, In: *Veröffentlichungen des Geographischen Instituts der Karl-Marx Universität Leipzig,* Vol. XVIII/ 3, Akademie Verlag, Berlin, pp. 280–380.

Castenholz, R.W. and Garcia-Pichel, F. (2000) Cyanobacterial responses to UV-radiation, In: B.A. Whitton and M. Potts (eds.) *Ecology of Cyanobacteria. Their Diversity in Time and Space.* Kluwer, Dordrecht, pp. 591–611.

Chróst, R.J. (1990) Microbial ectoenzymes in aquatic environments, In: J. Overbeck and R.J. Chróst (eds.) *Aquatic Microbial Ecology: Biochemical Molecular Approaches.* Springer, New York, pp. 47–48.

Cohen, Y. (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities.* American Society for Microbiology, Washington, DC, pp. 22–36.

Cohen, Y. and Rosenberg, E. (eds.) (1989) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities.* American Society for Microbiology, Washington, DC.

Cole, J.J. (1982) Interaction between bacteria and algae in aquatic ecosystems. Annu. Rev. Ecol. Syst. **13**: 291–314.

Connor, M.S., Teal, J.M. and Valiela, I. (1982) The effect of feeding by mud snails, *Ilyanassa obsoleta* (Say), on the structure and metabolism of a laboratory benthic algal community. J. Exp. Mar. Biol. Ecol. **65**: 39–45.

Cook, P.L.M., Revill, A.T., Clementson, L.A. and Volkman, J.K. (2004) Carbon and nitrogen cycling in intertidal mudflats of a temperate Australian estuary. III Sources of organic matter. Mar. Ecol. Progr. Ser. **280**: 55–72.

Cottrell, M.T. and Kirchman, D.L. (2000) Natural assemblages of marine Proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. **66**: 1692–1697.

Eisma, D. (1998) Wind flats deposits, In: D. Eisma, P.L. Boer, G.C. de Cadee, K. Dijkema, H. Ridderinkhof and C. Philippart (eds.) *Intertidal Deposits: River Mouths, Tidal Flats and Coastal Lagoons.* CRC Press LLC, Washington, DC, pp. 309–316.

Garcia-Pichel, F., Prufert-Bebout, L. and Muyzer, G. (1996) Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. Appl. Environ. Microbiol. **62**: 3284–3292.

Herndl, G.J. (1992) Marine snow in the Northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. Mar. Microb. Food Webs **6**: 149–172.

Heyl, K. (2007) Hydrolytische Aktivität in einer mikrobiellen Matte im Windwatt "Bock" der südlichen Ostsee. Diploma-thesis, University of Rostock.

Javor, B.J. and Castenholz, R.W. (1984) Productivity studies of microbial mats, Laguna Guerrero Negro, Mexico, In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites.* Alan R. Liss, New York, pp. 149–170.

Jensen, L.M. (1983) Phytoplankton release of extracellular organic carbon, molecular weight composition, and bacterial assimilation. Mar. Ecol. Progr. Ser. **11**: 39–48.

Kamjunke, N., Köhler, B., Wannicke, N. and Tittel, J. (2008) Algae as competitors for glucose with heterotrophic bacteria. J. Phycol. **44**: 616–623.

Karsten, U. (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (Cyanobacteria) from benthic microbial mats: response to salinity change. J. Phycol. **19**: 501–506.

Karsten, U., Maier, J. and Garcia-Pichel, F. (1998) Seasonality in UV-absorbing compounds of cyanobacterial mat communities from an intertidal mangrove flat. Aquat. Microb. Ecol. **16**: 37–44.

Kern, C. (2008) Untersuchung zum Vorkommen und Grazing heterotropher Protozoen in einer mikrobiellen Matte im Windwatt "Bock", Zingst. Diploma-thesis, University of Rostock.

Kube, J. (1992) Das Makrozoobenthos des Windwatts am Bock – Szenario des Jahres 1991. Diploma-thesis, University of Rostock.

Lass, H.U. and Magaard, L. (1996) Wasserstandsschwankungen und Seegang, In: G. Rheinheimer (ed.) *Meereskunde der Ostsee.* Springer, Berlin, pp. 68–74.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

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

Lodders, N., Stackebrandt, E. and Nübel, U. (2005) Frequent genetic recombination in natural populations of the marine cyanobacterium *Microcoleus chthonoplastes*. Environ. Microbiol. **7**: 434–442.

Ludwig, R., Pringault, O., de Wit, R., de Beer, D. and Jonkers, H.M. (2006) Limitation of oxygenic photosynthesis and oxygen consumption by phosphate and organic nitrogen in a hypersaline microbial mat: a microsensor study. FEMS Microbiol. Ecol. **57**: 9–17.

Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009) *Brock Biology of Microorganisms*, 12th ed. Pearson/Benjamin Cumings, San Fransisco, CA.

Marshall, K.C. (1989) Cyanobacterial-heterotrophic bacterial interaction, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities.* American Society for Microbiology, Washington, DC, pp: 239–245.

Martinez, J., Smith, D.C., Steward, G.F. and Azam, F. (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquat. Microb. Ecol. **10**: 223–230.

Martinez-Alonso, M., Mir, J., Caumette, P., Gaju, N., Guerrero, N. and Esteve, I. (2004) Distribution of phototrophic populations and primary production in a microbial mat from the Ebro Delta, Spain. Int. Microbiol. **9**: 19–25.

Matthäus, W. (1996) Temperatur, Salzgehalt und Dichte, In: G. Rheinheimer (ed.) *Meereskunde der Ostsee*. Springer, New York, pp. 75–81.

Montuelle, B. and Volat, B. (1998) Impact of wastewater treatment plant discharge on enzyme activity in freshwater sediments. Ecotoxicol. Environ. Safety **40**: 154–159.

Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., van Beusekom, J.E.E., de Beer, D., Dublier, N. and Amann, R. (2006) Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømo basin, Wadden Sea. Syst. Appl. Microbiol. **7**: 333–348.

Mußmann, M., Kouske, I., Rabus, R. and Amann, R. (2005) Diversity and vertical distribution of cultured and uncultured *Deltaproteobacteria* in an intertidal mudflat of the Wadden Sea. Environ. Microbiol. **7**: 405–418.

Pattanaik, B., Roleda, M.Y., Schumann, R. and Karsten, U. (2008) Isolate-specific effects of ultraviolet radiation on photosynthesis, growth and mycosporine-like amino acids in the microbial mat-forming cyanobacterium *Microcoleus chthonoplastes*. Planta **227**: 907–916.

Pinckney, J.L., Paerl, H.W. and Fitzpatrick, M. (1995) Impacts of seasonality and nutrients on microbial mat community structure and function. Mar. Ecol. Progr. Ser. **123**: 207–216.

Quesada, A. and Vincent, W.F. (1997) Stratagies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. Eur. J. Phycol. **32**: 335–342.

Redfield A.C. (1934) On the proportions of organic derivations in sea water and their relation to the composition of plankton, In: R.J. Daniel (ed.) *James Johnstone Memorial Volume.* University Press of Liverpool, pp. 177–192.

Reinhard, R. (1953) *Der Bock, Entwicklung einer Sandbank zur neuen Ostsee-Insel,* VEB Geographisch-Kartographische Anstalt Gotha, Gotha.

Rengefors, K., Pettersson, K., Blenckner, T. and Anderson, T.M. (2001) Species-specific alkaline phosphatase activity in freshwater spring phytoplankton: application of a novel method. J. Plankton Res. **23**: 435–443.

Rippe, H. and Dierschke, V. (1997) Picking out the plum jobs: feeding ecology of curlews *Numenius arquata* in a Baltic Sea wind flat. Mar. Ecol. Progr. Ser. **159**: 239–247.

Schlungbaum, G. and Baudler, H. (2001) Die Darß-Zingster Bodden, eine Studie. Schriftenreihe des Landesamtes für Umwelt. Nat. Geol. Mecklenburg-Vorpommern **1**: 53–60.

Schwarzer, K. (1996) Dynamik der Küste, In: G. Rheinheimer (ed.) *Meereskunde der Ostsee.* Springer, Berlin, pp. 25–33.

Severin, I. (2005) Interaktion zwischen heterotrophen Bakterien und dem mattenbildenden Cyanobakterium *Microcoleus chthonoplastes* aus Nord- und Ostsee. Diploma-thesis, University of Rostock.

Siegesmund, M., Johansen, J.R., Karsten, U. and Friedl, T. (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J. Phycol. **44**: 1572–1585.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R. and Zeglin, L.H. (2008) Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. **11**: 1252–1264.

Sirová, D., Vrba, J. and Rejmankova, E. (2006) Extracellular enzyme activities in benthic cyanobacterial mats: comparison between nutrient-enriched and control sites in marshes of northern Belize. Aquat. Microb. Ecol. **44**: 11–20.

Stal, L. (1994) Microbial mats in coastal environments, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats – Structure, Development and Environmental Significance.* Springer, Heidelberg, pp. 21–32.

Stal, L. (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol. **131**: 1–32.

Stal, L. (2003) Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. Geomicrobiol. J. **20**: 463–478.

Stal L. and Krumbein, W.E. (1985) Isolation and characterization of cyanobacteria from a marine microbial mat. Bot. Mar. **28**: 351–365.

Stal, L. and Caumette, P. (eds.) (1994) *Microbial Mats – Structure, Development and Environmental Significance.* Springer, Heidelberg.

Stal, L., van Gemerden, H. and Krumbein, W.E. (1985) Structure and development of a benthic marine microbial mat. FEMS Micobiol. Ecol. **74**: 305-312.

Sundbäck, K. (1983) Microphytobenthos on sand in shallow brackish water, Öresund, Sweden. Ph.D. thesis, University of Lund, Sweden.

Underwood, G.J.C., Paterson, D.M. and Parkes, R.J. (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnol. Oceanogr. **40**: 1243–1253.

van Gemerden, H. (1993) Microbial mats: a joint venture. Mar. Geol. **113**: 3–25.

Villbrandt, M., Stal, L.J. and Krumbein, W.E. (1990) Interactions between nitrogen-fixation and oxygenic photosynthesis in a marine microbial mat. FEMS Microbiol. Ecol. **74**: 59–72.

Wetzel, R.G. (1991) Extracellular enzymatic interactions: storage, redistribution and interspecific communication, In R.J. Chróst (ed.) *Microbial Enzymes in Aquatic Environments.* Springer, New York, pp. 6–28.

Witkowski, A. (1990) Fossilization processes of the microbial mat developing in clastic sediments of the Puck Bay (Southern Baltic Sea, Poland). Acta Geol. Pol. **40**: 1–27.

Witkowski, A. (1991) Diatoms of the Puck Bay coastal shallows (Poland, Southren Baltic). Nordic J. Bot. **11**: 689–701.

Witte, K. (2005) Untersuchungen zur Entwicklung von Ökotypen in dem Cyanobakterium *Microcoleus chthonoplastes* entlang der Salinitätsgradienten der Ostsee. Ph.D. thesis, University of Rostock.

Witte, K., Woelfel, J. and Karsten, U. (2004) Das Vorkommen mikrobieller Matten an der Deutschen Ostseeküste am Beispiel des Windwatts "Großer Werder". Rostocker Meeresbiologische Beiträge **12**: 61–70.

Woelfel, J. (2004) Saisonale Sukzession in der photoautotrophen Schicht mikrobieller Matten des Windwattes der Darß-Zingster Boddenkette. Diploma-thesis, University of Rostock.

Woelfel, J., Schumann, R., Adler, S., Hübener, T. and Karsten, U. (2007) Diatoms inhabiting a wind flat of the Baltic Sea: species diversity and seasonal succession. Estuar. Coast. Shelf Sci. **75**: 296–307.

Zubkov, M.V., Tarran, G.A. and Fuchs, B.M. (2004) Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. FEMS Microbiol. Ecol. **50**: 153–161.

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DIAZOTROPHIC MICROBIAL MATS

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1. Introduction

Microbial mats have been the focus of scientific research for a few decades. These small-scale ecosystems are examples of versatile benthic communities of microorganisms, usually dominated by phototrophic bacteria (e.g., Krumbein et al., 1977; Jørgensen et al., 1983). They develop as vertically stratified populations of functionally different groups of microorganisms along physicochemical gradients (Fig. 1). The stratification of these functional groups of microorganisms has been attributed to the prevailing gradients of oxygen, sulfide, and light, which are generated and maintained by the metabolic activities of the community members (Revsbech et al., 1983; van Gemerden, 1993). Microbial mats are found in a wide variety of different environments such as, for example, marine intertidal flats, hypersaline and alkaline environments, hot springs, and hot and cold deserts. The diversity in environments supporting the growth of microbial mats is only exceeded by the genetic and metabolic diversity of mat-inhabiting organisms.

In most cases, cyanobacteria form the main structural element of microbial mats. As primary colonizers on bare substrate they are the prerequisite for the development of this microbial ecosystem. Cyanobacteria have low nutritional requirements and because of their capability of oxygenic photosynthesis, CO_2 and N_2 fixation, anaerobic dark fermentation, and the production of extracellular polymeric substances (EPS) these organisms seem to be predestined for this task (Stal, 2001). The ability to fix atmospheric N_2 (dinitrogen) represents a distinctive advantage that allows cyanobacteria to colonize the often nutrient-poor and particularly nitrogen-depleted environments in which microbial mats thrive. This ability is widespread not only among cyanobacteria but also among other *Bacteria* and *Archaea*.

Among the taxonomically diverse group of N_2 fixing organisms (diazotrophs) cyanobacteria deserve special attention because they combine the fixation of CO_2 and N_2, providing them with basically unlimited access to the two quantitatively most important elements for living biomass. Cyanobacteria are the only oxygenic phototrophic prokaryotes and possess a plant-type photosynthetic apparatus. They are also special because they combine the incompatible processes of oxygenic photosynthesis and the oxygen-sensitive N_2 fixation and hence they evolved special strategies to make this possible. For a detailed account on this topic the reader is

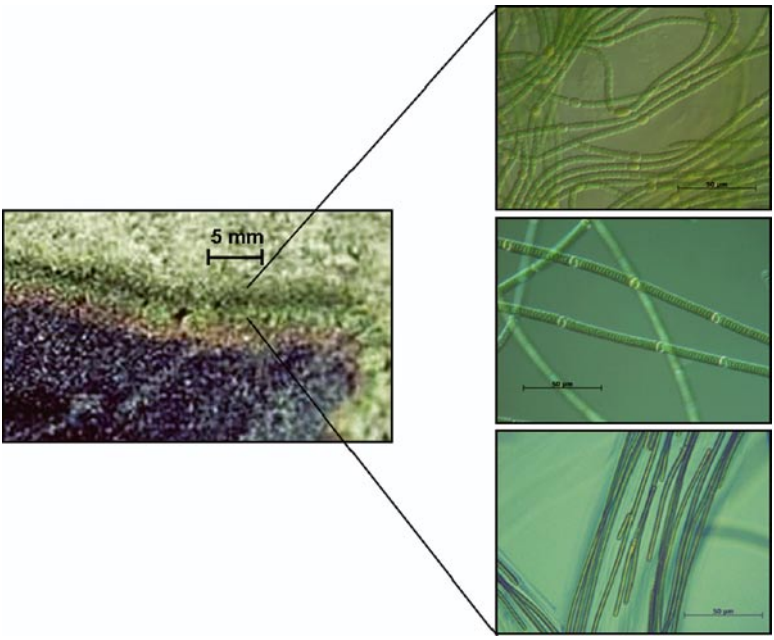

Figure 1. Diazotrophic microbial mat (*left*) and micrographs of diazotrophic cyanobacteria that can be found in intertidal mats (*right panels*) (From *top* to *bottom*: *Anabaena*, *Nodularia* and *Lyngbya*).

referred to the many extensive reviews (e.g., Bergman et al., 1997; Berman-Frank et al., 2003; Fay, 1992; Gallon, 1992). A summary of the main strategies (after Stal, 1995) is given here because diazotrophic cyanobacteria usually determine the pattern of N_2 fixation in microbial mats.

One group of filamentous diazotrophic cyanobacteria is exceptional because their representatives differentiate a special cell, the heterocyst, which is the site of N_2 fixation in these organisms. The heterocyst provides an anoxic environment for nitrogenase by losing the oxygenic photosystem II and by having a multilayered glycolipid cell envelope that serves as a gas diffusion barrier limiting the flux of oxygen (see also reviews by, e.g., Haselkorn, 1978; Böhme, 1998). This cell imports carbohydrate from the neighboring vegetative cells in exchange for fixed nitrogen. Hence, in heterocystous cyanobacteria N_2 fixation is spatially separated from oxygenic photosynthesis. Heterocystous cyanobacteria are common in many freshwater and terrestrial environments but rare in microbial mats. Non-heterocystous diazotrophic cyanobacteria evolved a variety of other strategies. Many filamentous and unicellular diazotrophic cyanobacteria fix N_2 only under anaerobic conditions (avoidance). This type of cyanobacteria may thrive under anoxic conditions in sulfidic microbial mats. Other mat-forming diazotrophic cyanobacteria fix N_2 aerobically by confining it to the night and hence separating it temporally from oxygenic photosynthesis.

2. Occurrence of Microbial Mats

Microbial mats are distributed globally (Fig. 2) and can be found in a wide variety of environments, particularly in extreme habitats. Extreme environments are usually characterized by highly selective conditions often excluding higher (eukaryotic) organisms (Paerl et al., 2000). Microbial mats can be found in cold polar regions (Arctic and Antarctic), in the dry and hot desert, in hypersaline environments, hot springs, and in coastal environments. The latter are often characterized by strongly fluctuating environmental conditions such as large variations in water availability and desiccation, and salinity, temperature, oxygen, and sulfide gradients. Investigations of microbial mats have focused on the structural aspects, biogeochemical cycles, and on the biodiversity of the functional groups of microorganisms that comprise the mat as well as the diversity of their metabolic capabilities.

This overview will discuss diazotrophic microbial mats from a variety of different environments and describe the particularities with respect to N_2 fixation and the diazotrophic community members. It will be based on examples rather than aiming at completeness. The apparent abundance of occurrences of microbial mats in the northern hemisphere is obviously biased by the higher research input. Habitats that support the development of microbial mats are reported from the poles to the tropics. That triggers a number of questions that we like to discuss in this chapter. Since microbial mats are found in such different geographic locations and in such diverse habitats, which factors are important for their largely similar structure and functioning? Are all microbial mats capable of

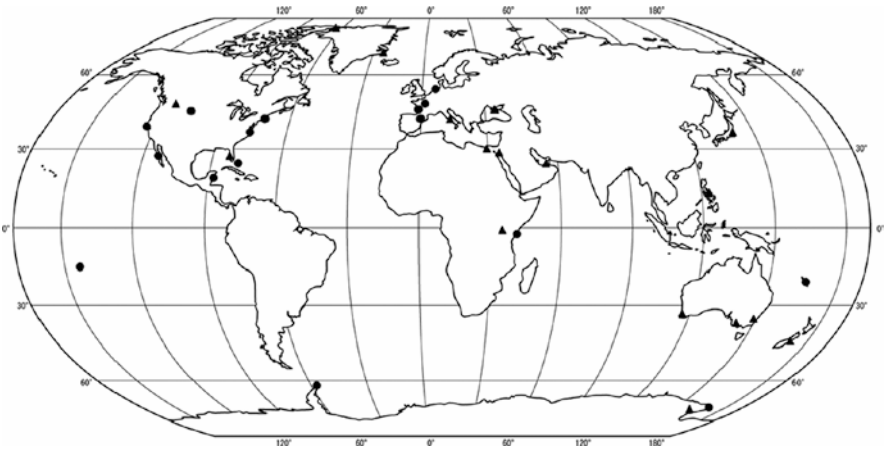

Figure 2. Global distribution of microbial mats. Indicated are sites of microbial mats that have been investigated in depth. Mats reported to possess diazotrophic activity are labeled with filled circles (•), those that were not investigated for diazotrophy are depicted by a closed triangle (▲). All mats that were investigated for the capability of N_2 fixation possessed this property.

fixing N_2? What factors determine N_2 fixation and are selective for the type of diazotroph? We will discuss these questions with the help of some selected and well-studied habitats.

2.1. INTERTIDAL MICROBIAL MATS

Microbial mats develop on intertidal sediments in a variety of coastal environments where the conditions are harsh due to strongly fluctuating physicochemical gradients and environmental parameters. These environments largely exclude grazing organisms that would otherwise prevent the accumulation of microorganisms necessary to form a microbial mat. Especially, coastal tidal flats with low slopes and fine sandy sediment serve as excellent habitats for mats (Stal, 2000).

The beaches of the barrier islands in the Netherlands, Germany and Denmark, separating the North Sea from the Wadden Sea, are a good example of an environment that allows the development of microbial mats (e.g., Severin and Stal, 2008; Stal et al., 1984; Villbrandt et al., 1990). Several types of microbial mats can be found on the northwest banks where the deposition of fine sand on the extended and shallow tidal flats provides a suitable substrate for the settlement of cyanobacteria. *Microcoleus chthonoplastes* and *Lyngbya aestuarii* were the predominant cyanobacteria in these mats but also other genera (e.g., *Spirulina*, *Gloeothece* and *Merismopedia*) were present. The diversity of the different mat types varied as did the daily pattern of nitrogenase activity. Mats in an early stage of development showed the highest biomass (chlorophyll *a*) specific nitrogenase activity (NA). This can be explained by the high demand for nitrogen in the rapidly growing mats. In such mats NA was almost entirely confined to the night. Since these mats did not turn anoxic during the night, the energy for night time NA was supplied by aerobic respiration. NA measurements of a well-developed mat showed a small sunset- and a big sunrise-peak, obviously due to favorable conditions for N_2 fixation at these times. Light, photosynthetic activity and hence oxygen concentrations were still low but sufficient for energy supply of nitrogenase. No NA was observed in the dark, presumably because the mat turned anoxic and did not allow for respiration to supply energy needed for NA. Light and oxygen appeared to have a major impact on N_2 fixation dynamics in these mats (Villbrandt et al., 1990). A more complex pattern was found in mats on the intertidal sandy beach on the island Schiermonnikoog, the Netherlands (Severin and Stal, 2008). A microbial mat characterized by a mixed cyanobacterial community showed activity maxima at sunset and sunrise. In contrast to the well-developed mat mentioned above, considerable NA was also detected during the night. This was attributed to a shift in the actively N_2 fixing microbial community. Another mat, dominated by the non-heterocystous *L. aestuarii*, exhibited a diel NA pattern characteristic for aerobically N_2-fixing non-heterocystous cyanobacteria (Fig. 3).

Microbial mats developing in the subtropics were intensively studied on North Carolina's Outer Banks barrier islands (e.g., Shackleford Banks). These mats

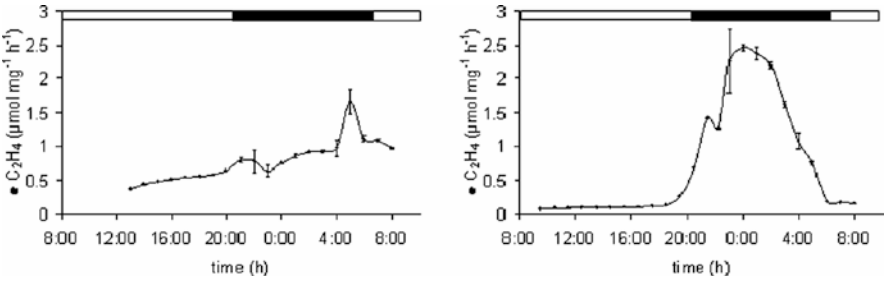

Figure 3. Daily cycle of nitrogenase activity (NA) for a mixed cyanobacterial community with heterocystous cyanobacteria present (*left panel*) and a microbial mat dominated by *Lyngbya aestuarii* (*right panel*).

were widely distributed throughout the intertidal zone where they grow on mud and sand flats, experiencing mostly full seawater salinity or even hypersaline conditions in addition to high irradiances and low nutrient supply (Paerl et al., 2000). The mats were most of the time exposed and only immersed for a few hours during high tide. The dominant cyanobacteria in the Shackleford microbial mats were *M. chthonoplastes*, *L. aestuarii* and representatives of the genera *Oscillatoria*, *Phormidium*, and *Synechocystis*. Bebout et al. (1987) observed an inverse relationship of CO_2 and N_2 fixation and suggested energy competition between the two processes and/or inhibition of NA by photosynthetically produced oxygen as a possible cause. They also reported a tight energy coupling based on the observation that a lower nighttime NA followed low photosynthetic activity during the preceding day. In addition, these mats also exhibited seasonal dynamics of NA (Paerl et al., 1996). From spring to fall NA peaked during the night whereas in winter maximum NA was observed at midday. Such seasonal changes may be caused by a succession of different groups of diazotrophic bacteria that have different strategies for fixing N_2. In winter, conditions might not be optimal for cyanobacteria, which could lead to a competitive advantage for chemotrophic N_2-fixing organisms. This is supported by observations made by Zehr et al. (1995) who found that in these mats *nifH* sequences, which code for nitrogenase reductase of the non-heterocystous cyanobacterium *Lyngbya* were present only in summer whereas no cyanobacterial *nifH* sequences were retrieved in winter. It is possible that the low availability of light limits the growth of cyanobacteria in winter. The role of heterotrophic diazotrophs in this microbial mat was further investigated by Steppe and Paerl (2002, 2005). Clone libraries of the *nifH* gene yielded sequences similar to certain sulfate-reducing bacteria (SRB). The possible involvement of SRB in N_2 fixation was supported by the inhibitory effect of molybdate, a structural analogue of sulfate and a potent inhibitor of sulfate reduction.

Intertidal microbial mats are also known from various tropical environments (e.g., Pinckney et al., 1995b). Coastal intertidal and subtidal stromatolites are found in Shark Bay (Western Australia) and on the Exuma Cays (Bahamas). Stromatolites are formed by lithifying microbial mats and have been considered

as the living examples of fossil Precambrian stromatolites. The laminated structure of the stromatolites is generated by the lithification of the cyanobacterial mat, which is affected by seasonal influences and sometimes by erratic events. Mats on the Exuma Cays are found in the back reef intertidal and subtidal zones (Pinckney et al., 1995c). The upper 10–30 cm is only exposed at low tide for a few hours and the mats therefore do not experience desiccation. A horizontal zonation of several mat types with varying community composition was observed but cyanobacteria generally dominated. The most prominent cyanobacterium in the Exuma Cays stromatolites was *Schizothrix gracilis*. Also present were the filamentous non-heterocystous *Lyngbya*, *Oscillatoria*, and *Phormidium* as well as the heterocystous *Calothrix* and the unicellular *Gloeothece*. These mats exhibited NA, which was found to be higher during the day (Pinckney et al., 1995c). However, on another occasion NA was found to be maximal during the night (Steppe et al., 2001). This discrepancy may have been due to a shift in community structure or unrecorded differences in environmental conditions. The level of NA in these mats was reported to be dependent on photosynthetic activity and availability of organic carbon. The daily patterns were the same in spring and summer but the mechanism by which NA was maintained varied with the seasons. *De novo* synthesis of nitrogenase was not detected in March whereas NA in August almost entirely depended on newly synthesized nitrogenase. *NifH* sequences from cyanobacteria, Alphaproteobacteria, and obligate anaerobic bacteria were found in the stromatolites (Steppe et al., 2001). The relative contribution of these groups to N_2 fixation under different conditions could explain differences in NA patterns.

For a range of different tropical microbial mats that have been investigated, nighttime as well as daytime NA has been reported. For instance, nighttime NA has been observed in microbial mats in Puerto Rico (Diaz et al., 1990) and in the non-heterocystous *Hydrocoleum*-dominated microbial mats in New Caledonia (Charpy et al., 2007). The heterocystous cyanobacterium *Nodularia*-dominated microbial mats in New Caledonian exhibited higher daytime activity (Charpy et al., 2007). Daytime activity also occurred in mats harboring heterocystous as well as non-heterocystous cyanobacterial diazotrophs in French Polynesia (Charpy-Roubaud et al., 2001; Charpy-Roubaud and Larkum, 2005) and in microbial mats found on Zanzibar, Tanzania (Bauer et al., 2008). The Zanzibar microbial mats were dominated by filamentous *Oscillatoriales* and unicellular *Chroococcales* and were therefore expected to show NA in the dark. However, these mats did not reveal cyanobacterial *nifH* sequences and confirmed similar observations from other habitats that cyanobacteria might not necessarily represent the major diazotrophs in microbial mats.

Structurally similar microbial mats built by cyanobacteria are found from the tropics to the polar regions indicating that temperature is not a selecting factor against these microbial ecosystems. From the limited knowledge available we might conclude that the community composition may be different and also that different functional groups are involved in N_2 fixation in polar and tropical microbial mats.

2.2. ANTARCTIC MICROBIAL MATS

Microbial mats in the Arctic are mostly found in tundra environments, but they have been much less investigated than those found on the Antarctic continent. Antarctica is the coldest, driest, and windiest continent and is therefore considered an extreme habitat, limiting microbial growth. Cyanobacteria in polar regions are known to inhabit exposed rock surfaces, fissures and interstitial spaces, and form mats on the bottom of streams, lakes, and ponds. Antarctic microbial mats have been studied on the McMurdo Ice Shelf. This habitat is characterized by a temperature range from −60°C in winter to 0°C to −40°C in summer, light extremes (total darkness in winter and permanent light in summer), and low precipitation. Due to heating and melting in summer, water availability increases and soil particles accumulate in cracks, creating substrates for cyanobacteria. In general, two types of microbial mats can be found: *Oscillatoriales*-dominated mats and those mainly composed of *Nostoc commune* (Vincent, 2000). The genera *Oscillatoria*, *Schizothrix*, and *Phormidium* form mats of several millimeter thickness and with a red-orange surface that serves as a UV screen. The cyanobacteria occur as a blue-green layer at the bottom. *Nostoc commune* mats may be up to 20 mm thick and are found in rather ephemeral environments.

Microbial mats associated with the ice cover of Lake Bonney exhibited NA but with lower rates than those reported for mats in temperate and tropical regions. Moreover, NA was mainly confined to the light period (Olson et al., 1998). Cyanobacterial mats colonizing the bottom of a variety of small lakes and ponds were layered similar to mats from temperate regions and dominated by *Oscillatoriales* (Fernandez-Valiente et al., 2001). NA in these mats was within the range of rates reported from temperate mats but, similar to the Lake Bonney ice cover mats, activity was also much higher at daytime. This hints at a major contribution of heterocystous cyanobacteria to N_2 fixation although such organisms were only represented by less than 10% of the counts. *NifH* sequences revealed the presence of cyanobacteria as well as chemotrophic organisms. The latter would be independent from light unless they receive their substrate directly from the cyanobacteria as proposed by Steppe et al. (1996).

Temperature is not necessarily a factor that prohibits N_2 fixation (within physiological limits) but it will affect metabolic rates of organisms. However, there are other factors that have a potential impact on microbial performance in polar regions. Melt water ponds revealed the highest cyanobacterial diversity at lowest salinities (Jungblut et al., 2005). *Oscillatoriales*, including *Oscillatoria*, *Lyngbya*, and *Phormidium*, and heterocystous *Nostocales*, represented by *Nostoc* and *Nodularia*, were the predominant organisms, confirming their endemic nature in Antarctic environments.

In conclusion, against the odds of this hostile environment, diazotrophic microbial mats develop and seem to be common in Antarctica. Low temperature for growth, freeze–thaw cycles, solar radiation with a high level of UV, a range of salinities, and prolonged dormancy evoked appropriate adaptations and are apparently selected for a specific group of mat-forming cyanobacteria.

2.3. HOT SPRING MICROBIAL MATS

Hot springs represent an environment that occupies the high end of the temperature spectrum. Microbial mats in the Octopus and Mushroom Springs in Yellowstone National Park (USA) are among the best-studied examples. The microbial mats at these alkaline siliceous hot springs are situated in the effluent channel with temperatures between 42°C and 74°C. These mat communities are dominated by *Chloroflexus*-like bacteria and *Synechococcus*-like cyanobacteria (Ward et al., 1998) but community composition changed horizontally along the temperature gradient (Ferris et al., 1996) and vertically according to the gradients of light and chemical conditions (Ramsing et al., 2000). Denaturing gradient gel electrophoresis (DGGE)-profiles of the 16S rRNA gene confirmed the stability of the community composition at temperature-defined sites independent from the season (Ferris and Ward, 1997). This suggests the presence of ecologically specialized groups with adaptations corresponding to the prevailing temperature and light conditions as well as to the chemical gradient (Ward et al., 2006). For a long time, N_2 fixation has been considered to be absent or irrelevant in these hot spring microbial mats. However, whole genome sequencing of the major cyanobacterial players in these microbial mats, *Synechococcus* spp., showed the presence of a fully operational *nif* operon. Subsequently, it was shown that *Synechococcus nifH* expression and NA occurred in a daily manner as is the case in other non-heterocystous microbial mats (Steunou et al., 2006, 2008). NA peaked in the early evening and, much more pronounced, in the early morning. This was attributed to the favorable combination of low light intensities and low oxygen concentrations, confirming earlier work in coastal microbial mats (Villbrandt et al., 1990) and in cultures of the non-heterocystous cyanobacterium *L. aestuarii* (Stal and Heyer, 1987). *Nif*-transcripts accumulated in the evening and declined during the night while nitrogenase was stable throughout the night, suggesting that *de novo* synthesis of nitrogenase did not occur during that period. Thermal adaptation of diazotrophy in these mats was concluded from the temperature dependence of NA which was in approximate agreement with the one previously measured for growth and photosynthesis of hot spring *Synechococcus* isolates. Ambient temperatures of 60–65°C supported highest mat NA.

Major impacts on bacterial growth in hot spring microbial mats are gradients of temperature, chemical conditions, and light, and communities have been proven to adapt to niches defined by these factors.

2.4. HYPERSALINE MICROBIAL MATS

Hypersaline lakes, ponds, and sabkhas are other examples of extreme environments exhibiting a low diversity of mostly prokaryotic life that adapted to the hostile conditions. The Salins-de-Giraud saltern in the Camargue, France, has been extensively studied. Cyanobacteria were the major components of microbial mats

at 70–150% salinity (Fourçans et al., 2004). DGGE revealed a diverse community that comprised of the filamentous non-heterocystous cyanobacteria *Microcoleus*, *Oscillatoria*, *Leptolyngbya*, *Phormidium*, and the unicellular *Pleurocapsa* and *Gloeothece*. SRB, sulfur-oxidizing and anoxygenic phototrophic bacteria were also present and the populations were vertically stratified according to microgradients of oxygen, sulfide, and light. Filamentous cyanobacteria were mainly present at the surface, followed by sulfur-oxidizing bacteria and anoxygenic phototrophs. SRB were distributed throughout the whole mat. Depth-related differences in the community structure were reported to be of greater importance than temporal variations during a day–night cycle (Villanueva et al., 2007). NA showed seasonal changes and was higher in the dark during summer and higher in the light in winter (Bonin and Michotey, 2006). This was attributed to seasonal changes in the diazotrophic community as has been proposed for other microbial mats (Paerl et al., 1996). Moreover, in winter, denitrification was slightly higher than N_2 fixation but in summer this situation inversed turning these mats into an overall net source of combined nitrogen.

Another well-studied hypersaline habitat is La Salada de Chiprana, Spain, the only permanent natural hypersaline inland lake in Western Europe. This lake is characterized by a salinity of ~70% and high concentrations of magnesium and sulfate. The microbial mats in this lake had a peculiar inverted stratification with *Chloroflexus*-like bacteria on top and cyanobacteria (*Microcoleus*- and *Pseudanabaena*-like) underneath (Jonkers et al., 2003). This phenomenon was explained by the downwards migration of the cyanobacteria in order to escape UV damage. Below a calcium carbonate layer another cyanobacterial layer was present, dominated by *Microcoleus*-like filamentous cyanobacteria. In addition, unicellular *Halothece*- and *Gloeocapsa*-like cyanobacteria, purple and colorless sulfur bacteria and aerobic heterotrophs contributed to the community. NA was present throughout the day and night, although the rates were slightly (10%) higher at night (Camacho and de Wit, 2003). NA was low compared to activities measured in other microbial mats and the use of metabolic inhibitors suggested methanogens and aerobic heterotrophs as the major diazotrophs (De Wit et al., 2005). Hence, although these mats were dominated by cyanobacteria, they were apparently not the major contributors to N_2.

Hypersaline diazotrophic microbial mats with a high diversity were also found in the salterns of Guerrero Negro, Mexico. The community appeared much more diverse than previously expected, showing an uneven distribution of bacteria in the upper layers (Ley et al., 2006). In this mat, persisting under a cover of brine of 80% salinity, *Chloroflexi* dominated when whole mat community was taken into account. Cyanobacteria predominated the upper (oxic) 2 mm. Diversity differed according to oxygen and sulfide concentrations and it was implied that related bacteria occupy similar chemical niches because they share physiological properties. The cyanobacterial components of this microbial mat were stable (Green et al., 2008). Experimental decrease of salinity or manipulations using sulfate additions did not affect community composition. It is not known how this

community remained stable after such dramatic changes of the environmental conditions. Microbial mats dominated by *Lyngbya* or *Microcoleus* were investigated with respect to community composition and N_2 fixation (Omoregie et al., 2004a, b). The *Lyngbya*-dominated type was 2–3 cm thick, fibrous, and had a rough surface. It was periodically flooded, desiccated, and physically relocated. This mat was assumed to represent a pioneer mode of microbial mat with a high external nitrogen demand which would explain the higher NA compared to *Microcoleus*-dominated mat. The latter showed a thickness of 4–5 cm, a cohesive structure and a smoother surface. The lamination was more apparent and the diversity higher than in the *Lyngbya*-mat. A lower external nitrogen demand of this supposedly established mat could explain the lower NA. Sequencing of the *nifH* gene revealed that delta- and gamma proteobacteria were more prominently present in the clone libraries than cyanobacteria.

In hypersaline mats in different regions of the world, cyanobacteria and *Chloroflexus*-like bacteria are the dominant mat-building organisms, although a variety of other bacteria are present and may be responsible for N_2 fixation. All these members of the community are facing high salinities, solar radiation, and high temperatures. These factors affect community composition and metabolic performance but do not prevent the formation of these highly diverse and complex microecosystems.

3. Factors Controlling Occurrence and Performance of Diazotrophic Microbial Mats

All environments in which microbial mats develop are characterized by extreme conditions such as temperature, solar (UV) irradiation, salinity, alkalinity, desiccation, or their fluctuations. However, the limited range of environmental conditions under which higher organisms are able to survive is an important factor for the development of microbial mats in these extreme environments. This is mainly due to the absence of higher grazing organisms.

Cyanobacteria are especially well adapted as primary colonizers and are therefore often found as the builders of the matrix of the mat that forms the prerequisite for the subsequent settlement of other microorganisms (see introduction). However, certain environmental factors may prevent microbial growth and mat development. These include turbulence, as present in areas with high wave energy, and grazing pressure, for example, under less extreme conditions (Vitousek and Howarth, 1991). The high energy costs of N_2 fixation might also constrain diazotrophic activity and nutrient (other than nitrogen) limitations could prevent microbial mats from developing. Major environmental factors playing a role in all the above-mentioned microbial mats, and probably determining the occurrence of certain mat organisms, are temperature and salinity. Nevertheless, they do not appear to prevent the development and activity of diazotrophic microbial mats. Diazotrophs have obviously adapted to a large range of environmental conditions.

3.1. TEMPERATURE

Diazotrophic microbial mats have been found in all climatic zones on earth, and cyanobacteria are clearly dominating in habitats at either end of the temperature range from the polar regions to the tropics. Although they are thought to have optimal growth temperatures of above 20°C, cyanobacterial mats can be found in environments ranging in temperature from 0°C to 60°C. However, isolated strains of cyanobacteria from polar regions were not psychrophilic (i.e., temperature optimum of growth below 15°C) but had on average an optimal growth temperature of approximately 20°C (ranging from 15°C to 35°C) and were therefore considered to be psychrotolerant (Tang et al., 1997). The most prominent effect of temperature on a living cell is that it determines the rate of metabolic reactions within the physiological limits of the organism. It is therefore not surprising that N_2 fixation rates are much lower in polar regions than in warmer environments. Moreover, one has to take into account that polar regions are characterized by a multitude of other harsh conditions besides low temperature (e.g., UV-stress, desiccation, and long dormancy periods). The slow growth rate also demands much lower rate of nitrogen supply.

Growth and metabolic activity is possible only when liquid water is present. Microbial mats are found at the bottom of Antarctic lakes where most of the water is frozen except just above the bottom. In cracks and fissures in rocks the temperature may rise above zero allowing the growth of cyanobacteria. In pockets in sea ice, brines are present due to exclusion of sea salt from the frozen water. These brines have very low freezing points. It is well known that algae and cyanobacteria, including diazotrophic species, grow in these pockets at temperatures well below zero (Rysgaard et al., 2001). Hence, these cyanobacteria probably experience the high salinity as a more serious stress than the low temperature. The compatible solutes that are required for life at high salinity (see below) also serve as an antifreeze protecting the proteins of the cell in case of freezing. Ice crystals forming inside the cytoplasm are a serious threat for living cells. Compatible solutes will lower the freezing point of the cytoplasm and also protect enzymes. The accumulation of ice nucleation proteins in the outer membrane is another way the cell protects itself from damage through ice crystals in the cytoplasm.

While Oscillatoriales and Nostocales are the dominant cyanobacteria in polar regions, hardly any other cyanobacteria than the *Synechococcus* morphotype is found at temperatures above 55°C (Ward et al., 1998). Thermal environments with lower temperatures (~45°C) are known as sites where heterocystous cyanobacteria such as *Fischerella* sp. and Oscillatoriales form diazotrophic microbial mats. Highest N_2 fixation rates were measured at ambient but not at experimentally lowered temperatures. Hence, adaptation to the effect of high temperature on cell metabolism has occurred. The most broadly observed negative effects of elevated temperatures on organisms are denaturation of proteins, for example, enzymes and transport systems, and the disintegration of membranes. One way of adaptation to high

temperature is the increase of chaperones that serve as heat shock proteins by facilitating and maintaining the correct folding of proteins under extreme conditions. Furthermore, the proportion of saturated fatty acids in membrane structures can be increased to achieve an elevated rigidity. The effect of elevated temperature on N_2 fixation has been studied for the chemotrophic bacterium *Klebsiella pneumoniae* (Hennecke and Shanmugam, 1979), as well as for the heterocystous cyanobacterium *Anabaena cylindrica* and the unicellular cyanobacterium *Gloeothece* sp. (Gallon et al., 1993). In both cyanobacteria NA was inhibited but this was not a result from thermal inactivation of the enzyme but due to an increased O_2 sensitivity of nitrogenase. Although the mechanism was reported to be different for both organisms, no inhibition occurred under anoxic conditions. It can therefore be concluded that, unlike in polar regions, organisms fixing N_2 in thermal environments must have evolved specific adaptations.

Habitats within the temperature range marked by polar and hot spring environments can often be found in intertidal areas which are characterized by a strong fluctuation of environmental parameters, including temperature. Daily and seasonal temperature changes are common for temperate and subtropical areas whereas in tropical habitats seasonal variability is usually smaller but daily changes can be more pronounced. In these environments organisms either have to be tolerant to the temperature regime or occupy niches according to their temperature preferences. In any case, microbial mat communities have been shown to persist in intertidal areas and exhibit high N_2 fixation rates. So at least for cyanobacteria, a distribution according to temperature preferences seems likely and the occupation of adequate temperature niches by certain forms allows for distribution in a wide range of habitat temperatures. Nitrogenase does not seem to be subjected to thermal inactivation by a moderate increase of ambient temperature but oxygen sensitivity might be elevated and thus limit N_2 fixation under aerobic conditions (Gallon et al., 1993). Moreover, a decrease of temperature will decrease the rate of respiration which could result in the inability of the N_2-fixing cell to maintain anaerobic conditions. An increase in temperature would not have the opposite effect (when the N_2-fixing cell is already anaerobic) although it might select for diazotrophic cyanobacteria that were unable to fix N_2 due to the low rate of respiration at the lower temperature.

Based on these observations, temperatures within physiological limits do not prevent mat-building diazotrophic organisms from (extreme) habitats all over the world. Furthermore, N_2 fixation has been found in environments at both ends of the observed temperature range and suggests the existence of well-adapted diazotrophs.

3.2. SALINITY

As is the case with temperature, salinity can be a highly variable environmental factor in many habitats. And similar to temperature, various niches within these habitats allow the existence of a diverse community of mat-building and diazotrophic

organisms. These organisms are adapted to thrive in high–salinity environments, analogous to the high temperature adapted *Synechococcus*-morphotype in hot spring microbial mats. Such organisms must withstand the general problem of water loss in hypertonic habitats causing the damage of plasma membranes and renders the cell metabolically inactive and eventually prohibits growth. Commonly found cyanobacteria in hypersaline microbial mats are filamentous non-heterocystous species, mostly Oscillatoriales. Among these, *M. chthonoplastes* is particularly important. *M. chthonoplastes* is common in marine microbial mats and hypersaline environments but also dominates desert crusts which can experience extreme desiccation.

Hypersaline cyanobacteria are adapted to the high osmotic pressure to which they are exposed. It has been suggested that the common sheath that encloses bundles of trichomes of *Microcoleus* serves as a means of water retention (Dor and Danin, 2001). This strategy may also be used by other cyanobacteria. However, cyanobacteria in hypersaline microbial mats, as is the case in microbial mats in other environments, are embedded in a gelatinous matrix of EPS and this seems in general important as a strategy to protect the organisms from desiccation. It is therefore not specific for hypersaline conditions.

The accumulation of compatible solutes is probably the major adaptation to hypersaline conditions. Compatible solutes increase the osmolarity of the cytoplasm thereby alleviating osmotic stress. Compatible solutes are low molecular organic compounds that are highly soluble and do not interefere with enzyme activity not even at high concentrations. The higher the salinity the organisms are exposed to the higher concentration of compatible solutes is required and this puts specific demands to the properties of the solute. Hence, there is a suite of compatible solutes each type particularly suitable for a specific range of salinities.

Halophiles from different habitats are known to modify the structure of their membranes when exposed to high concentrations of sodium. Despite the stress imposed on the organisms by high salinities, communities were diverse and harbored unicellular cyanobacteria as well as other microorganisms such as SRB and sulfur-oxidizing bacteria. One prominent member of the green non-sulfur bacteria in hypersaline microbial mats was *Chloroflexus* which is also known from hyperthermal environments.

Although salinity has been proposed as a major environmental factor determining microbial community composition (Lozupone and Knight, 2007), the occurrence of microbial mats is not restricted by high or rapidly changing salinities. But when it comes to functioning, salinity might actually be a limiting factor, even for N_2 fixation. Pinckney et al. (1995a) investigated the effect of salinity on microbial mats from a hypersaline environment on the Bahamas. A decrease of salinity (to half the original value) caused a considerable increase in both CO_2 and N_2 fixation rates. The addition of dissolved organic carbon and nutrients had no effect. These findings suggest that, in contrast to high temperatures, high salinities suppress both processes carried out by the indigenous microbial mat community and may outweigh other potentially limiting factors such as low nutrient concentrations. Organisms that are exposed to high salinities must invest a considerable part of their metabolic

capacities to the accumulation of compatible solutes and to the pumping of sodium ions. It is not surprising that this impacts the performance of the organism and in particular N_2 fixation, which has a high demand for energy and reducing equivalents, and competes for the same resources. Therefore, high salinities do not prevent settlement of microbial mats but may limit biological processes including N_2 fixation. N_2 fixation is difficult under hypersaline conditions and may be absent under very high salinities.

4. Summary

For all habitats that were briefly discussed in this chapter it can be concluded that microbial mats are present in a variety of environments, many of which are among the most hostile on this planet, and span a wide range of conditions. The large (metabolic) diversity of mat organisms enables these microscale ecosystems to persist under a wide variety of conditions. These conditions, as much as they differ from habitat to habitat, select for certain habitat-specific communities and also shape their metabolic performance. Temperature and salinity are important parameters influencing the occurrence and overall productivity of microbial mats as well as diazotrophy. N_2 fixation was an important process in all mats that were investigated for it. Temperature and salinity are proposed to be of major importance for the degree of mat development and diazotrophy but do not exclude mat organisms from thermal and hypersaline environments. There are many factors that influence N_2 fixation. These include nutrient and trace metal availability as well as energy supply and oxygen inhibition. The latter two have repeatedly been shown to play a key role in controlling N_2 fixation (e.g., Bebout et al., 1987; Villbrandt et al., 1990; Steunou et al., 2008). Energy supply and oxygen show strong spatial, daily and seasonal, as well as year-to-year variations and therefore shape the whole community's N_2 fixation, but due to the small-scale variability of these factors, a correlation to the global distribution of diazotrophic microbial mats is difficult.

5. References

Bauer, K., Diez, B., Lugomela, C., Seppala, S., Borg, A.J. and Bergman, B. (2008) Variability in benthic diazotrophy and cyanobacterial diversity in a tropical intertidal lagoon. FEMS Microbiol. Ecol. **63**: 205–221.

Bebout, B.M., Paerl, H.W., Crocker, K.M. and Prufert, L.E. (1987) Diel interactions of oxygenic photosynthesis and N_2 fixation (acetylene reduction) in a marine microbial mat community. Appl. Environ. Microbiol. **53**: 2353–2362.

Bergman, B., Gallon, J.R., Rai, A.N. and Stal, L.J. (1997) N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol. Rev. **19**: 139–185.

Berman-Frank, I., Lundgren, P. and Falkowski, P. (2003) Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res. Microbiol. **154**: 157–164.

Böhme, H. (1998) Regulation of nitrogen fixation in heterocyst-forming cyanobacteria. Trends Plant Sci. **3**: 346–351.

Bonin, P.C. and Michotey, V.D. (2006) Nitrogen budget in a microbial mat in the Camargue (Southern France). Mar. Ecol. Prog. Ser. **322**: 75–84.

Camacho, A. and de Wit, R. (2003) Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat. Microb. Ecol. **32**: 261–273.

Charpy, L., Alliod, R., Rodier, R. and Golubic, S. (2007) Benthic nitrogen fixation in the SW New Caledonia lagoon. Aquat. Microb. Ecol. **47**: 73–81.

Charpy-Roubaud, C. and Larkum, A.W.D. (2005) Dinitrogen fixation by exposed communities on the rim of Tikehau atoll (Tuamotu Archipelago, French Polynesia). Coral Reefs **24**: 622–628.

Charpy-Roubaud, C., Charpy, L. and Larkum, A.W.D. (2001) Atmospheric dinitrogen fixation by benthic communities of Tikehau Lagoon (Tuamotu Archipelago, French Polynesia) and its contribution to benthic primary production. Mar. Biol. **139**: 991–997.

De Wit, R., Falcon, L.I. and Charpy-Roubaud, C. (2005) Heterotrophic dinitrogen fixation (acetylene reduction) in phosphate-fertilised *Microcoleus chthonoplastes* microbial mat from the hypersaline inland lake 'la Salada de Chiprana' (NE Spain). Hydrobiologia **534**: 245–253.

Diaz, M.R., Corredor, J.E. and Morell, J.M. (1990) Nitrogenase activity of *Microcoleus lyngbyaceus* mat communities in a eutrophic, tropical marine environment. Limnol. Oceanogr. **35**: 1788–1795.

Dor, I. and Danin, A. (2001) Life strategies of *Microcoleus vaginatus*: a crust-forming cyanophyte on desert soils. Nova Hedwigia, Beiheft **123**: 317–339.

Fay, P. (1992) Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol. Rev. **52**: 340–373.

Fernandez-Valiente, E., Quesada, A., Howard-Williams, C. and Hawes, I. (2001) N_2-fixation in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Microb. Ecol. **42**: 338–349.

Ferris, M.J. and Ward, D.M. (1997) Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **63**: 1375–1381.

Ferris, M.J., Muyzer, G. and Ward, D.M. (1996) Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. Appl. Environ. Microbiol. **62**: 340–346.

Fourçans, A., de Oteyza, T.G., Wieland, A., Sole, A., Diestra, E., van Bleijswijk, J., Grimalt, J.O., Kühl, M., Esteve, I., Muyzer, G., Caumette, P. and Duran, R. (2004) Characterization of functional bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue, France). FEMS Microbiol. Ecol. **51**: 55–70.

Gallon, J.R. (1992) Reconciling the incompatible: N_2 fixation and O_2. New Phytol. **122**: 571–609.

Gallon, J.R., Pederson, D.M. and Smith, G.D. (1993) The effect of temperature on the sensitivity of nitrogenase to oxygen in the cyanobacteria *Anabaena cylindrica* (Lemmermann) and *Gloeothece* (Nägeli). New Phytol. **124**: 251–257.

Green, S.J., Blackford, C., Bucki, P., Jahnke, L.L. and Prufert-Bebout, L. (2008) A salinity and sulfate manipulation of hypersaline microbial mats reveals stasis in the cyanobacterial community structure. ISME J. **2**: 457–470.

Haselkorn, R. (1978) Heterocysts. Annu. Rev. Plant. Physiol. Plant Mol. Biol. **29**: 319–344.

Hennecke, H. and Shanmugam, K.T. (1979) Temperature control of nitrogen fixation in *Klebsiella pneumoniae*. Arch. Microbiol. **123**: 259–265.

Jonkers, H.M., Ludwig, R., de Wit, R., Pringault, O., Muyzer, G., Niemann, H., Finke, N. and de Beer, D. (2003) Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol. Ecol. **44**: 175–189.

Jørgensen, B.B., Revsbech, N.P. and Cohen, Y. (1983) Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. **28**: 1075–1093.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ. Microbiol. **7**: 519–529.

Krumbein, W.E., Cohen, Y. and Shilo, M. (1977) Solar Lake (Sinai), 4. Stromatolitic cyanobacterial mats. Limnol. Oceanogr. **22**: 635–656.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

Lozupone, C.A. and Knight, R. (2007) Global patterns in bacterial diversity. Proc. Natl. Acad. Sci. USA **104**: 11436–11440.

Olson, J.B., Steppe, T.F., Litaker, R.W. and Paerl, H.W. (1998) N_2-fixing microbial consortia associated with the ice cover of Lake Bonney, Antarctica. Microb. Ecol. **36**: 231–238.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004a) Comparison of diazotroph community structure in *Lyngbya* sp. and *Microcoleus chthonoplastes* dominated microbial mats from Guerrero Negro, Baja, Mexico. FEMS Microbiol. Ecol. **47**: 305–318.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004b) Determination of nitrogen-fixing phylotypes in *Lyngbya* sp. and *Microcoleus chthonoplastes* cyanobacterial mats from Guerrero Negro, Baja California, Mexico. Appl. Environ. Microbiol. **70**: 2119–2128.

Paerl, H.W., Fitzpatrick, M. and Bebout, B.M. (1996) Seasonal nitrogen fixation dynamics in a marine microbial mat: potential roles of cyanobacteria and microheterotrophs. Limnol. Oceanogr. **41**: 419–427.

Paerl, H.W., Pinckney, J.L. and Steppe, T.F. (2000) Cyanobacterial–bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ. Microbiol. **2**: 11–26.

Pinckney, J., Paerl, H.W. and Bebout, B.M. (1995a) Salinity control of benthic microbial mat community production in a Bahamian hypersaline lagoon. J. Exp. Mar. Biol. Ecol. **187**: 223–237.

Pinckney, J., Paerl, H.W. and Fitzpatrick, M. (1995b) Impacts of seasonality and nutrients on microbial mat community structure and function. Mar. Ecol. Prog. Ser. **123**: 207–216.

Pinckney, J., Paerl, H.W., Reid, R.P. and Bebout, B. (1995c) Ecophysiology of stromatolitic microbial mats, Stocking-Island, Exuma-Cays, Bahamas. Microb. Ecol. **29**: 19–37.

Ramsing, N.B., Ferris, M.J. and Ward, D.M. (2000) Highly ordered vertical structure of *Synechococcus* populations within the one-millimeter-thick photic zone of a hot spring cyanobacterial mat. Appl. Environ. Microbiol. **66**: 1038–1049.

Revsbech, N.P., Jørgensen, B.B., Blackburn, T.H. and Cohen, Y. (1983) Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles of a microbial mat. Limnol. Oceanogr. **28**: 1062–1074.

Rysgaard, S., Kühl, M., Glud, R.N. and Hansen, J.W. (2001) Biomass, production and horizontal patchiness of sea ice algae in a high-Arctic fjord (Young Sound, NE Greenland). Mar. Ecol. Prog. Ser. **223**: 15–26.

Severin, I. and Stal, L.J. (2008) Light dependency of nitrogen fixation in a coastal microbial mat. ISME J. **2**: 1077–1088.

Stal, L.J. (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol. **131**: 1–32.

Stal, L.J. (2000) Cyanobacterial mats and stromatolites, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria: Their Diversity in Time and Space.* Kluwer, Dordrecht, pp. 61–120.

Stal, L.J. (2001) Coastal microbial mats: the physiology of a small-scale ecosystem. S. Afr. J. Bot. **67**: 399–410.

Stal, L.J. and Heyer, H. (1987) Dark anaerobic nitrogen fixation (acetylene reduction) in the cyanobacterium *Oscillatoria* sp. FEMS Microbiol. Ecol. **45**: 227–232.

Stal, L J., Grossberger, S. and Krumbein, W.E. (1984) Nitrogen-fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. Mar. Biol. **82**: 217–224.

Steppe, T.F. and Paerl, H.W. (2002) Potential N_2 fixation by sulfate-reducing bacteria in a marine intertidal microbial mat. Aquat. Microb. Ecol. **28**: 1–12.

Steppe, T.F. and Paerl, H.W. (2005) Nitrogenase activity and *nifH* expression in a marine intertidal microbial mat. Microb. Ecol. **49**: 315–324.

Steppe, T.F., Olson, J.B., Paerl, H.W., Litaker, R W. and Belnap, J. (1996) Consortional N_2 fixation: a strategy for meting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol. Ecol. **21**: 149–156.

Steppe, T.F., Pinckney, J.L., Dyble, J. and Paerl, H.W. (2001) Diazotrophy in modern marine Bahamian stromatolites. Microb. Ecol. **41**: 36–44.

Steunou, A.S., Bhaya, D., Bateson, M.M., Melendrez, M.C., Ward, D.M., Brecht, E., Peters, J.W., Kühl, M. and Grossman, A.R. (2006) *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. Proc. Natl. Acad. Sci. USA **103**: 2398–2403.

Steunou, A.S., Jensen, S.I., Brecht, E., Becraft, E.D., Bateson, M.M., Kilian, O., Bhaya, D., Ward, D.M., Peters, J.W., Grossman, A.R. and Kühl, M. (2008) Regulation of nif gene expression and the energetics of N_2 fixation over the diel cycle in a hot spring microbial mat. ISME J. **2**: 364–378.

Tang, E.P.Y., Tremblay, R. and Vincent, W.F. (1997) Cyanobacterial dominance of polar freshwater ecosystems: are high-latitude mat-formers adapted to low temperature? J. Phycol. **33**: 171–181.

van Gemerden, H. (1993) Microbial mats: a joint venture. Mar. Geol. **113**: 3–25.

Villanueva, L., Navarrete, A., Urmeneta, J., White, D.C. and Guerrero, R. (2007) Analysis of diurnal and vertical microbial diversity of a hypersaline microbial mat. Arch. Microbiol. **188**: 137–146.

Villbrandt, M., Stal, L.J. and Krumbein, W.E. (1990) Interactions between nitrogen-fixation and oxygenic photosynthesis in a marine cyanobacterial mat. FEMS Microbiol. Ecol. **74**: 59–72.

Vincent, W.F. (2000) Cyanobacterial dominance in the polar regions, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria: Their Diversity in Time and Space.* Kluwer, Dordrecht, The Netherlands, pp. 321–340.

Vitousek, P.M. and Howarth, R.W. (1991) Nitrogen limitation on land and in the sea – how can it occur. Biogeochemistry **13**: 87–115.

Ward, D.M., Ferris, M.J., Nold, S.C. and Bateson, M.M. (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol. Mol. Biol. Rev. **62**: 1353–1370.

Ward, D.M., Bateson, M.M., Ferris, M.J., Kühl, M., Wieland, A., Koeppel, A. and Cohan, F.M. (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. Philos. Trans. R. Soc. Lond. B. **361**: 1997–2008.

Zehr, J.P., Mellon, M., Braun, S., Litaker, W., Steppe, T. and Paerl, H.W. (1995) Diversity of heterotrophic nitrogen-fixation genes in a marine cyanobacterial mat. Appl. Environ. Microbiol. **61**: 2527–2532.

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ARCHITECTURES OF BIOCOMPLEXITY: LICHEN-DOMINATED SOIL CRUSTS AND MATS

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1. Introduction

Higher plant cover is generally sparse or absent in extreme environments, but the soil is rarely bare. It is covered instead by complex communities known as biological soil crusts (BSCs). These components of open vegetation are found in all climatic zones. Currently, BSCs are prone to threat in many habitats. While climatic change is a general factor altering the composition of open vegetation, human activities and landscape management (e.g. overgrazing livestock) can lead to destruction of biological soil crust communities, including the loss of species diversity. Biological soil crusts have only recently come into focus as ecologically important components of terrestrial ecosystems. BSCs reduce wind and water erosion of the soils while serving the ecosystem by fixation of atmospheric nitrogen and contribution to soil organic matter (Eldridge and Greene, 1994).

The delimitation of the term BSCs is not precise, as some restrict it to particular cyanobacteria-dominated crust types, while most textbooks keep the term in a wider sense and include crusts formed by a wider variety of organisms, including lichens. The structure of these crusts is varied and its development depends mostly on climatic parameters, which determine humidity levels and mechanic forces. The interplay of the organismic life with environmental conditions leads to typical crust morphologies. Rather smooth crusts, with higher proportion of cyanobacteria, are typically present in hot deserts. With increasing coolness of habitats, the profile of soil crust structures becomes more pronounced, either with pinnacled shapes of up to 15 cm height, or developing rolling surfaces as a consequence of freeze–thaw effects. These highly structured crusts also support a higher percentage of lichen and moss cover, and display a complex bioarchitecture (Belnap et al., 2001a; Fig. 1).

Thread-forming cyanobacteria often dominate early successional stages in biological soil crusts. Thereafter, many organisms of diverse phylogenetic groups associate in complex communities. Usually, one phenotypic group dominates in the crusts and are used to classify those, either by color or shape. Dark colors often signify cyanobacterial crusts, while green algal crusts are visible as green casts on moist soil surfaces. Moss-dominated crusts form greenish fur-like carpets, whereas

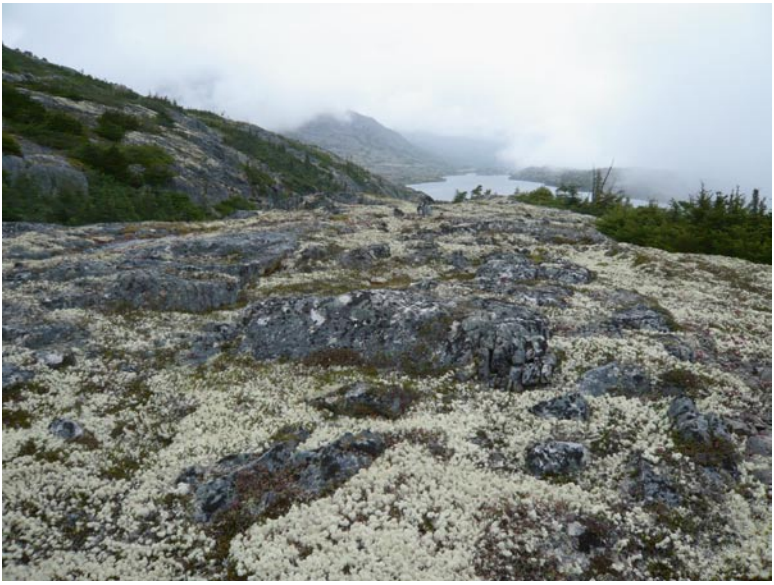

Figure 1. Ground-covering mats formed by *Cladonia* spp. White Pass near Skogway, Alaska (Photograph kindly provided by Toby Spribille).

increased morphological diversity is developed by lichen crusts. Lichens emerge from the association of fungi with compatible algae to form a unique joint structure, the thallus (Grube and Hawksworth, 2007). The resulting architectures of crusts and mats are among the most elaborate and long-living in the microbial realm.

Many lichens are particularly well adapted to endure lasting periods of drought. They are therefore more prominent than mosses in habitats with strong humidity and temperature oscillations and exposure to high light conditions. In these situations, soil is a challenging substratum for slow-growing filamentous fungi. The fragility of soil still allows lichens to establish and to develop diverse morphologies which help to stabilize soil. Crustose lichens form a more or less closed flat layer on the soil surface and are intricately fused to the substratum with the entire lower side. Together with their co-occurring organisms, crustose lichens permeate and stabilize the top few centimeters of soil (Belnap et al., 2001b). Foliose lichens are leaf-like and loosely attached to the substratum, usually by specialized structures, whereas fruticose lichens are three-dimensional and brush- or thread-like. The latter develop variably organized morphologies, which are among the most complex structures developed in the fungal kingdom (Grube and Hawksworth, 2007). Fruticose lichens can form extensive mats on the ground at higher altitudes and latitudes. The three main types of lichen-growth types are linked by intermediate forms, such as rosette-like (e.g., *Buellia asterella*, Fig. 2) or squamulose (scale-like) phenotypes.

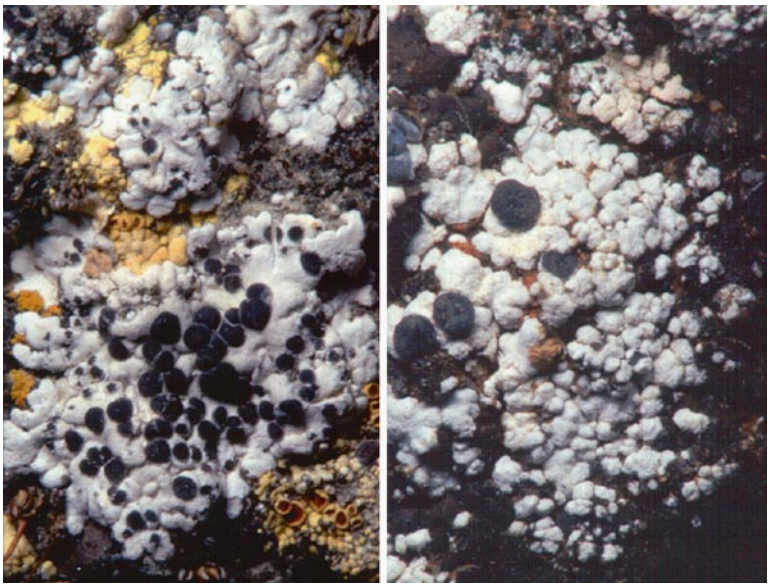

Figure 2. Variation in growth style among related soil crust lichens in the genus *Buellia*. *Left*: rosette-like growth in *Buellia asterella. Right*: squamulose-like habit of *Buellia dijiana*.

In this chapter, we present a brief review of lichen-dominated soil crusts and mats. Specifically, we highlight architectural principles in lichen-dominated soil crusts and the advanced structures in mats that highlight the cohabitation of unrelated organisms. We will denote as "crusts" those structures primarily developed by crustose to squamulose lichens, and recognize those communities as "mats" that are primarily composed of fruticose lichens.

2. From Lichen Crusts to Lichen Mats on Exposed Soils

Soil crust- and mat-forming lichens do not comprise a particular systematic group, but rather originate from diverse evolutionary lineages without strict habitat preferences. Not surprisingly, some of the species may also occur on rock habitats. Species of lichen-forming fungi on soil can rather frequently be found also to creep over decaying moss parts enriched by minerals or superficially eroded rocks. Substratum chemistry and texture are key to determining the composition in lichen-dominated BSCs. Some lichens are adapted to more or less unstable soils such as sands or eroding clay (e.g. *Placynthiella oligotropha*, *Dibaeis baeomyces*). A greater diversity of species, however, prefers rather stable, well packed soils (among other *Stereocaulon* spp., *Psora* spp., *Fulgensia* spp.).

Once compatible algae are recognized by a lichen fungus in the soil, a genetic reprogramming initiates the establishment of the fungal-species-specific

thallus (Trembley et al., 2002). Fungal hyphae coil around algal cells before the thallus differentiates into its typical layers. At the same time, the production of secondary metabolites and pigments is initiated and the thallus proceeds with typical growth strategies, which may differ among species in the same genus.

The thallus of most lichen-forming fungal species comprises several functionally distinct layers. Such layered, or heteromeric, thalli comprise at least an algal-rich layer and an upper surface layer that is devoid of living algal cells. In contrast, species with homoiomeric thalli, i.e., without stratification, have an even distribution of photobionts in the entire lichen structure. Usually lichen thalli are exposed to full light at the surface layer of the soil habitat; suffused thalli in the soil are rarer (*Eolichen*, *Thrombium*).

The lichen–soil interface differs substantially among crust and mat-forming lichens. Soil crust lichens often develop multiple bundles of hyphae (rhizines) which grow among the soil particles, thereby binding them more or less tightly together. Others anchor their areoles by a dark-colored central hyphal structure (*Lecanora himalayae*; Poelt and Grube, 1993a), or species can develop a central felt of individual hyphae (rhizohyphal felt) that penetrates deep into the soil substratum (*Lecanora pachyrrhiza*; Poelt and Grube, 1993a). Asta et al. (2001) studied the interface of three soil-inhabiting lichens by transmission electron microscopy. Two of the lichens studied, *Baeomyces rufus* and *Dibaeis baeomyces*, are phenotypically similar crusts. Both form hyphal bundles, which attach to mineral soil particles to form larger aggregates. Individual hyphae grow around soil particles. Sometimes organic debris is also caught in the mycelial net below the thallus, including conifer needles, moss leaves, pollen grains, and faecal pellets of the associated microfauna.

Initial stages of soil colonization by microbes are characterized by high spatial variation due to chemical heterogeneity of soil particles (Carson et al., 2009), while later stages tend to consolidate a frequency distribution with some dominating strains in the microbial communities. In the case of lichens, stability of the community structure could be achieved by means of intricate interactions in the thallus structure. The lichen cover may also influence the hygric conditions of the underlying soil habitat (hypothallosphere). Water conditions could also be affected by the increasing density of fungal hyphae and by soil particle conglutination. It has been noticed that particle binding by microbes or by their excreted materials can contribute to substratum porosity. This led to the concept of self-organization in soil–microbe systems (Young and Crawford, 2004). The mycelial architecture of the substratum–lichen interface contributes to the phenomenon of higher organization in these communities.

Crustose lichens can radially expand as a single unit of growth, or they may result from the fusion of independent minute thallus areoles that arise at the periphery (Fig. 2). Growth patterns are specific for lichen-forming fungal species, and even closely related species can differ in growth strategy. While lateral extension of the thallus is the strategy most frequently found, a somewhat unusual growth type is displayed by the Himalayan soil lichen *Tephromela siphulodes*. Apparently due to frequent microscopic perturbation of the substratum, this lichen develops a sort of

intercalary growth between the attachment points with the substratum, which results in elevated worm-like structures (Poelt and Grube, 1993b).

By the emergence of the thallus from the surface to form three-dimensional architectures, crust lichens increase biomass and to some extent can avoid competition for space; indeed, in some cases the development of aerial structures enables them to overgrow other lichens. Upright growing branched thalli can grow in ground-covering mats which proceed to form distinct vegetation types (Fig. 1). These represent perhaps also the tallest microbial mats, growing in the range of several centimeters. Species of the genera *Cladonia* (subgenus *Cladina*) and *Cetraria* s.l. are in fact very abundant over large areas at high altitudes and latitudes. Many species of *Cladonia* are commonly known as "reindeer lichens." They survive and slowly grow in tundra heaths, which is too nutrient-poor to sustain more substantial cover by faster-growing vascular plants. It is generally believed that lichens receive nutrients from precipitation only. However, these fruticose lichens may add ca. 15% of the biomass during one growing season (Bjerke, personal communication, 2008), indicating that their relative growth rate is substantial. One additional source of nutrient supply might be epiphytic cyanobacteria growing on the outer surface of the lichens that are not visible to the human eye. As described in detail below, their success might be enhanced by other bacterial players as well. Incidence of cyanobacteria and other microbial species in lichen mats may be influenced by microclimatic conditions in the dense arrangement of thallus branches, but this has not yet been investigated in detail. However, biomass can also be differentially distributed among fungal and algal partners depending on climatic conditions. Adaptation to a wider range of thermal regimes by altering relative abundance of partners to reconcile the different temperature–efficiency curve of photosynthesis and respiration has been described by the community adaptation hypothesis (Friedman and Sun, 2005). As would be predicted by this hypothesis, Sun and Friedman (2005) observed that algal biomass in mat-forming *Cladonia* species is greater with increasing habitat temperatures (and decreasing geographic latitude).

Reindeer lichens growing in mats are not tightly bound to the soil substrata (Asta et al., 2001). Their basal parts start to senesce and begin to breakdown and these lichens do not form hyphal networks in the soil. Especially in species of the genus *Cladonia* the evolution of this growth strategy is obvious. *Cladonia* is a cosmopolitan genus of mostly soil-inhabiting lichens, with two main morphological groups distinguished as the subgenera *Cladonia* and *Cladina*. Members of the subgenus *Cladonia* develop primary scale-like thalli that are partly attached to the ground, and from which erect branches, the podetia, emerge. Species in the subgenus *Cladina* grow only as erect podetia (after they initially emerge from a crust). *Cladonia* species form hollow cylinders, which are mechanically ideal forms to build tall structures, but there are perhaps other functions involved with this architecture, for example, to create an internal habitat for bacterial growth (Cardinale et al., 2008). With the lack of the primary thallus, *Cladina* species also sacrifice a close contact with the substratum. The disentanglement of the substratum is found in many fruticose lichens growing on soil substrata.

While reindeer lichens tend to keep their compact mat-like structure, detaching soil lichens in flat open areas of gravelly and sandy soils in arid and semiarid regions can ultimately develop vagrant forms. Their thalli can form tight balls that are driven as "tumble lichens" by the wind over large distances. However, vagrancy is not restricted to fruticose or foliose lichens. Crust lichens that can grow around small gravel or compact soil grains tend to become vagrant especially after the central soil fragment gets lost. *Sphaerothallia esculenta*, sometimes interpreted as the biblical manna, is a good example for this type of thallus development.

3. Community Structure of Lichen Crusts and Mats on Soils

Lichen soil crusts vary in their composition. Species may assemble into colorful multispecies communities, but extensive monomorphic mat synusia also exist and these may comprise often only one or few morphologically similar species. Communities formed by slow-growing crust lichens on undisturbed soils usually exhibit species diversity at microscales. The composition of soil crust communities has been classified and named in various syntaxonomical approaches. Although composition is variable and taxonomic entities can be difficult to separate, the names accorded to these crust assemblages represent the ecological parameters of the habitats fairly well.

Individuals in soil-inhabiting communities have fairly different sizes, as molecular studies revealed in *Cladonia* communities. *Cladonia chlorophaea* s.l. is a representative species on siliceous ground which develops extensive thalli with multiple podetia. Various chemotypes of this lichen are sometimes considered as separate species; however, rRNA gene variation indicates that each podetium in a mat can represent a distinct genotype, and that chemotypes do not consistently correlate with genotypes. This high amount of genetic diversity is contrasted by findings with members in the subgenus *Cladina* (reindeer Cladonias), where neighboring podetia of *Cladonia* (*Cladina*) *subtenuis* of the same cushion have been shown to be genetically homogeneous (DePriest, 1994; Beard and DePriest, 1996).

4. Interior Design of Lichen-Dominated Soil Crusts

Lichen-dominated communities consist of fungal mycelia and algae, both lichenized and non-lichenized, along with mosses, protists, and bacteria (including *Archaea*). The entire community, which is structurally dominated by the lichen thallus, fits the concept of a symbiome, as outlined by Sapp (2003, 2004). The lichens cover much of the soil surface and create a fine-scale spatial gradient of unique microniches from the upper lichen surface to the soil depths (in the range of millimeters to centimeters). Within this system, the primary carbon fixers are eukaryotic alga, cyanobacteria, and mosses. Heterotrophic bacteria perform a variety of little studied

functions such as nutrient mobilization and N-fixation. Feeding on these is a variety of microbial eukaryotes (protists), whose roles in BSCs are poorly understood. These unicellular or plasmodial species are probably the main bacterial predators in crusts, and they likely have a substantial impact on the composition of the bacterial community (Ronn et al., 2002). Also, diverse species of fungi are present in lichens (Harutyunyan et al., 2008), or associated with them (Fig. 2). Even in apparently simple substrata, vast fungal diversity can be present, including still largely unknown major lineages, as shown by Vandenkoornhuyse et al. (2002).

Though considerable work has focused on the microbial composition of crusts in warm-arid environments, much less is known about crust community structure and diversity in arctic–alpine habitats. However, these habitats are important potential sources of novel organisms and sensitive early indicators of climatic change (Johnson et al., 2002; Nemergut et al., 2005). They also appear to harbor a poorly estimated and untapped well of microbial diversity (Neufeld and Mohn, 2005; Bates and Garcia-Pichel, 2009).

As there are differences in the growth strategies and velocities of crustose lichens, species may also overgrow or invade neighboring thalli. Invasive growth inside other thalli is also typical for parasitic lichens that seem to be quite common under the scenario of the struggle for surface cover. *Diploschistes muscorum* is a typical case of parasitism in soil crusts. It invades the thalli of *Cladonia* species and anatomically restructures their thalli over time. This behavior is accompanied by a switch of the algal partner in the parasitized parts (Friedl, 1987).

When such parasitic lichens become independent at later ontogenetic stages, they are called juvenile parasites. Early stages of the greenish-yellow lichen *Arthrorhaphis citrinella* invade *Baeomyces rufus*, an early colonizer of acid soils with high clay content and of weathered siliceous rocks. This type of exploitation of preexisting symbioses might be more common in lichen-dominated crusts than was thought earlier. Hyperparasitism on juvenile crustose lichen parasites may be displayed by multiple species on the same host species, for example, on *Arthrorhaphis* (*Cercidospora trypetheliza*, *C. soror*, *Stigmidium arthrorhaphidis*; Hafellner and Obermayer, 1995). These highly host-specific lichenicolous fungi are often not aggressive parasites but have apparently adapted more commensalistic lifestyles (Fig. 3). One particular case of parasitism is represented by *Dacampia engeliana*, a non-lichenized fungus on leafy *Solorina* species, which grow on calciferous soil. The species reshapes the thallus in the early stages to build small cushions of connected scales (de los Ríos A and Grube, 2000).

Apart from biotrophic lichenicolous interactions, decaying crust lichens can be infected by more saprotrophic fungi, for example, *Niesslia kununguakii* on dead *Arthroraphis alpina* (Alstrup and Hansen, 2001). The total diversity of fungi in lichens is still poorly known, apart from the visible forms which develop characteristic spore-producing structures. Culture techniques reveal a high diversity of lichen-associated fungi in soil-inhabiting lichens (Petrini et al., 1990; Arnold et al., 2009), and culture-independent techniques will add further fungi to this diversity. The biological relationship of these fungi to the host lichens

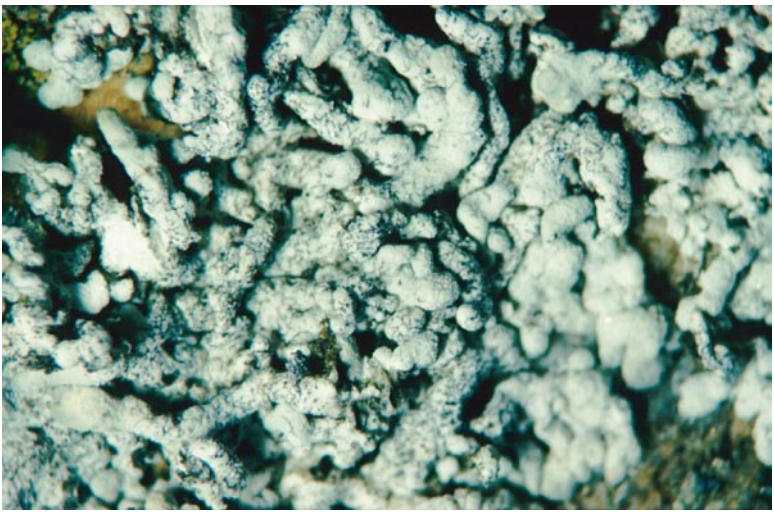

Figure 3. *Lichenostigma immersa*, a lichenicolous fungus forms dark discolorations specifically on the crust lichen *Buellia elegans*.

is still unclear. However, the presence of specific fungi in necrotic basal parts of matforming lichens suggests specialization to different developmental stages of the hosts (Aptroot and Alstrup, 1999).

5. Accessory Cyanobacteria: Cyanotrophy and Cephalodia

There is still little knowledge about diversity of algal species associated with crust- and mat-forming lichens. Usually, one algal species dominates in lichen thalli, although other strains of the same ecological group of photobionts can be found in the same species. About 10% of all lichens have cyanobacteria as their only photobiont partners, and among the remaining 90% of green algal lichens, several unrelated lichen-forming fungal species form additional interactions with cyanobacteria. They may frequently grow in the proximity of free-living or partly lichenized cyanobacteria (often *Stigonema* spp.). These facultative interactions (depending on habitat nutrition) to obligate relationships have been termed cyanotrophy (Poelt and Mayrhofer, 1988). In obligate cyanotrophic associations, cyanobacteria are covered by hyphae giving rise to structures termed paracephalodia.

In lichens with higher thallus complexity, for example, fruticose *Stereocaulon* species, interactions with cyanobacteria are localized in well-delimited thallus stuctures. These parts, named cephalodia, contain mainly *Nostoc* as additional partner in a tripartite lichen association. The primary engagement of these cyanobacterial associates in dinitrogen fixation is well supported by the higher

percentage of heterocysts in cephalodia than found in related lichens which possess only cyanobacteria as photoautotrophic partners. The phenotypic plasticity regarding cyanobacterial partners in lichens has both been observed and modelled. Hyvärinen et al. (2002) suggested that lichens behave rather as optimal harvesters of their symbiont's functions than as mutualistic partners.

6. Selective Assemblages

It is only recently that symbiotic association patterns have been studied in greater detail. Lichen fungi do not unspecifically associate with microscopic algae. In pioneering studies, Ahmadjian et al. (1980) and Ahmadjian and Jacobs (1981) observed that isolates of *Rhizoplaca chrysoleuca* and the soil-inhabiting *Cladonia cristatella* ("British Soldier Lichen") associate with several lichen photobionts in vitro and may form well differentiated thalli, but only undifferentiated, sorediate prethallus stages of development were achieved with distantly related photobiont species or with free-living algae. Similar results were obtained by Schaper (2003) with *Fulgensia bracteata*, another typical soil crust lichen-forming fungal species. A proper lichen thallus developed only in association with the genuine algal partner, while coculturing with other lichen photobionts resulted in delays or failure to develop the lichenized stage.

ITS nrDNA polymorphism of the algal bionts was studied in a population of *Fulgensia fulgida* growing in a lichen community called *Toninio–Psoretum decipientis*. *F. fulgida* grows on calcareous soil or over mosses in open grassland in the Mediterranean. The thalli of *F. fulgida* contained two strains of *Trebouxia asymmetrica*, one of them occurred also in a thallus of *Toninia sedifolia*. Other co-occurring lichens, namely *Squamarina lentigera*, *Catapyrenium michelii*, and *Collema cristatum* contained other algae (*Asterochloris irregularis*, *Myrmecia*, and *Nostoc*, respectively). *F. fulgida* and *T. sedifolia* apparently share the same photobiont pool (Beck et al., 2002), which could partly explain their co-occurrence.

The phylogenetic range of compatible partners can be called symbiont specificity, while the frequency of association among partners is termed symbiont selectivity. In other words, if several compatible photobionts are available, the lichen fungus will select one of the strains with higher frequency. Comparative studies revealed that fungal species can be grouped into three significantly different specificity classes: photobiont specialists, intermediates, and generalists (Yahr et al., 2006). This gross classification may be helpful in understanding ecological patterns of lichens.

Association patterns with organisms other than photobionts have so far been poorly studied. More work has yet to be done on soil crust-forming lichens to understand the bacterial communities associated in lichens. Previously, both Obermayer (1994), using light microscopy, and Asta et al. (2001), using transmission electron microscopy, have demonstrated bacteria in soil crust lichens, and at the lichen–soil interface. Their taxonomic composition has not yet been studied in detail.

Lecidoma demissum forms cushion-like thalli and is often found in clearings of alpine grasslands and in places with prolonged snow cover. Figure 4 shows that the part below the superficial thallus is densely colonized by other microorganisms. In particular, bacteria occur in great abundance. Initial analyses of the fungal

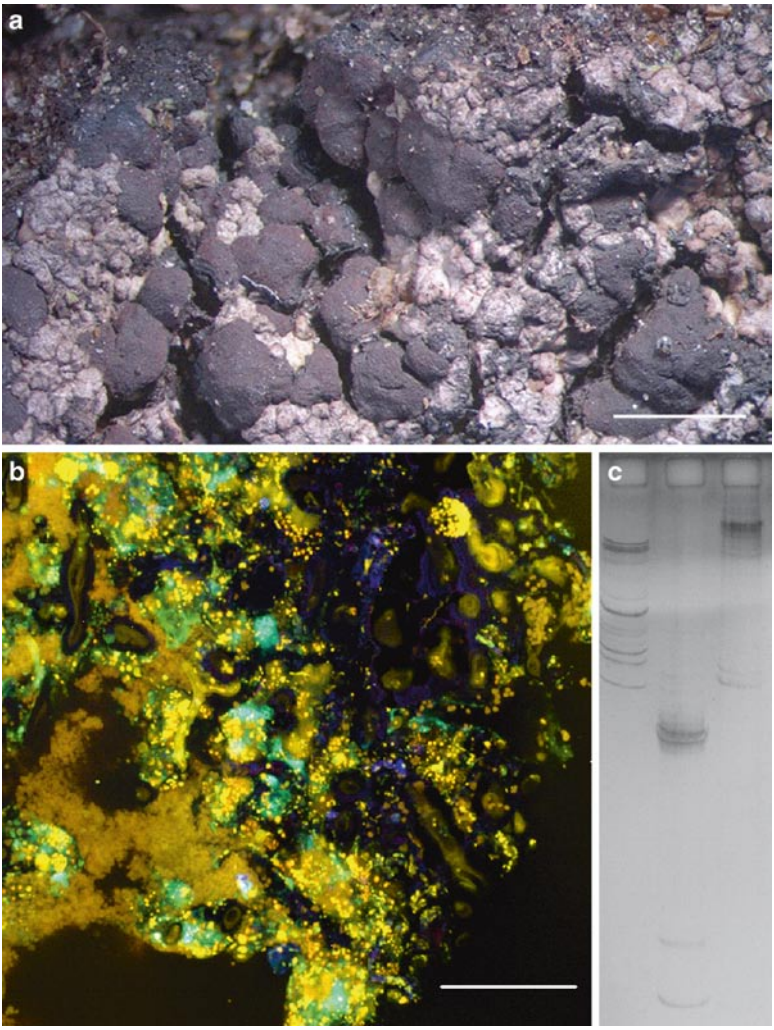

Figure 4. (**a**) *Lecidoma demissum*. Habit. Bar = 2 mm. (**b**) Section through soil showing dense bacterial community underneath alpine soil-inhabiting lichen *Lecidoma demissum.* Dark delimited structures are sectioned hyphae, yellow spots are bacteria visualized by acridine orange staining. Bar = 30 µm. (**c**) SSCP banding pattern using fungal ITS PCR products, from *left* to *right*: *Lecidoma demissum*, *Baeomyces rufus*, *Arthrorhaphis citrinella*. In all cases, bands of varying length and intensity indicate multiple fungi of different abundance in the hypothallosphere of these lichens.

communities by single strand conformation polymorphism analysis reveal that the lichen–soil interface of this species as well as that of *Baeomyces* and *Arthrorhaphis* is colonized by various additional fungi (Muggia and Grube, 2010; Fig. 4c). The analysis of bacterial communities will certainly be more complex. The structure of bacterial communities in soil is influenced by the mineral substrata in their microhabitat and mineral heterogeneity in soil contributes to the spatial variation in bacterial communities (Carson et al., 2009). The presence of diverse fungi in soil crusts adds to the parameters which influence microbial diversity. Recent studies of lichen-associated communities isolated directly from lichens indicate that lichens also host a wide taxonomic diversity of bacteria, including novel lineages (Cardinale et al., 2006, 2008; Grube et al., 2009).

The abundance and specific location of bacteria in the symbiotic structures of mat-forming reindeer lichens can be high on appropriate surfaces (Cardinale et al., 2008). Most bacteria are detected on the inner surfaces of the internally hollow podetia of *Cladonia*. There, the bacterial communities form biofilm-like communities dominated by *Alphaproteobacteria*, while *Actinobacteria* and *Betaproteobacteria* are less frequent. The latter often form small colonies that grow adjacent to or are mixed with larger colonies of *Alphaproteobacteria*. *Firmicutes* were rarely detected, and no *Gammaproteobacteria* were present. Phenotypically different bacterial colonies are arranged in a biofilm-like, continuous layer on the internal surface of the *C. arbuscula* podetia, mainly occurring in small colonies of a few to a few hundred cells. Further analyses showed that the alphaproteobacterial fraction from *Cladonia arbuscula* comprise primarily Acetobacteraceae (Cardinale et al., 2008). Other bacteria, which occur at lower frequencies, could contribute important biological roles, including the production of bioactive metabolites: A *Streptomyces* strain isolated from *Cladonia uncialis* produced enedyines, which are potent antibiotics at very low concentrations, and novel antibiotics (Davies et al., 2005; Williams et al., 2008).

Highly abundant bacterial communities are also present on other lichens comprising reindeer lichen mats. *Cetraria islandica*, commonly known also as "Icelandic moss" ("Isländisches Moos," "Brødmose," etc.), develops brownish cushions with erect branches in appropriate habitats, where it occurs intermingled with other lichens. The species forms extensive carpets preferentially over acidic soil and occurs in Boreal to subarctic tundra or above the tree-line in the Alps. It is therefore mostly found in wind-swept habitats, where the lichen is exposed to rapid and often drastic climatic fluctuations, ranging from over-hydration to almost complete desiccation and extreme daily temperature changes. At these sites *Cetraria* is an important stabilizer of alpine soil. In preliminary analyses, we observed a dense biofilm of bacteria on the entire outer surface of *C. islandica*. As in *Cladonia*, members of the *Alphaproteoabcteria* dominate the community (Fig. 5).

C. islandica is still in use today as an herbal and pharmaceutical, for example, in the form of teas, to cure infections of the upper respiratory tract, among other illnesses. The species contains several secondary metabolites, including the butyrolactones protolichesterinic acid and roccellaric acid (Horhant et al., 2007), and

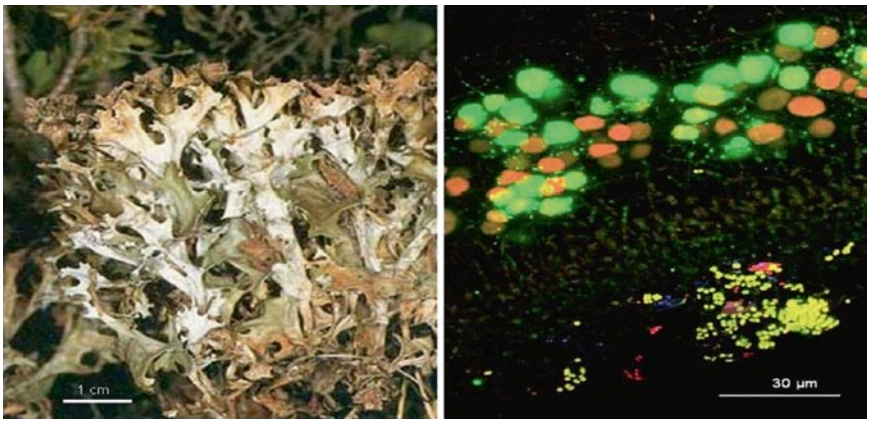

Figure 5. *Cetraria islandica*. *Left*: habit with perpetually growing tip and senescing basal part. *Right*: Microscopic section through the symbiotic structure. Large green and red cells are eukaryotic algae in different stages, faint green is fungal cytoplasm, small yellow dots are *Alphaproteobacteria*, red dots are other eubacteria. FISH-CLSM image.

the depsidone fumarprotocetratric acid (and traces of protocetraric acid), as well as reddish quinone-like compounds at the senescing bases. All of these could influence the structure of associated bacterial communities. Protolichesterinic acid, the main representative of the small class *o*-paraconic acids, exhibits antitumor, antibiotic, and anti-inflammatory properties (Boustie and Grube, 2005; Ingólfsdóttir et al., 1997). Preliminary observations indicate that this compound may actually impair bacterial quorum sensing (K. Riedel, personal communication, 2009). In this context, it is also remarkable that a chemical gradient exists between the growing tips and the senescing bases, which might influence bacterial colonization. The tips have been observed to contain more paraconic acids, whereas the base contains higher amounts of fumarprotocetraric acid (W. Obermayer, personal communication, 2009). Interestingly, the lower parts of this lichen contain lesser amounts of *Alphaproteobacteria*. To what extent the microclimatic conditions arising in the dense stands of the species could contribute to differences in chemistry or bacterial community structure remains to be studied.

The growth of mat-forming reindeer lichens is indeterminate and not limited by specific age-related death rates under undisturbed environmental conditions (e.g., absence of grazing animals or air pollution). Basal parts of a lichen thallus can locally senesce and detach from the underlying substratum, while biomass increases in actively growing parts. In very old stage, however, the senescing portions may catch up to slow new growth, which may cause the entire individual to die after a century or more. Recycling and translocation of components are of key importance for robustness and longevity of lichens in nutrient-poor habitats. Such frugal management of resources will be of particular importance for

surface-detached lichens. Experimental studies have demonstrated the translocation of fixed nitrogen compounds from the base to the tip in mat-forming reindeer lichens (Ellis et al., 2005), which contributes to nutritional autarchy.

Rainfall could be an interesting selective force shaping the bacterial communities in fruticose lichens. In our studies we observed that washing of lichens removes a significant portion of the surface-attached bacterial fraction. Only those bacterial strains which tightly bind or intrude into the intercellular polysaccharide matrix remain on the lichens. Growing colonies of such strains have a competitive advantage and will persist and become dominant. Thus, washing by intense rainfall could lead to more specific interactions (irrespective of whether these are beneficial or detrimental).

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8. References

Ahmadjian, V. and Jacobs, J.B. (1981) Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck. Nature **289**: 169–172.

Ahmadjian, V., Russell, L.A. and Hildreth, K.C. (1980) Artificial reestablishment of lichens. I. Morphological interactions between the phycobionts of different lichens and the mycobionts of *Cladonia cristatella* and *Lecanora chrysoleuca*. Mycologia **72**: 73–89.

Alstrup, V. and Hansen, E.S. (2001) New lichens and lichenicolous fungi from Greenland. Graphis Scripta **12**: 41–50.

Aptroot, A. and Alstrup, V. (1999) Three hyphomycetes isolated from the necrotic lichen *Cladonia rangiformis*. Cryptogamie, Mycologie **20**: 189–191.

Arnold, A.E., Miadlikowska, J., Higgins, K.L., Sarvate, S.D., Gugger, P., Way, A., Hofstetter, V., Kauff, F. and Lutzoni, F. (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Syst. Biol. **58**: 283–297.

Asta, J., Orry, F., Toutain, F., Souchier, B. and Villemin, G. (2001) Micromorphological and ultrastructural investigations of the lichen-soil interface. Soil Biol. Biochem. **33**: 323–337.

Bates, S.T. and Garcia-Pichel, F. (2009) A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: their diversity and relative contribution to microbial biomass. Envion. Microbiol. **11**: 56–67.

Beard, K.H. and DePriest, P.T. (1996) Genetic variation within and among mats of the reindeer lichen, *Cladina subtenuis*. Lichenologist **28**: 171–182.

Beck, A., Kasalicky, T. and Rambold, G. (2002) Myco-photobiontal selection in a Mediterranean cryptogam community with *Fulgensia fulgida*. New Phytol. **153**: 317–326.

Belnap, J., Kaltenecker, J.H., Rosentreter, R., Williams, J., Leonard, S. and Eldridge, D. (2001a) *Biological Soil Crusts: Ecology and Management*. Technical Reference, 1730-2, U.S. Dept. of the Interior, Bureau of Land Management, U.S. Geological Survey, Denver, Colorado, 110 pp.

Belnap, J., Büdel, B. and Lange, O.L. (2001b) Biological soil crusts: characteristics and distribution, In: J. Belnap and O.L. Lange (eds.) *Biological Soil Crusts: Structure, Function, and Management.* Springer, Berlin, pp. 3–30.

Boustie, J. and Grube, M. (2005) Lichens, a promising source of bioactive secondary metabolites. Plant Gen. Res. **3**: 273–287.

Cardinale, M., Puglia, A.M. and Grube, M. (2006) Molecular analysis of lichen-associated bacterial communities. FEMS Microbiol. Ecol. **57**: 484–495.

Cardinale, M., Müller, H., Berg, G., de Castro, J. and Grube, M. (2008) *In situ* analysis of the bacterial community associated with the reindeer lichen *Cladonia arbuscula* reveals predominance of *Alphaproteobacteria*. FEMS Microbiol. Ecol. **66**: 63–71.

Carson, J.K., Campbell, L., Rooney, D., Clipson, N. and Gleeson, D.B. (2009) Minerals in soil select distinct bacterial communities in their microhabitats. FEMS Microbiol. Ecol. **67**: 381–388.

Davies, J., Wang, H., Taylor, T., Warabi, K., Huang, X.-H. and Andersen, R.J. (2005) Uncialamycin, a new enediyne antibiotic. Org. Lett. **7**: 5233–5236.

de los Ríos, A. and Grube, M. (2000) Host–parasite interfaces of some lichenicolous fungi in the Dacampiaceae (Dothideales, Ascomycota). Mycol. Res. **104**: 1348–1353.

DePriest, P.T. (1994) Variation in the *Cladonia chlorophaea* complex II: ribosomal DNA variation in a Southern Appalachian population. The Bryologist **97**: 117–126.

Eldridge, D.J. and Greene, R.S.B. (1994) Microbiotic soil crusts: a review of their roles in soil and ecological processes in the rangelands of Australia. Aust. J. Soil Res. **32**: 389–415.

Ellis, C.J., Crittenden, P.D., Scrimgeour, C.M. and Ashcroft, C.J. (2005) Translocation of ^{15}N indicates nitrogen recycling in the mat-forming lichen *Cladonia portentosa*. New Phytol. **168**: 423–434.

Friedl, T. (1987) Thallus development and phycobionts of the parasitic lichen *Diploschistes muscorum*. Lichenologist **19**: 183–191.

Friedman, I. and Sun, H.J. (2005) Communities adjust their temperature optima by shifting producer-to-consumer ratio, shown in lichens as models: I. Hypothesis. Microb. Ecol. **49**: 523–527.

Grube, M. and Hawksworth, D.L. (2007) Trouble with lichen: the re-evaluation and re-interpretation of thallus form and fruit body types in the molecular era. Mycol. Res. **111**: 1116–1132.

Grube, M., Cardinale, M., Vieira de Castro, J., Müller, H. and Berg, G. (2009) Species-specific structural and functional diversity of bacterial communities in lichen symbiosis. ISME J. **3**: 1105–1115.

Hafellner, J. and Obermayer, W. (1995) *Cercidospora trypetheliza* und einige weitere lichenicole Ascomyceten auf *Arthrorhaphis*. Cryptogamie, Bryologie-Lichénologie **16**: 177–190.

Harutyunyan, S., Muggia, L. and Grube, M. (2008) Black fungi in lichens from seasonally arid habitats. Stud. Mycol. **61**: 83–90.

Horhant, D., Le Lamer, A.C., Boustie, J., Uriac, P. and Gouault, N. (2007) Separation of a mixture of paraconic acids from *Cetraria islandica* (L.) Ach. employing a fluorous tag-catch and release strategy. Tetrahedron Lett. **48**: 6031–6033.

Hyvärinen, M., Härdling, R. and Tuomi, J. (2002) How to maximize carbon gain in a cyanolichen: an optimization model. Oikos **98**: 498–504.

Ingólfsdóttir, K., Hjálmarsdóttir, M.Á., Guðjónsdóttir, G.A., Brynjolfsdóttir, A., Sigurðsson, A. and Steingrímsson, Ó. (1997) *In vitro* susceptibility of *Helicobacter pylori* to protolichesterinic acid from *Cetraria islandica*. Antimicrob. Agents Chemother. **41**: 215–217.

Johnson, D., Campbell, C.D., Lee, J.A., Callaghan, T.V. and Gwynn-Jones, D. (2002) Arctic microorganisms respond more to elevated UV-B radiation than CO_2. Nature **416**: 82–83.

Muggia, L. and Grube, M. (2010) Fungal composition of licher thalli assessed by single strand conformation polymorphism. Lichenologist (in press).

Nemergut, D.R., Costello, E.K., Meyer, A.F., Pescador, M.Y., Weintraub, M.N. and Schmidt, S.K. (2005) Structure and function of alpine and arctic soil microbial communities. Res. Microbiol. **156**: 775–784.

Neufeld, J.D. and Mohn, W.W. (2005) Unexpectedly high bacterial diversity in arctic tundra relative to boreal forest soils, revealed by serial analysis of ribosomal sequence tags. Appl. Environ. Microbiol. **71**: 5710–5718.

Obermayer, W. (1994) Die Flechtengattung *Arthrorhaphis* (Arthrorhaphidaceae, Ascomycotina) in Europa und Grönland. Nova Hedwigia **58**: 275–333.

Petrini, O., Hake, U. and Dreyfuss, M.M. (1990) An analysis of fungal communities isolated from fruticose lichens. Mycologia **82**: 444–451.

Poelt, J. and Grube, M. (1993a) Beiträge zur Kenntnis der Flechtenflora des Himalaya VIII – *Lecanora* subgen. *Placodium*. Nova Hedwigia **57**: 305–352.

Poelt, J. and Grube, M. (1993b) Beiträge zur Kenntnis der Flechtenflora des Himalaya VI – Die Gattung *Tephromela* (mit Bemerkungen zum Genus *Heppsora*). Nova Hedwigia **57**: 1–17.

Poelt, J. and Mayrhofer, H. (1988) Über Cyanotrophie bei Flechten. Plant Syst. Evol. **158**: 265–281.

Ronn, R., McCaig, A.E., Griffiths, B.S. and Prosser, J.L. (2002) Impact of protozoan grazing on bacterial community structure in soil microcosms. Appl. Environ. Microbiol. **68**: 6094–6105.

Sapp, J. (2003) *Genesis: The Evolution of Biology*. Oxford University Press, New York.

Sapp, J. (2004) The dynamics of symbiosis: an historial overview. Can. J. Bot. **82**: 1046–1056.

Schaper, G.M. (2003) *Komplexe Interaktionsmuster und die Dynamik von Entwicklungsprozessen in Flechtenökosystemen*. Unpublished DPhil Thesis, Heinrich-Heine-Universität, Düsseldorf.

Sun, H.J. and Friedman, E.I. (2005) Communities adjust their temperature optima by shifting producer-to-consumer ratio, shown in lichens as models: II. Verification. Microb. Ecol. **49**: 528–535.

Trembley, M.L., Ringli, C. and Honegger, R. (2002) Morphological and molecular analysis of early stages in the resynthesis of the lichen *Baeomyces rufus*. Mycol. Res. **106**: 768–776.

Vandenkoornhuyse, P., Baldauf, S.L., Straczek, J., Leyval, C. and Young, J.P.W. (2002) Extensive and novel fungal diversity in plant roots. Science **295**: 2051.

Williams, D.E., Davies, J., Patrick, B.O., Bottriell, H., Tarling, T., Roberge, M. and Andersen, R.J. (2008) Cladoniamides A-G, tryptophan-derived alkaloids produced in culture by *Streptomyces uncialis*. Org. Lett. **10**: 3501–3504.

Yahr, R., Vilgalys, R. and DePriest, P.T. (2006) Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. New Phytol. **171**: 847–860.

Young, I.M. and Crawford, J.W. (2004) Interactions and self-organization in the soil-microbe complex. Science **304**: 1634–1637.

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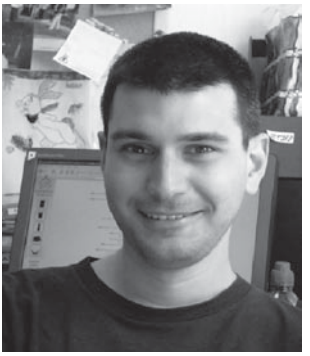

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IRON AND BACTERIAL BIOFILM DEVELOPMENT

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1. Introduction

Iron is an essential element for nearly all organisms on earth including most bacteria, which have to acquire iron to maintain growth. Iron is an important cofactor of many enzymes, serving as a cofactor in electron carrying proteins, and is also important for RNA and DNA metabolism. Although iron is required for growth, high concentrations can be toxic as excess iron promotes generation of free radicals via the Fenton reaction, radicals that damage DNA, proteins, and the cell membrane (Touati, 2000). At the beginning of life on earth, iron was readily available and soluble. However, as our planet matured the levels of oxygen in the atmosphere increased, resulting in dramatically reduced iron solubility, exacerbating the toxic effects associated with this element. Consequently, bacteria had to develop sophisticated mechanisms to scavenge iron from dilute environmental sources and in parallel regulate tightly cellular iron homeostasis. It is interesting to note that as life on earth continues to evolve, the role of iron as an essential element is maintained. Although the microbial growth requirement for iron has been known for many years, it was discovered only recently that this metal serves also as a signal for bacterial biofilm development. In this chapter, we will review the most recent findings concerning iron regulation of biofilm formation within the more general context of the relationship between iron and bacteria in the environment.

2. Iron Acquisition and Regulation in Bacteria

In nature, under aerobic conditions the soluble form of iron (Fe^{2+}) is scarce (10^{-8} M) and most iron is found in the insoluble ferric form (Fe^{3+}), and therefore unavailable for use by biological systems. Thus bacteria have had to evolve sophisticated and versatile iron acquisition systems to promote Fe^{3+} uptake and its conversion to Fe^{2+}.

2.1. IRON UPTAKE BY SIDEROPHORES

One mechanism bacteria utilize to scavenge Fe^{3+} from the environment involves secretion and uptake of siderophores (iron carriers in Greek), which are low

molecular weight compounds (<1,000 Da) that chelate iron. They are produced by bacteria and released into the surrounding environment under limiting iron conditions. Siderophores have high affinity for Fe^{3+}, dissociation constants being in the 10^{22}–10^{50} M^{-1} range. Typically, the siderophore backbone comprises amino acids, particularly the non-protein D-amino acids ornithine and citrulline (Pohlmann and Marahiel, 2008), and an iron binding moiety. Siderophores are categorized according to their iron binding moiety: (i) hydroxamate, (ii) catechol, or (iii) hydroxyacid (Orsi, 2004).

Due to their molecular weight, siderophores are not able to diffuse freely through general porins present in the outer membrane of Gram negative bacteria (which can only transport solutes smaller than 600 Da) and thus require special energy-dependent outer membrane-bound receptors and uptake systems for their transport. In Gram negative bacteria, the cell wall necessitates two uptake stages, initially across the outer membrane into the periplasm and then from the periplasm across the inner membrane into the cytoplasm. Similar to other outer membrane receptors, siderophore receptors form a β-barrel structure comprising 22 transmembranal β-sheets, which are connected by large extracellular and short periplasmic loops. The barrel is "corked" by a globular domain derived from the first 160 amino acids of the N-terminal sequence. Ferri-siderophores bind with high affinity to the external loops and thus trigger a conformational change in the region that mediates contact between the TonB Box, a highly conserved section of the "cork" domain, and the periplasmic part of TonB protein. TonB protein resides within a "TonB complex" (TonB:ExbB:ExbD), which is integrated in the inner membrane and uses the proton gradient as an energy source. The conformational change in the TonB box region induced by the Ferri-siderphore binding to external loops "energizes" TonB such that in turn, Ton B induces a conformational change in the siderophore receptor that promotes transport of the Ferri-siderophore into the periplasm. Once in the periplasm, the Ferri-siderophore is bound by specialized periplasmic binding proteins (PBP) in order to prevent the production of potentially damaging reactive oxygen species via the Fenton reaction. PBPs are classified into nine groups according to sequence similarity (Tam and Saier, 1993; Claverys, 2001). Class 8 PBPs are responsible for Ferri-siderophore binding, with a different PBP binding each category of siderophore. For example, in *Escherichia coli* FhuD binds to hydroxymate and FepB to catachol type siderophores (Sprencel et al., 2000). Transport of the Ferri-siderophore from the periplasm to the cytoplasm is mediated by an inner membrane transporter of the ATP-binding cassette (ABC) class, a multi-protein complex. Upon delivery of the Ferri-siderophore-PBP complex to the ABC transporter, cytoplasmic ATP is hydrolyzed and the Ferri-siderophore alone is moved into the cytoplasm (Fig. 1). In Gram positive bacteria, which have a different cell wall than Gram negative bacteria, siderophore uptake is much simpler. Siderophores are recognized by membrane-anchored binding proteins and then transported directly into the cytoplasm by ABC transporter systems (Andrews et al., 2003) (Fig. 2).

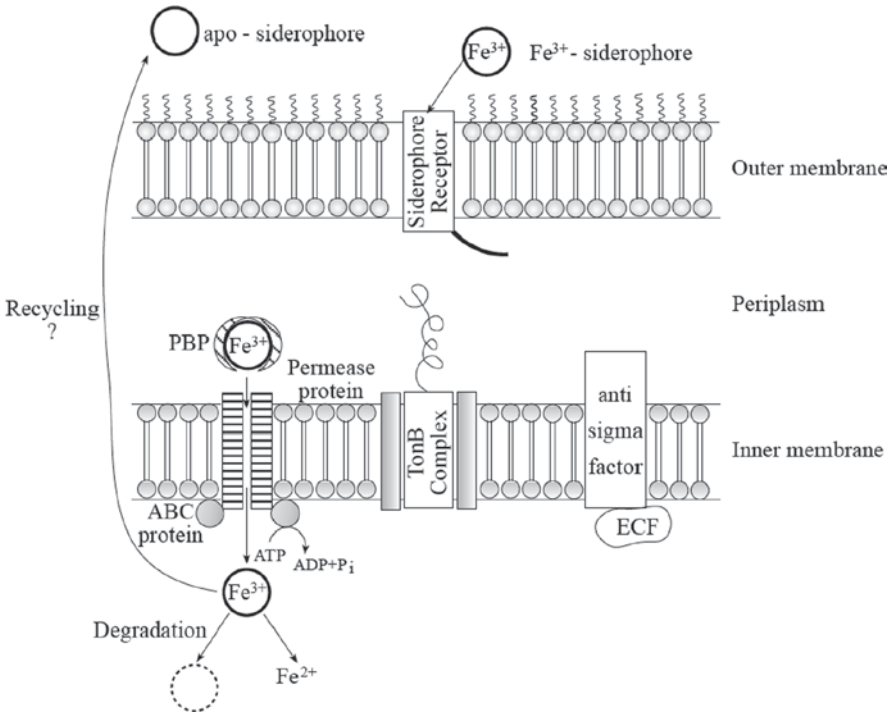

Figure 1. Iron uptake by siderophores in Gram negative bacteria.

Once inside the cell, iron has to be released from the siderophore in order to be available for assimilation. There are currently two known mechanisms by which this is achieved (Miethke and Marahiel, 2007):

1. Reduction – Fe^{3+} is reduced to Fe^{2+} either enzymatically by cytoplasmic or inner membrane-bound reductases (Schröder et al., 2003) or via a nonenzymatic reaction using intracellular free electron donors, such as NADPH or NADH (Wandersman and Delepelaire, 2004). Since siderophores have a much lower affinity for the Fe^{2+} ion, this ferrous ion is quickly sequestered by iron binding proteins in the cell. Subsequently, the apo-siderophore is believed to be "recycled," although a siderophore recycling system has yet to be identified.
2. Degradation – The siderophore is degraded, resulting in release of Fe^{3+} into the cytoplasm where it is reduced to Fe^{2+} by cytoplasmic reductases. Examples of proteins that degrade siderophores include Fes, a cytoplasmic esterase that degrades the *E. coli* siderophore enterobactin (Larsen et al., 2006; Raymond et al., 2003) and YuiI, a cytoplasmic trilactone hydrolase responsible for degrading the *Bacillus subtilis* siderophore bacillibactin (Miethke et al., 2006).

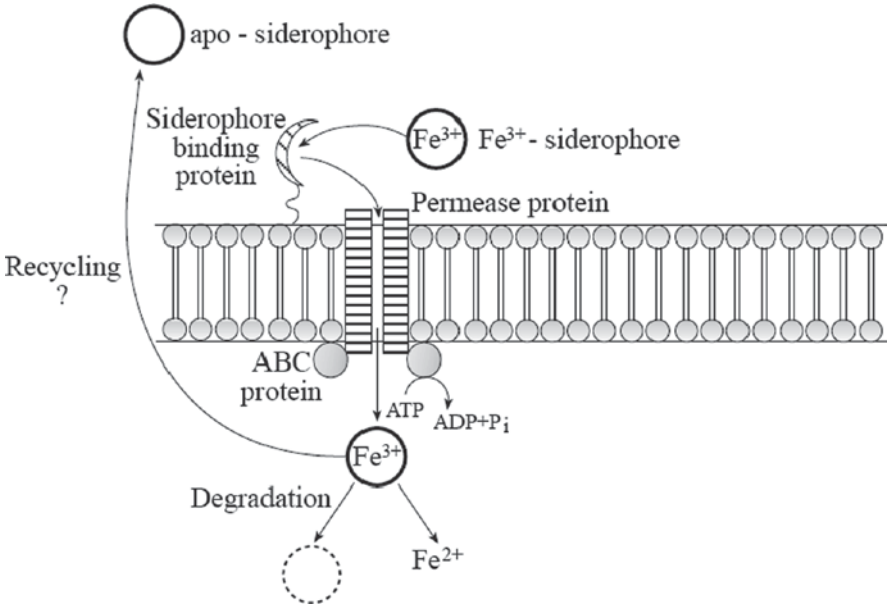

Figure 2. Iron uptake by siderophores in Gram positive bacteria.

Many bacteria can scavenge iron from the environment using, in addition to their own, siderophores produced by other bacteria. For example, the Gram positive bacterium *Staphylococcus aureus* produces the siderophores staphylopherrin and aureochelin but can also utilize the siderophore enterobactin synthesized by the Gram negative bacterium *E. coli* (Sebulsky and Heinrichs, 2001). In this way, certain bacteria have the ability to "steal" iron from other microorganisms, perhaps providing them with a competitive advantage in some environments.

2.2. NON-SIDEROPHORE IRON UPTAKE

Some bacteria are capable of non-siderophore iron uptake. For example, *E. coli* expresses ferric citrate receptors that allow them to use ferric citrate as an iron source (e.g., FecA). This transport system also uses TonB to promote movement of ferric citrate into the periplasm (Schröder et al., 2003; Braun and Herrmann, 2007).

During infection, pathogenic bacteria encounter extremely low iron concentrations since most iron is unavailable, bound within organic compounds (heme) or to carrier proteins such as transferrin and lactoferrin. Accordingly, pathogenic bacteria have evolved special mechanisms to survive in such an iron-deprived environment. Some pathogenic bacteria exploit host iron-binding proteins, for example, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Moraxella catarrhalis* can utilize lactoferrin or transferrin as iron sources. Generally, the host iron-binding

protein in complex with iron atoms are bound by special bacterial membrane receptors and free iron is transported into the cytoplasm using periplasmic binding proteins and an ABC transporter (Ekins et al., 2004; Miller et al., 2008). However, even in the absence of bacterial receptors to bind particular host iron-binding proteins, iron can still be obtained from these host proteins via their degradation. Examples of enzymes expressed by pathogenic bacteria that degrade host iron-binding proteins include a serine protease from *B. subtilis* and an alkaline protease from *Pseudomonas aeruginosa* (Kim et al., 2006; Park et al., 2006).

Some pathogenic bacteria make use of heme as an iron source. Certain Gram negative bacteria release heme-binding proteins, hemophores, which capture free heme and enable its uptake via special receptor systems. Such systems have been identified in *P. aeruginosa*, *Pseudomonas fluorescens*, *Yersinia pestis*, *Yersinia enterolitica*, *Haemophilus influenzae*, and *Serratia marcescens* (Cescau et al., 2007). Due to their high affinity for heme, (e.g., Ka = 5.3×10^{10} M^{-1} for HasA from *S. marcescens*), the hemophores can even scavenge heme from within organic compounds, such as hemopexin and hemoglobin (Cescau et al., 2007; Wolff et al., 2008). To date, hemophores have been identified only in Gram negative bacteria. However, recent research has revealed that the Gram positive bacterium *Bacillus anthracis* secretes two proteins, IsdX1 and IsdX2, which remove heme molecules from hemoglobin (Maresso et al., 2008) and thus, may be considered hemophores. Nonetheless, some Gram positive bacteria express special membrane receptors that bind heme-containing proteins or free heme, which facilitate exploitation of host heme even in the absence of hemophores. For example the Gram positive pathogen *S. aureus* expresses the haptoglobin-binding protein, HarA (Dryla et al., 2003).

2.3. IRON STARVATION SIGMA FACTOR

Iron uptake is an energetically "expensive" process that can bear a "costly price" due to the toxic affects associated with this element. Consequently, bacterial iron homeostasis is strictly regulated. One mechanism by which bacteria coordinate gene expression with iron concentration involves extracytoplasmic function (ECF) sigma factors. ECF sigma factors are a subclass of the σ^{70} sigma factors (Brooks and Buchanan, 2008). Under normal conditions ECF sigma factors are held by the "antisigma factor" close to the inner membrane, and thus inactivated. The antisigma factor is an integral inner membrane protein, the periplasmic part of which is bound to siderophore receptors. When an iron-loaded siderophore binds the siderophore receptor, an event indicative of low iron conditions, this triggers the antisigma factor to recruit the RNA polymerase (RNAP) machinery to the inner membrane and then release it in complex with the ECF sigma factor. Subsequently, this RNAP–ECF sigma factor complex binds specific promoters and directs their expression in line with limited iron conditions, ensuring a rapid transcriptional response to iron starvation (IS). One of the most studied iron starvation ECF sigma factors is PvdS from the human pathogen *P. aeruginosa.* When iron-loaded pyoverdine binds its cognate receptor FpvA, the sigma factor PvdS

in complex with RNAP is released from the antisigma factor FpvR. Then PvdS guides RNAP to PvdS-dependent promoters, which contain a conserved sequence called the iron starvation box (IS Box) (Visca et al., 2002). The genes under PvdS regulation can be divided into two classes. The first class includes genes responsible for iron acquisition, such as pyoverdine biosynthesis genes and the gene encoding a transferrin degrading enzyme, AprA (Shigematsu et al., 2001). The second class are genes commonly referred to as virulence factors, such as the proteolyic enzyme prpL (Wilderman et al., 2001) and endotoxin A (ToxA) (Hunt et al., 2002). In summary, these environmentally responsive transcription factors enable bacteria to monitor the presence of specific siderophores and respond to the level of iron in the environment.

2.4. FUR AND sRNA REGULATION

An important regulator responsible for iron homeostasis inside the cell is the Ferric Uptake Regulon (Fur) protein. Fur is a dimeric metaloprotein that acts as intracellular iron "sensor." When iron concentrations surpass a certain threshold, iron binds to the Fur protein and activates it. Activated fur binds to the 19 nucleotide sequence, GATAATGATAATCATTATC, known as the "Fur box," typically located between −10 and −35 in promoter regions (Rudolph et al., 2006). Fur box binding represses gene expression from that promoter. Fur homologs are found in diverse bacteria. Exceptionally, in *N. meningitidis* the Fur box is located upstream to the −35 region in a few promoters and Fur binding activates instead of repressing (Delany et al., 2004). The genes repressed by Fur are very diverse and depend on the bacterial type, and even bacterial strain. In *P. aeruginosa* for example, Fur is an essential protein and is known to repress directly genes involved in iron uptake, such as the pyoverdine operon, heme uptake systems, proteases, and toxins (Vasil, 2007).

In addition, Fur binding to the Fur Box can mediate indirectly activation of gene expression via regulation of sRNAs. sRNAs are short noncoding RNAs, typically 50–500 nucleotides long. sRNAs base pair with a target mRNA, forming an RNA–RNA complex that is recognized and degraded by RNaseE (Pichon and Felden, 2007). In *E. coli* the sRNA responsible for iron regulation is RyhB, which is expressed constantly. Under iron replete conditions, Fur represses RyhB transcription, resulting in enhanced expression of RyhB target mRNAs, previously degraded due to RyhB. Many RhyB-dependent transcripts encode "nonessential iron-binding proteins," their expression is desirable only when iron is readily available as these proteins lower the intracellular iron pool and accordingly increase the cellular demand for iron (Jacques et al., 2006). Other RhyB-dependent transcripts are either directly or indirectly involved in cellular metabolism. For example, RhyB influences directly cellular metabolism by regulating the expression of genes involved in the TCA cycle such as *sdhABCD* (succinate dehydrogenase complex), *frdABCD* (fumarate reductase) and *acnAB* (the stationary phase aconitase and exponential phase aconitase, respectively). RhyB indirectly regulates metabolism by affecting expression of Fe–S cluster synthesis enzymes and genes involved in

oxidative stress such as *sodB* (Masse et al., 2005). In *P. aeruginosa* there are two sRNAs involved in iron metabolism, PrrF1 and PrrF2 (Wilderman et al., 2004). Notably, these same sRNAs were demonstrated recently also to control quorum sensing, a cell density-dependent signaling process, via inhibition of *Pseudomonas* quinolone quorum sensing signal (PQS) biosynthesis (Oglesby et al., 2008). As quorum sensing is an important social behavior required for successful biofilm formation in *P. aeruginosa*, this finding hints at the complex relationship between iron and biofilm formation (Davies et al., 1998).

3. Biofilms and Corrosion

The biofilm lifestyle is a protected mode of growth that facilitates bacterial survival in hostile environments. It is now well recognized that microbial cells undergo profound changes during the transition from free-living to matrix-embedded communities (Whiteley et al., 2001; Hall-Stoodley and Stoodley, 2005). Biofilm development occurs in a series of complex but discrete and tightly regulated steps (O'Toole et al., 2000; Hall-Stoodley and Stoodley, 2005): (i) microbial attachment to the surface; (ii) growth, and aggregation of cells into microcolonies; (iii) maturation; and (iv) dissemination of progeny cells for new colony formation.

Bacterial biofilms, which represent the primary colonizers of surfaces in the environment, play an important role in corrosion. Corrosion is an electrochemical process that results in deterioration of a metal due its interaction with the environment (Hamilton, 1985). Corrosion-related costs exceed 276 billion dollars a year in the USA alone (Koch et al., 2001). Rusting of iron materials in the presence of oxygen and water is probably the most familiar and common form of corrosion. A reaction on the metal surface couples iron oxidation (anodic reaction) to reduction of the metal (cathodic reaction). The ferrous ion (Fe^{2+}) is oxidized further to ferric ion (Fe^{3+}) that forms amorphous solid $Fe(OH)_3$ under neutral conditions. Bacterial biofilms influence corrosion by altering the chemistry around a metal. This process is defined as biocorrosion or microbiologically influenced corrosion (Jones and Amy, 2000). Most biocorrosion research has focused on sulfate-reducing bacteria (SRB) that reduce sulfate to sulfide. In the absence of oxygen, sulfide products (such as FeS) act as strong cathodes and accelerate the oxidation of Fe(O) (Lee et al., 1995; Hamilton, 1998, 2003).

The extracellular polymeric matrix, an integral part of biofilms, may also contribute to biocorrosion. The matrix of certain bacterial species can bind metals via anionic functional groups such as phosphate, sulfate, and carboxyl groups (Rohwerder et al., 2003). In particular, the affinity of the matrix for multivalent cations such as Ca^{2+}, Mg^{2+}, and Fe^{3+} can be very strong, sometimes shifting the standard reduction potentials of the metals. For example, Fe^{2+}/Fe^{3+} redox potentials vary with different ligands, from +1.2 to −0.4 V. Thus, metals bound to the matrix can act as electron "shuttles" and facilitate novel redox reactions, for example, direct electron transfer from iron or FeS. In the presence of oxygen or another suitable electron acceptor, such a redox reaction depolarizes the cathode

and promotes corrosion (reviewed in [Beech and Sunner, 2004]). Chan and colleagues have reported recently that bacterial exopolymers, most notably acidic polysaccharides, can serve as a template for assembling FeOOH crystals. The observed mineralization was shown to result from the contact between the extracellular polymeric substance and oxidized iron, via ferric iron binding with carboxylic groups. This oxidation of ferrous ions and mineralization was noted to incur proton release and a decrease in extracellular pH, which the authors suggested should increase the proton motive force and encourage metabolic energy generation by the cells (Chan et al., 2004). Since the presence of ferrous metal and iron oxyhydroxide in the biofilm matrix enhances oxidation of the ferrous ion, overall the cathodic reaction is promoted and accordingly, corrosion.

Microbial iron respiration is also considered to play an important role in biocorrosion and under some conditions inhibits biocorrosion (reviewed by Lee and Newman (2003). During initial biofilm formation on the metal surface, oxygen is available and is consumed by aerobic respiration. This promotes localized anodic and cathodic reactions, which accelerate electrochemical corrosion. But as oxygen is depleted due to bacterial growth, respiration promotes reduction of the ferric ion, resulting in diffusion of ferrous ion into the surrounding fluid. In this way, under static conditions, iron respiration creates a protective shield of ferrous ions that serve to "soak up" any of the limited oxygen that might be diffusing into the fluid. Any ferrous ions oxidized back to ferric ions are reduced again by respiration. Therefore, the biofilm serves to create locally an anoxic environment that inhibits biocorrosion. Notably, this anoxic environment is exquisitely sensitive to fluid movements. Under flow conditions, Fe^{2+} ions become diluted and oxygen readily available, similar to the situation during initial biofilm formation, which accelerates corrosion on the surface (Dubiel et al., 2002).

The discovery that biofilms exert a protective affect and inhibit corrosion under certain conditions was unexpected (reviewed in Zuo, 2007). Three mechanisms have been proposed:

1. Removal of corrosive-promoting agents due to bacterial physiological activities – The most obvious corrosive agent is oxygen. Potekhina et al. have shown that biofilm-forming bacteria restrict corrosion under aerobic conditions by utilizing oxygen during respiration (Potekhina et al., 1999). Similarly, Jayaraman and colleagues demonstrated that thicker biofilms protect the surface better, showing that the protection required a viable biofilm and was not due to cellular metabolites secreted by the biofilm, concluding that oxygen consumption and the consequent anoxic environment close to the metal surface reduces corrosion (Jayaraman et al., 1998).
2. Secretion of antimicrobial agents that inhibit growth of corrosion causing bacteria – Jayaraman et al. genetically engineered *B. subtilis* to express antimicrobials and found that biofilms of this transgenic strain inhibit the growth of corrosion causing SRBs and reduce corrosion rates (Jayaraman et al., 1999a). Furthermore, biofilms of *Brevibacillus brevis* that naturally secrete gramicidin S have

been shown to inhibit SRB colonization and reduce corrosion of mild steel and stainless steel (Jayaraman et al., 1999b). The advantage of this approach to reducing corrosion is that the antimicrobial agents are produced within the biofilm and consequently, do not face the diffusion barriers of the biofilm matrix. Moreover, it is presumed that the limited diffusion through the biofilm matrix ensures a relatively high local concentration of the antimicrobial agent in the biofilm (Jayaraman et al., 1999a).

3. Generation of a layer by the biofilm that protects the surface from corrosion – *Bacillus licheniformis* biofilms were shown to produce a sticky protective layer of γ-polyglutamate on metal surfaces that reduce corrosion by 90% (Ornek et al., 2002).

Natural biofilms comprise various microbial species with diverse metabolic traits making the study of biocorrosion and biofilm physiology a challenging task. Nonetheless, recent advances in genomic analyses (e.g., metagenomics) and innovative molecular tools should progress our understanding of the mechanisms that promote microbe-induced corrosion, facilitating development of novel approaches to control biocorrosion.

4. Biofilm Formation and Iron Regulation

Research from the last decade has revealed several key cellular processes to be important for biofilm formation including: cell–cell communication (Davies et al., 1998), surface motility (O'Toole and Kolter, 1998), and extracellular polysaccharide production (Matsukawa and Greenberg, 2004). Despite this insight, our current understanding of how bacteria regulate biofilm formation is still limited. In the last few years, an expanding body of work suggests that iron is a major player in the regulation and formation of biofilms. Most studies concerning iron regulation of biofilms have focused on bacterial pathogens as maintaining low free iron concentrations is one of the first lines of defense of the innate immune system. In the following section we will review, species by species, the recent literature describing the effects of iron on biofilm formation.

4.1. *STAPHYLOCOCCUS AUREUS*

S. aureus, a nonmotile Gram positive coccus, is one of the most frequently isolated pathogens associated with nosocomial infections (Johnson et al., 2005). The ability of *S. aureus* to form biofilms on biotic as well as abiotic surfaces, such as medical devices, contributes greatly to its pathogenicity and capacity to colonize new hosts (Beenken et al., 2004). Staphylococcal biofilms are influenced by various environmental cues such as osmotic stress, anaerobic growth, glucose availability (Johnson et al., 2008), and iron, the latter essential for survival of *S. aureus*. Accordingly, the bacterium possesses several different iron uptake pathways.

It produces three siderophores, termed staphylopherrin A and B and aureochelin (Maresso and Schneewind, 2006). The uptake of these iron-carrying molecules into the bacteria is hypothesized to be mediated by four ABC transporters found in the cytoplasmic membrane of *S. aureus*. With respect to regulation of iron uptake, the systems identified to date include Fur and a Fur homolog termed *perR* (Maresso and Schneewind, 2006).

The effects of both replete and restricted iron conditions on biofilm formation by *S. aureus* have been studied. *S. aureus* strains display increased biofilm formation in iron-depleted conditions. Addition of 50 μM $Fe_2(SO_4)_3$ to the growth medium represses biofilm formation. To investigate the involvement of Fur in iron-regulated biofilm formation, an *S. aureus* Newman strain and an isogenic NewmanΔ *fur::tet* mutant were assayed for biofilm formation in restricted and replete iron media (Johnson et al., 2005). The *fur* mutant, like the wild-type strain, was observed to display reduced biofilm formation in the presence of iron, indicating that Fur is not required to repress biofilm formation in iron replete conditions. However, the *fur* mutant displayed a fourfold decrease in biofilm production relative to the wild type strain in low iron conditions following a 24 h growth period. This finding suggests that Fur regulates positively biofilm formation under restricted iron conditions (Johnson et al., 2005). But when the strains were grown for a shorter period of time (6 h) in low iron conditions, the *fur* mutant exhibited more biofilm production than the wild type, pointing to a negative regulatory role for Fur initially in low iron conditions (Johnson et al., 2005). Summarily, Fur appears to have a complex role in the regulation of biofilm formation in iron-depleted conditions. Fur-dependent regulation may be direct, perhaps via binding the promoters of genes involved in biofilm formation, or it may be indirect, via intermediary molecules, like small RNAs (Johnson et al., 2005).

The polymeric *N*-acetylglucosamine polysaccharide (PNAG) is considered critical for biofilm formation by *S. aureus* (Cramton et al., 1999). PNAG is synthesized by the products of the *ica* operon, which comprises the *ica*R regulatory gene as well as the *ica*ADBC biosynthesis genes (Götz, 2002). Biofilm formation assays show that in low iron conditions a Newman *ica* mutant produces 93% less biofilm than a wild-type strain (Johnson et al., 2008). However, since wild-type strains grown in iron-rich (low biofilm production) versus iron-poor (high biofilm production) media (Johnson et al., 2005) produce the same amount of PNAG, the aforementioned effect of the *ica* locus on biofilm formation in low iron is likely mediated by factors other than PNAG. Indeed, this reasoning led to the discovery of two *S. aureus* secreted proteins, Eap and Emp (Johnson et al., 2005). The levels of these two proteins are reduced significantly in surface protein extracts prepared from the *ica* mutant grown in low iron media, compared to their levels in extracts taken from the wild-type strain grown similarly. Eap and Emp are bacterial adhesins, noncovalently linked to the *S. aureus* cell surface. In general, host cell injury, such as a tissue wound or a vessel wall injury, exposes various adhesive glycoproteins including fibronectin, fibrinogen, collagen, and vitronectin, which promote attachment of other eukaryotic cells (Hussain et al., 2001). However, in addition, these

exposed glycoproteins serve as substrates for bacterial adhesins such as Eap and Emp. Notably, the interaction between bacterial adhesins and exposed host glycoproteins is considered important for bacterial tissue colonization and biofilm formation inside the host (Hussain et al., 2001). Therefore, it is perhaps not surprising that two bacterial adhesions (Emp and Eap) are regulated by the *ica* locus and appear to be involved in low iron-induced biofilm formation by *S. aureus*. Additionally, recent studies have shown that Fur regulates positive expression of these adhesins, which could explain the repressed biofilm formation of the *fur* mutant observed after sustained growth in low iron conditions (Johnson et al., 2008).

4.2. *STAPHYLOCOCCUS EPIDERMIDIS*

Another biofilm forming bacterium belonging to the *Staphylococcus* genus is *Staphylococcus epidermidis*. This nonmotile Gram positive bacterium is a common cause of foreign body-associated infections, such as infections of prosthetic valves, pacemakers, and cerebrovascular shunts. The formation of *S. epidermidis* biofilms (or "slime") is largely dependent on production of a polysaccharide called PIA. PIA synthesis is regulated by icaR (Vuong et al., 2005). One study demonstrated that iron-depleted conditions, generated by the addition of the iron chelator EDDA, promote slime production by most clinically relevant strains of *S. epidermidis* and are associated with the presence of extracellular polysaccharides such as PIA (Deighton and Borland, 1993). The induced PIA production observed in low iron conditions, and concomitant increase in biofilm formation, appears to be related to the functionality of TCA cycle. According to Vuong et al. (2005), a reduction in available iron hinders the functionality of TCA cycle enzymes such as aconitase and fumarase. Consequently, the TCA cycle is disturbed and metabolites are shunted into PIA production (Vuong et al., 2005).

4.3. *PSEUDOMONAS AERUGINOSA*

P. aeruginosa is a motile Gram negative, opportunistic pathogen, associated with nosocomial infections. It is resistant to many types of antibiotic treatments and is able to induce an array of diseases in various hosts. *P. aeruginosa* is a major player in the chronic lung infections suffered by cystic fibrosis patients and is partially responsible for the high mortality rates of these subjects (Williams et al., 2007). So far, two siderophores have been identified in *P. aeruginosa*, pyoverdine and pyochelin. The gene products of the *pvd* locus are responsible for synthesis of pyoverdine, which is effective at acquiring iron from transferrin and lactoferrin (Poole and McKay, 2003). The gene products of two separate operons, *pchDCBA* and *pchEFGHI*, determine synthesis of pyochelin, which has a much lower affinity for iron than pyoverdine, but is still effective at acquiring iron from transferrin. A specific receptor transports each of these siderophores, fpvA for pyoverdine and fptA for pyochelin.

Pyochelin expression is regulated directly by Fur while the regulation of pyoverdine is mediated by the pvdS sigma factor which is, in turn, regulated by Fur (Poole and McKay, 2003). Additionally, *P. aeruginosa* can utilize a range of heterologous siderophores originating from various bacteria or fungi and exploit natural iron chelators, like citrate or desferrioxamine, explaining its capacity to thrive in metabolically diverse environments (Poole and McKay, 2003).

In the presence of low iron conditions induced by an iron chelator, like lactoferrin, *P. aeruginosa* forms an irregular biofilm, characterized as a thin layer of cells. In the absence of lactoferrin, that is, in the presence of sufficient iron concentrations, a normal mushroom-shaped biofilm is formed, comprising a thick layer of cells (Singh et al., 2002). While researching the role of iron in *P. aeruginosa* aberrant biofilm formation, a novel phenomenon was observed, namely that iron limitation promotes a form of surface motility called twitching (Singh et al., 2002; Patriquin et al., 2008). Twitching is mediated by type IV pili and reflects the spreading of *P. aeruginosa* over the surface of a substratum during the initial stages of biofilm formation. It is believed that iron limitation causes the cells to "wander" constantly across the surface, thus disrupting their ability to settle and form structured communities (Singh et al., 2002; Singh, 2004). Twitching is also thought to be involved in generating the "stalk" arrangement beneath the mushroom caps of structured biofilms (Klausen et al., 2003) (Fig. 3).

To investigate the role of iron in biofilm development, several mutant strains of *P. aeruginosa* PAO1 have been constructed: pyoverdine mutant (PAO.*1ΔpvdA*),

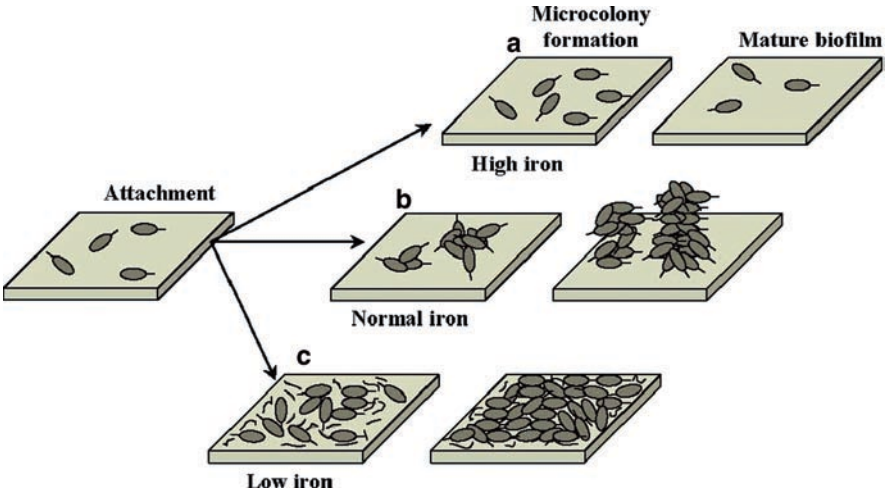

Figure 3. The role of iron in *Pseudomonas aeruginosa* biofilm formation. (**a**) In high iron conditions cells attach normally and multiply but do not remain attached to the glass surface and biofilms do not form. (**b**) In replete (normal) iron conditions (1–100 μM) bacteria attach, multiply and develop into microcolonies that mature into structured mushroom-like biofilms. (**c**) Low iron conditions promote the twitching phenomenon, cells attach and multiply but daughter cells move away from the point of replication disrupting the formation of structured biofilms.

pyochelin mutant (PAO1*pchA*:Tc^{R+}), and a pyoverdine pyochelin double mutant (*pvdA,pchA*). Biofilm formation by each of these mutant strains was analyzed carefully in the absence of lactoferrin (replete iron conditions). The pyochelin mutant, like the wild-type strain, produces a normal mushroom-like biofilm. In contrast, the pyoverdine mutant forms an abnormal biofilm, similar to the biofilm formed by wild type in the presence of lactoferrin. However, after exposure to pyoverdine-conditioned medium, the pyoverdine mutant was found to produce a normal biofilm. The pyoverdine pyochelin double mutant also produces an abnormal biofilm, even in the presence of very high iron concentrations. Nevertheless, addition of ferric dicitrate or desferrioxamine, two natural iron chelators that can be exploited by *P. aeruginosa*, to the growth medium of the pyoverdine mutant was observed to allow normal biofilm development. Taken together, these results evidence that ongoing iron acquisition from the environment is essential for *P. aeruginosa* to be able to develop a normal mushroom-shaped biofilm (Banin et al., 2005). But iron-regulated biofilm development seems to proceed through "check points." One check point is that in response to low iron twitching is stimulated. Another, seemingly master Fur-dependent check point is indicated by the finding that a Fur mutant produces a normal biofilm even in the presence of lactoferrin (Banin et al., 2005).

Recent findings suggest a relationship between the iron regulon and the quorum sensing regulon (Bollinger et al., 2001; Cornelis and Aendekerk, 2004; Lequette et al., 2006). This connection highlights the importance of iron in biofilm production since quorum sensing is known to play a key role in biofilm development (Davies et al., 1998; Singh et al., 2000). *P. aeruginosa* possesses two HSL-mediated quorum sensing systems. The first one consists of LasR, a transcriptional regulator, and LasI, a protein responsible for the synthesis of the *las* system autoinducer, 3-oxo-C12-HSL. The second quorum sensing system comprises the transcriptional regulator RhlR, and *rhlI*, a gene responsible for the synthesis of the *rhl* system autoinducer, C4-HSL. These *las* and *rhl* systems regulate the expression of several virulence factors, including alkaline protease, rhamnolipids, elastase, phospholipase C, pyocyanin, as well as biofilm formation (Duan and Surette, 2007). A connection between the iron and quorum sensing regulons was discovered when it was observed that a mutation in the *rhlI* gene of the *P. aeruginosa* K2589 strain causes a reduction in twitching and is characterized by normal biofilm formation despite low iron conditions (Patriquin et al., 2008). Specifically, limiting iron conditions induce *rhlI* expression, resulting in increased amounts of the autoinducer signal C4-HSL, which in turn promotes twitching and affects biofilm formation.

Iron levels influence not only establishment but also maintenance of *P. aeruginosa* biofilms. High iron concentrations perturb biofilm formation and promote dissociation of a preformed biofilm (Musk et al., 2005; Yang et al., 2007). Specifically, Musk et al. reported that iron salts (ammonium ferric citrate, ferric chloride, ferric sulfate, and ferrous sulfate), at iron concentrations >100 μM, inhibit *P. aeruginosa* biofilm formation without any effect on growth. This inhibition is not due to reduced adhesion of cells to the surface as initial biofilm formation, the first 10 h

of development, is unaffected. Rather the excess iron seems to disrupt the later stages of biofilm development, such that very few cells are adhering to the surface by 48 h (Musk et al., 2005) (Fig. 3). Yang et al. found that elevated iron conditions reduce the amount of extracellular DNA (ecDNA), an important component of biofilm matrices (Yang et al., 2007). Further study implicated that this fluctuation in ecDNA levels is mediated by a third quorum sensing system found in *P. aeruginosa*, the *pqs* system (Yang et al., 2007). Briefly, the autoinducer of the *pqs* system is 2-heptyl-3-hydroxy-4-quinolone, commonly referred to as PQS (Pesci et al., 1999). Two mutant strains deficient in PQS production, mutated at the *pqsA* and *pqsR* loci, display iron-independent reductions in ecDNA, supporting that PQS regulates ecDNA production but importantly, revealing that the regulatory effect of iron concentration on ecDNA production is mediated by the *pqs* system. Further evidence for this link between quorum sensing and iron-regulated biofilm maintenance is provided by the finding that biofilms grown in flow cell chambers with low iron conditions express *pqs* highly, whereas biofilms grown in high iron conditions express *pqs* poorly (Yang et al., 2007).

4.4. *ESCHERICHIA COLI*

E. coli is a motile Gram negative bacterium belonging to the family *Enterobacteriaceae*. Certain strains of *E. coli* are involved in urinary tract infections (UTIs). Two phenotypic traits of *E. coli* contribute to colonization of the urinary tract, namely fast growth and superior biofilm formation (Roos et al., 2006; Hancock et al., 2007). *E. coli* possesses three iron uptake systems, a low affinity aerobactin system and two high affinity systems, yersiniabactin (Ybt) and enterobactin. Synthesis of the Ybt siderophore and its receptor FyuA is dependent on proteins encoded within the high pathogenicity island (HPI). Microarray analysis of the urinary tract isolate *E. coli* VR50 has shown that during biofilm growth HPI is among the most upregulated gene clusters (Hancock et al., 2008), supporting that iron plays a role in biofilm formation. In order to investigate further a role for iron in biofilm formation, using human urine as the growth medium, *E. coli* VR50 and a *fyuA* deletion mutant, VR50*fyuA*, were grown in a flow chamber system and their biofilm formation monitored. The *fyuA* deletion mutant displays impaired biofilm formation, with respect to biomass and biofilm structure, forming small, scattered patches of biofilm whereas the wild type strain covers the entire surface of the glass slide. Biofilm formation by another urinary tract *E. coli* strain 83972 was also examined. A mutation in the *fyuA* gene of this strain also results in significantly reduced biofilm formation, specifically, a 53% reduction in microtitre plates when compared to the parent strain (Hancock et al., 2008). Taken together, these data indicate that FyuA expression affects biofilm formation and support the premise that iron regulates biofilm formation by UTI *E. coli* strains. However, it was necessary to demonstrate explicitly that the role of *fyuA* in biofilm formation relates to its ability to transport iron into the cell via Ybt-Fe binding, for

the position of FyuA in the outer membrane raises the possibility that FyuA contributes to biofilm formation by affecting cellular adhesiveness. Therefore, to clarify the role of FyuA in biofilm formation Hancock et al. examined the affect of iron addition to the growth medium on biofilm formation by *fyuA* deletion mutant strains and found it sufficient to promote biofilm formation. Moreover, three UTI wild-type strains were shown to produce less biofilm in the presence of iron chelators and more biofilm in the presence of added iron. Summarily, these observations corroborate the premise that iron serves as an important signal for biofilm formation by UTI *E. coli* strains (Hancock et al., 2008).

4.5. *VIBRIO CHOLERAE*

Vibrio cholerae is a Gram negative, rod-shaped bacterium. It thrives naturally in fresh and salty waters and when invading a human host colonizes the gastrointestinal tract causing cholera. The capacity of *V. cholerae* to form biofilms is crucial for both its survival in aquatic environments and its ability to colonize human hosts (Hall-Stoodley and Stoodley, 2005). As with numerous other bacteria, discussed above, it seems that iron is an important player in biofilm formation by *V. cholerae*. Biofilm formation by the E1 Tor *V. cholerae* strain N16961 has been examined under different conditions. In the presence of iron chelators (low iron conditions) N16961 produces little biofilm and represses rugose switching, a sort of colony morphology switch that relates to the expression level of exopolysaccharide. This phenotype accords with earlier data as typically rugose switching indicates increased exopolysaccharide expression and is associated with more biofilm formation (Wai et al., 1998). The effect of iron on biofilm formation appears to be mediated by RyhB, a small regulatory RNA responsible for down-regulating expression of sodB, TCA cycle enzymes, and energy metabolism proteins. *ryhB* transcription is controlled by a Fur and iron-responsive promoter, induced >tenfold in low iron conditions and repressed by Fur in replete iron conditions. To validate that iron regulates biofilm formation by *V. cholerae*, a N16961 strain mutated in the *rhyB* locus was generated and examined. The *ryhB* mutant is unable to form normal biofilms in low iron conditions, but addition of excess iron restores normal biofilm formation, suggesting that the *rhyB* mutant is iron stressed. Notably, the *rhyB* mutant was observed to exhibit decreased chemotaxis in low iron conditions implying that RhyB is required also for normal cellular iron metabolism. Although these data seemingly support iron as a player in biofilm formation, RhyB could influence biofilm formation in low iron conditions via mechanisms independent of iron metabolism. Since RyhB expression is induced in limiting iron conditions and RyhB represses energy metabolism, it is possible that in the *ryhB* mutant certain energy metabolism enzymes are inappropriately expressed. Indeed, it has been shown that in the *rhyB* mutant under low iron conditions the succinate dehydrogenase operon exhibits up-regulated expression, which presumably lowers substantially the cellular levels of succinate, a component of the exopolysaccharide

produced by *V. cholerae* during biofilm growth. Therefore, RyhB involvement in biofilm regulation under limiting iron conditions may be mediated by repression of succinate dehydrogenase (Mey et al., 2005). Furthermore, the role of RyhB in biofilm regulation in low iron conditions may be via its effect on motility, which is considered important for biofilm formation. Microarray analyses reveal decreased expression of several flagellar genes in the *ryhB* mutant. In conclusion, the iron-regulated repressor small RNA RyhB influences biofilm formation in low iron conditions most likely via several different mechanisms, including iron metabolism, energy metabolism and motility (Mey et al., 2005).

4.6. ORAL PATHOGENS

The relationship between iron and biofilm formation has been studied in three oral pathogens: *Streptococcus mutans*, *Actinomyces naeslundii*, and *Haemophilus actinomycetemcomitans*. *S. mutans* is a Gram positive bacterium and the major cause of dental cavities. Colonization of *S. mutans* on the tooth surface promotes adhesion of other oral pathogens, thus initiating formation of a mixed species biofilm that is dental plaque (Rolerson et al., 2006). A study of this pathogen concluded that aggregation, which eventually leads to biofilm formation, is induced in iron-restricted saliva and in normal saliva when the iron concentrations are kept within the range 0.1–1 μM. Increasing the iron concentration in saliva was found to cause a decrease in cell aggregation and biofilm formation (Francesca et al., 2004).

Similarly, it has been observed that low environmental iron conditions promote biofilm formation by the oral pathogen *A. naeslundii*, a Gram positive bacterium that is an early colonizer of the oral cavity. Accordingly, excess iron inhibits biofilm formation by this pathogen. An iron-dependent repressor thought to regulate biofilm formation by *A. naeslundii* in high iron concentrations is AmdR. For an *amdR* mutant produces biofilms even in a medium with increased metal ion concentration (Moelling et al., 2007).

The third oral pathogen for which iron regulation of biofilm development has been studied is *H. actinomycetemcomitans*, a Gram negative bacterium associated with periodontitis, an inflammatory disease that affects dental tissue. *H. actinomycetemcomitans* does not use siderophores to acquire iron from the environment. Rather, it uses mostly host haemin as an iron source. In agreement with the phenotypes of other Gram negative bacteria, it has been reported that the presence of DIP, an iron chelator, reduces biofilm formation by certain strains of *H. actinomycetemocomitans* (Rhodes et al., 2007).

4.7. *MYCOBACTERIUM SMEGMATIS*

Mycobacterium smegmatis, a saprophytic, motile, Gram positive bacterium is found mostly in the environment near large bodies of water (Brown-Elliott and Wallace, 2002). *M. smegmatis* serves as a model organism for the more pathogenic

species belonging to its genus, like *Mycobacterium leprae* and *Mycobacterium tuberculosis*. Biofilm development by *M. smegmatis* requires the presence of at least 1 μM iron in the growth medium (Ojha et al., 2005). This observation and the finding that iron acquisition genes are upregulated in biofilms relative to planktonic cells, suggest that iron is an important player in biofilm formation by *M. smegmatis*. Accordingly, mutations affecting synthesis of exochelin, one of the siderophores synthesized by *M. smegmatis*, or affecting its uptake, are associated with impaired biofilm formation. However, mutations in mycobactin, another siderophore synthesized by *M. smegmatis*, or in iron-ABC transporters, do not cause impaired biofilm formation. Thus, it appears that specifically the exochelin iron-uptake system is required for *M. smegmatis* biofilm formation. Notably, iron regulates also production of C_{56}–C_{68} fatty acids, which are building blocks for some biofilm matrix components. *M. smegmatis* grown in iron concentrations below 1 μM produce very low amounts of C_{56}–C_{68} fatty acids, which could explain the poor biofilm formation in these conditions (Ojha and Hatfull, 2007).

4.8. IRON AND THE BIOFILM MATRIX

As alluded above, changes in iron concentration can influence synthesis of the biofilm matrix. The building blocks of the biofilm matrix are polysaccharides, proteins, and DNA (Chen and Stewart, 2002; Whitchurch et al., 2002). For example, Yang et al. showed that the amount of ecDNA, an important matrix component of *P. aeruginosa* biofilms, is reduced in response to elevated iron concentrations (Yang et al., 2007). One of the forces thought to be involved in cohesion of the biofilm matrix is electrostatic interactions. In agreement with this, Chen and Stewart demonstrated that the viscosity of a biofilm suspension can be affected by changes in ionic strength and composition (Chen and Stewart, 2002). Also, treatment of *P. aeruginosa–Klebsiella pneumoniae* mixed biofilm suspensions with $FeCl_2$ or $Fe(NO_3)_3$ has been shown to increase biofilm viscosity, by 56% and 44% respectively (Chen and Stewart, 2002). Similarly, Banin et al. have found that chelation of iron, mediated by the addition of EDTA (50 mM), initiates detachment of cells from mature *P. aeruginosa* biofilms (Banin et al., 2006). Taken together, these results indicate that iron may not only be an important signal for biofilm development but also a cross-linking agent, which promotes covalent bonding between polymers and stabilizes the biofilm matrix.

5. Concluding Remarks

A few themes emerge from the data accumulated to date. First, there seems to be a general difference between Gram positive and Gram negative bacteria with regards to biofilm formation and iron. For the most part, biofilm formation by Gram positive bacteria increases in response to low iron conditions. This is true for both *S. aureus* and *Staphylococcus epidermidis* and two of the three oral pathogens

discussed here, *S. mutans* and *A. naeslundii*, all of which are Gram positive bacteria. In contrast, typically Gram negative bacteria, including *P. aeruginosa*, *E. coli*, and *V. cholerae*, repress biofilm formation in low iron conditions. However, this dichotomy is not universally accurate. The Gram positive bacterium *Mycobacterium smegmatis* demonstrates impaired biofilm formation in medium lacking a minimal iron concentration (Ojha et al., 2005) and the nonmotile Gram negative bacterium *Acinetobacter baumannii* exhibits increased biofilm formation in the presence of iron chelators (Tomaras et al., 2003).

Another theme concerns the role of motility in iron-regulated biofilm development. All nonmotile bacteria including *S. aureus*, *S. epidermidis*, *S. mutans*, *A. naeslundii*, and *A. baumannii* demonstrate increased biofilm formation in low iron conditions. In contrast, all motile bacteria including *P. aeruginosa*, *E. coli*, *V. cholerae*, and *M. smegmatis* display decreased biofilm formation in low iron conditions. Notably, in the case of *P. aeruginosa*, induction of surface motility has been suggested to explain the impaired biofilm formation observed under iron limiting condition (Singh et al., 2002; Singh, 2004). The only exception to this "motility classification" is the nonmotile, oral pathogen *H. actinomycetemcomitans*, which displays reduced biofilm formation in iron-chelated medium, but this microbe has not been studied extensively. An important question arising from this classification is whether iron regulates directly surface motility or controls cell–cell adhesion, which in turn affects motility and biofilm formation. Future work should characterize in more detail the role of motility in iron-regulated biofilm development in various bacterial species.

The biofilm mode of growth is an important feature of bacterial lifestyle that affects our daily life. These are exciting times in biofilm research as newly improved molecular and microscopy tools should allow us to begin to dissect the physiology of bacterial biofilms in the environment. A better understanding of biofilm growth is important not only for our intellectual appreciation of the living world, but also required urgently in order to control bacterial biofilm formation in industrial and medical settings. Specifically, the influence of iron on biofilm formation, the topic discussed in this review, represents a potential new approach to controlling biofilm formation.

6. References

Andrews, S.C., Robinson, A.K. and Rodriguez-Quinones, F. (2003) Bacterial iron homeostasis. FEMS Microbiol. Rev. **27**: 215–237.

Banin, E., Vasil, M.L. and Greenberg, E.P. (2005) Iron and *Pseudomonas aeruginosa* biofilm formation. Proc. Natl. Acad. Sci. USA **102**: 11076–11081.

Banin, E., Brady, K.M. and Greenberg, E.P. (2006) Chelator-induced dispersal and killing of *Pseudomonas aruginoa*. Appl. Environ. Microbiol. **72**: 2064–2069.

Beech, I.B. and Sunner, J. (2004) Biocorrosion: towards understanding interactions between biofilms and metals. Curr. Opin. Biotechnol. **15**: 181–186.

Beenken, K.E., Dunman, P.M., McAleese, F., Macapagal, D., Murphy, E., Projan, S.J., Blevins, J.S. and Smeltzer, M.S. (2004) Global gene expression in *Staphylococcus aureus* biofilms. J. Bacteriol. **186**: 4665–4684.

Bollinger, N., Hassett, D.J., Iglewski, B.H., Costerton, J.W. and McDermott, T.R. (2001) Gene expression in *Pseudomonas aeruginosa*: evidence of iron override effects on quorum sensing and biofilm-specific gene regulation. J. Bacteriol. **183**: 1990–1996.

Braun, V. and Herrmann, C. (2007) Docking of the periplasmic FecB binding protein to the FecCD transmembrane proteins in the ferric citrate transport system of *Escherichia coli*. J. Bacteriol. **189**: 6913–6918.

Brooks, B.E. and Buchanan, S.K. (2008) Signaling mechanisms for activation of extracytoplasmic function (ECF) sigma factors. Biochim. Biophys. Acta **1778**: 1930–1945.

Brown-Elliott, B.A. and Wallace, R.J. Jr. (2002) Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin. Microbiol. Rev. **15**: 716–746.

Cescau, S., Cwerman, H., Letoffe, S., Delepelaire, P., Wandersman, C. and Biville, F. (2007) Heme acquisition by hemophores. Biometals **20**: 603–613.

Chan, C.S., De Stasio, G., Welch, S.A., Girasole, M., Frazer, B.H., Nesterova, M.V., Fakra, S. and Banfield, J.F. (2004) Microbial polysaccharides template assembly of nanocrystal fibers. Science **303**: 1656–1658.

Chen, X. and Stewart, P.S. (2002) Role of electrostatic interactions in cohesion of bacterial biofilms. Appl. Microbiol. Biotechnol. **59**: 718–720.

Claverys, J.P. (2001) A new family of high-affinity ABC manganese and zinc permeases. Res. Microbiol. **152**: 231–243.

Cornelis, P. and Aendekerk, S. (2004) A new regulator linking quorum sensing and iron uptake in *Pseudomonas aeruginosa*. Microbiology **150**: 752–756.

Cramton, S.E., Gerke, C., Schnell, N.F., Nichols, W.W. and Gotz, F. (1999) The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun. **67**: 5427–5433.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. and Greenberg, E.P. (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **280**: 295–298.

Deighton, M. and Borland, R. (1993) Regulation of slime production in *Staphylococcus epidermidis* by iron limitation. Infect. Immun. **61**: 4473–4479.

Delany, I., Rappuoli, R. and Scarlato, V. (2004) Fur functions as an activator and as a repressor of putative virulence genes in *Neisseria meningitidis*. Mol. Microbiol. **52**: 1081–1090.

Dryla. A., Gelbmann. D., von Gabain, A. and Nagy, E. (2003) Identification of a novel iron regulated staphylococcal surface protein with haptoglobin-haemoglobin binding activity. Mol. Microbiol. **49**: 37–53.

Duan. K. and Surette, M.G. (2007) Environmental regulation of *Pseudomonas aeruginosa* PAO1 Las and Rhl quorum-sensing systems. J. Bacteriol. **189**: 4827–4836.

Dubiel, M., Hsu, C.H., Chien, C.C., Mansfeld, F. and Newman, D.K. (2002) Microbial iron respiration can protect steel from corrosion. Appl. Environ. Microbiol. **68**: 1440–1445.

Ekins, A., Khan, A.G., Shouldice, S.R. and Schryvers, A.B. (2004) Lactoferrin receptors in Gram-negative bacteria: insights into the iron acquisition process. Biometals **17**: 235–243.

Francesca, B., Ajello, M., Bosso, P., Morea, C., Andrea, P., Giovanni, A. and Piera, V. (2004) Both lactoferrin and iron influence aggregation and biofilm formation in *Streptococcus mutans*. Biometals **17**: 271–278.

Götz, F. (2002) *Staphylococcus* and biofilms. Mol. Microbiol. **43**: 1367–1378.

Hall-Stoodley, L. and Stoodley, P. (2005) Biofilm formation and dispersal and the transmission of human pathogens. Trends Microbiol. **13**: 7–10.

Hamilton, W.A. (1985) Sulphate-reducing bacteria and anaerobic corrosion. Annu. Rev. Microbiol. **39**: 195–217.

Hamilton, W.A. (1998) Bioenergetics of sulphate-reducing bacteria in relation to their environmental impact. Biodegradation **9**: 201–212.

Hamilton, W.A. (2003) Microbially influenced corrosion as a model system for the study of metal microbe interactions: a unifying electron transfer hypothesis. Biofouling **19**: 65–76.

Hancock, V., Ferrieres, L. and Klemm, P. (2007) Biofilm formation by asymptomatic and virulent urinary tract infectious *Escherichia coli* strains. FEMS Microbiol. Lett. **267**: 30–37.

Hancock, V., Ferrieres, L. and Klemm, P. (2008) The ferric yersiniabactin uptake receptor FyuA is required for efficient biofilm formation by urinary tract infectious *Escherichia coli* in human urine. Microbiology **154**: 167–175.

Hunt, T.A., Peng, W.T., Loubens, I. and Storey, D.G. (2002) The *Pseudomonas aeruginosa* alternative sigma factor PvdS controls exotoxin A expression and is expressed in lung infections associated with cystic fibrosis. Microbiology **148**: 3183–3193.

Hussain, M., Becker, K., von Eiff, C., Schrenzel, J., Peters, G. and Herrmann, M. (2001) Identification and characterization of a novel 38.5-kilodalton cell surface protein of *Staphylococcus aureus* with extended-spectrum binding activity for extracellular matrix and plasma proteins. J. Bacteriol. **183**: 6778–6786.

Jacques, J.F., Jang, S., Prevost, K., Desnoyers, G., Desmarais, M., Imlay, J. and Masse, E. (2006) RyhB small RNA modulates the free intracellular iron pool and is essential for normal growth during iron limitation in *Escherichia coli*. Mol. Microbiol. **62**: 1181–1190.

Jayaraman, A., Sun, A.K. and Wood, T.K. (1998) Characterization of axenic *Pseudomonas fragi* and *Escherichia coli* biofilms that inhibit corrosion of SAE 1018 steel. J. Appl. Microbiol. **84**: 485–492.

Jayaraman, A., Hallock, P.J., Carson, R.M., Lee, C.C., Mansfeld, F.B. and Wood, T.K. (1999a) Inhibiting sulfate-reducing bacteria in biofilms on steel with antimicrobial peptides generated in situ. Appl. Microbiol. Biotechnol. **52**: 267–275.

Jayaraman, A., Ornek, D., Duarte, D.A., Lee, C.C., Mansfeld, F.B. and Wood, T.K. (1999b) Axenic aerobic biofilms inhibit corrosion of copper and aluminum. Appl. Microbiol. Biotechnol. **52**: 787–790.

Johnson, M., Cockayne, A., Williams, P.H. and Morrissey, J.A. (2005) Iron-responsive regulation of biofilm formation in *Staphylococcus aureus* involves fur-dependent and fur-independent mechanisms. J. Bacteriol. **187**: 8211–8215.

Johnson, M., Cockayne, A. and Morrissey, J.A. (2008) Iron-regulated biofilm formation in *Staphylococcus aureus* Newman requires *ica* and the secreted protein Emp. Infect. Immun. **76**: 1756–1765.

Jones. D.A. and Amy, P.S. (2000) Related electrochemical characteristics of microbial metabolism and iron corrosion. Ind. Eng. Chem. Res. **39**: 575–582.

Kim, S.J., Park, R.Y., Kang, S.M., Choi, M.H., Kim, C.M. and Shin, S.H. (2006) *Pseudomonas aeruginosa* alkaline protease can facilitate siderophore-mediated iron-uptake via the proteolytic cleavage of transferrins. Biol. Pharm. Bull. **29**: 2295–2300.

Klausen, M., Aaes-Jorgensen, A., Molin, S. and Tolker-Nielsen, T. (2003) Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. Mol. Microbiol. **50**: 61–68.

Koch, G.H., Brongers, M.P.H., Thompson, N.G., Virmani, Y.P. and Payer, J.H. (2001) *Corrosion Costs and Preventative Strategies in the United States.* Report by CC Technologies Laboratories to the Federal Highway Administration. Report FHWA-RD-01-156. Office of Infrastructure Research and Development, Washington, DC.

Larsen, N.A., Lin, H., Wei, R., Fischbach, M.A. and Walsh, C.T. (2006) Structural characterization of enterobactin hydrolase IroE. Biochemistry **45**: 10184–10190.

Lee, A.K. and Newman, D.K. (2003) Microbial iron respiration: impacts on corrosion processes. Appl. Microbiol. Biotechnol. **62**: 134–139.

Lee, N.S., Kim, B.T., Kim, D.H. and Kobashi, K. (1995) Purification and reaction mechanism of arylsulfate sulfotransferase from *Haemophilus* K-12, a mouse intestinal bacterium. J. Biochem. **118**: 796–801.

Lequette, Y., Lee, J.H., Ledgham, F., Lazdunski, A. and Greenberg, E.P. (2006) A distinct QscR regulon in the *Pseudomonas aeruginosa* quorum-sensing circuit. J. Bacteriol. **188**: 3365–3370.

Maresso, A.W. and Schneewind, O. (2006) Iron acquisition and transport in *Staphylococcus aureus*. Biometals **19**: 193–203.

Maresso, A.W., Garufi, G. and Schneewind, O. (2008) *Bacillus anthracis* secretes proteins that mediate heme acquisition from hemoglobin. PLoS. Pathog. **4** :e1000132.

Masse, E., Vanderpool, C.K. and Gottesman, S. (2005) Effect of RyhB small RNA on global iron use in *Escherichia coli*. J. Bacteriol. **187**: 6962–6971.

Matsukawa. M. and Greenberg, E.P. (2004) Putative exopolysaccharide synthesis genes influence *Pseudomonas aeruginosa* biofilm development. J. Bacteriol. **186**: 4449–4456.

Mey, A.R., Craig, S.A. and Payne, S.M. (2005) Characterization of *Vibrio cholerae* RyhB: the RyhB regulon and role of *ryhB* in biofilm formation. Infect. Immun. **73**: 5706–5719.

Miethke, M. and Marahiel, M.A. (2007) Siderophore-based iron acquisition and pathogen control. Microbiol. Mol. Biol. Rev. **71**: 413–451.

Miethke, M., Klotz, O., Linne, U., May, J.J., Beckering, C.L. and Marahiel, M.A. (2006) Ferri-bacillibactin uptake and hydrolysis in *Bacillus subtilis*. Mol. Microbiol. **61**: 1413–1427.

Miller, C.E., Rock, J.D., Ridley, K.A., Williams, P.H. and Ketley, J.M. (2008) Utilization of lactoferrin-bound and transferrin-bound iron by *Campylobacter jejuni*. J. Bacteriol. **190**: 1900–1911.

Moelling, C., Oberschlacke, R., Ward, P., Karijolich, J., Borisova, K., Bjelos, N. and Bergeron, L. (2007) Metal-dependent repression of siderophore and biofilm formation in *Actinomyces naeslundii*. FEMS Microbiol. Lett. **275**: 214–220.

Musk, D.J., Banko, D.A. and Hergenrother, P.J. (2005) Iron salts perturb biofilm formation and disrupt existing biofilms of *Pseudomonas aeruginosa*. Chem. Biol. **12**: 789–796.

O'Toole, G.A. and Kolter, R. (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. **30**: 295–304.

O'Toole, G., Kaplan, H.B. and Kolter, R. (2000) Biofilm formation as microbial development. Annu. Rev. Microbiol. **54**: 49–79.

Oglesby, A.G., Farrow, J.M., 3rd, Lee, J.H., Tomaras, A.P., Greenberg, E.P., Pesci, E.C. and Vasil, M.L. (2008) The influence of iron on *Pseudomonas aeruginosa* physiology: a regulatory link between iron and quorum sensing. J. Biol. Chem. **283**: 15558–5567.

Ojha, A. and Hatfull, G.F. (2007) The role of iron in *Mycobacterium smegmatis* biofilm formation: the exochelin siderophore is essential in limiting iron conditions for biofilm formation but not for planktonic growth. Mol. Microbiol. **66**: 468–483.

Ojha, A., Anand, M., Bhatt, A., Kremer, L., Jacobs, W.R., Jr. and Hatfull, G.F. (2005) GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. Cell **123**: 861–873.

Ornek, D., Jayaraman, A., Syrett, B.C., Hsu, C.H., Mansfeld, F.B. and Wood, T.K. (2002) Pitting corrosion inhibition of aluminum 2024 by *Bacillus* biofilms secreting polyaspartate or γ-polyglutamate. Appl. Microbiol. Biotechnol. **58**: 651–657.

Orsi, N. (2004) The antimicrobial activity of lactoferrin: current status and perspectives. Biometals **17**: 189–196.

Park, R.Y., Sun, H.Y., Choi, M.H., Bai, Y.H., Chung, Y.Y. and Shin, S.H. (2006) Proteases of a *Bacillus subtilis* clinical isolate facilitate swarming and siderophore-mediated iron uptake via proteolytic cleavage of transferrin. Biol. Pharm. Bull. **29**: 850–853.

Patriquin, G.M., Banin, E., Gilmour, C., Tuchman, R., Greenberg, E.P. and Poole, K. (2008) Influence of quorum sensing and iron on twitching motility and biofilm formation in *Pseudomonas aeruginosa*. J. Bacteriol. **190**: 662–671.

Pesci, E.C., Milbank, J.B., Pearson, J.P., McKnight, S., Kende, A.S., Greenberg, E.P. and Iglewski, B.H. (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **96**: 11229–11234.

Pichon, C. and Felden, B. (2007) Proteins that interact with bacterial small RNA regulators. FEMS Microbiol. Rev. **31**: 614–625.

Pohlmann, V. and Marahiel, M.A. (2008) Delta-amino group hydroxylation of L-ornithine during coelichelin biosynthesis. Org. Biomol. Chem. **6**: 1843–1848.

Poole, K. and McKay, G.A. (2003) Iron acquisition and its control in *Pseudomonas aeruginosa*: many roads lead to Rome. Front. Biosci. **8**: 661–686.

Potekhina, J.S., Sherichevа, N.G., Povetkina, L.B., Pospelov, A.P., Rakitina, T.A., Warnecke, F. and Gottschalk, G. (1999) Role of microorganisms in corrosion inhibition of metals in aquatic habitats. Appl. Microbiol. Biotechnol. **52**: 639–646.

Raymond, K.N., Dertz, E.A. and Kim, S.S. (2003) Enterobactin: an archetype for microbial iron transport. Proc. Natl. Acad. Sci. USA **100**: 3584–3588.

Rhodes, E.R., Shoemaker, C.J., Menke, S.M., Edelmann, R.E. and Actis, L.A. (2007) Evaluation of different iron sources and their influence in biofilm formation by the dental pathogen *Actinobacillus actinomycetemcomitans*. J. Med. Microbiol. **56**: 119–128.

Rohwerder, T., Gehrke, T., Kinzler, K. and Sand, W. (2003) Bioleaching review Part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl. Microbiol. Biotechnol. **63**: 239–248.

Rolerson, E., Swick, A., Newlon, L., Palmer, C., Pan, Y., Keeshan, B. and Spatafora, G. (2006) The SloR/Dlg metalloregulator modulates *Streptococcus mutans* virulence gene expression. J. Bacteriol. **188**: 5033–5044.

Roos, V., Nielsen, E.M. and Klemm, P. (2006) Asymptomatic bacteriuria *Escherichia coli* strains: adhesins, growth and competition. FEMS Microbiol. Lett. **262**: 22–30.

Rudolph, G., Hennecke, H. and Fischer, H.M. (2006) Beyond the Fur paradigm: iron-controlled gene expression in rhizobia. FEMS Microbiol. Rev. **30**: 631–648.

Schröder, I., Johnson, E. and de Vries, S. (2003) Microbial ferric iron reductases. FEMS Microbiol. Rev. **27**: 427–447.

Sebulsky, M.T. and Heinrichs, D.E. (2001) Identification and characterization of *fhuD1* and *fhuD2*, two genes involved in iron-hydroxamate uptake in *Staphylococcus aureus*. J. Bacteriol. **183**: 4994–5000.

Shigematsu, T., Fukushima, J., Oyama, M., Tsuda, M., Kawamoto, S. and Okuda, K. (2001) Iron-mediated regulation of alkaline proteinase production in *Pseudomonas aeruginosa*. Microbiol. Immunol. **45**: 579–590.

Singh, P.K. (2004) Iron sequestration by human lactoferrin stimulates *P. aeruginosa* surface motility and blocks biofilm formation. Biometals **17**: 267–270.

Singh, P.K., Schaefer, A.L., Parsek, M.R., Moninger, T.O., Welsh, M.J. and Greenberg, E.P. (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature **407**: 762–764.

Singh, P.K., Parsek, M.R., Greenberg, E.P. and Welsh, M.J. (2002) A component of innate immunity prevents bacterial biofilm development. Nature **417**: 552–555.

Sprencel, C., Cao, Z., Qi, Z., Scott, D.C., Montague, M.A., Ivanoff, N., Xu, J., Raymond, K.M., Newton, S.M. and Klebba, P.E. (2000) Binding of ferric enterobactin by the *Escherichia coli* periplasmic protein FepB. J. Bacteriol. **182**: 5359–5364.

Tam, R. and Saier, M.H., Jr. (1993) Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol. Rev. **57**: 320–346.

Tomaras, A.P., Dorsey, C.W., Edelmann, R.E. and Actis, L.A. (2003) Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiology **149**: 3473–3484.

Touati, D. (2000) Iron and oxidative stress in bacteria. Arch. Biochem. Biophys. **373**: 1–6.

Vasil, M.L. (2007) How we learnt about iron acquisition in *Pseudomonas aeruginosa*: a series of very fortunate events. Biometals **20**: 587–601.

Visca, P., Leoni, L., Wilson, M.J. and Lamont, I.L. (2002) Iron transport and regulation, cell signalling and genomics: lessons from *Escherichia coli* and *Pseudomonas*. Mol. Microbiol. **45**: 1177–1190.

Vuong, C., Kidder, J.B., Jacobson, E.R., Otto, M., Proctor, R.A. and Somerville, G.A. (2005) *Staphylococcus epidermidis* polysaccharide intercellular adhesin production significantly increases during tricarboxylic acid cycle stress. J. Bacteriol. **187**: 2967–2973.

Wai, S.N., Mizunoe, Y., Takade, A., Kawabata, S.I. and Yoshida, S.I. (1998) *Vibrio cholerae* O1 strain TSI-4 produces the exopolysaccharide materials that determine colony morphology, stress resistance, and biofilm formation. Appl. Environ. Microbiol. **64**: 3648–3655.

Wandersman, C. and Delepelaire, P. (2004) Bacterial iron sources: from siderophores to hemophores. Annu. Rev. Microbiol. **58**: 611–647.

Whitchurch, C.B., Tolker-Nielsen, T., Ragas, P.C. and Mattick, J.S. (2002) Extracellular DNA required for bacterial biofilm formation. Science **295**: 1487.

Whiteley, M., Bangera, M.G., Bumgarner, R.E., Parsek, M.R., Teitzel, G.M., Lory, S. and Greenberg, E.P. (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. Nature **413**: 860–864.

Wilderman, P.J., Vasil, A.I., Johnson, Z., Wilson, M.J., Cunliffe, H.E., Lamont, I.L. and Vasil, M.L. (2001) Characterization of an endoprotease (PrpL) encoded by a PvdS-regulated gene in *Pseudomonas aeruginosa*. Infect. Immun. **69**: 5385–5394.

Wilderman, P.J., Sowa, N.A., FitzGerald, D.J., FitzGerald, P.C., Gottesman, S., Ochsner, U.A. and Vasil, M.L. (2004) Identification of tandem duplicate regulatory small RNAs in *Pseudomonas aeruginosa* involved in iron homeostasis. Proc. Natl. Acad. Sci. USA **101**: 9792–9797.

Williams, H.D., Zlosnik, J.E. and Ryall, B. (2007) Oxygen, cyanide and energy generation in the cystic fibrosis pathogen *Pseudomonas aeruginosa*. Adv. Microb. Physiol. **52**: 1–71.

Wolff, N., Izadi-Pruneyre, N., Couprie, J., Habeck, M., Linge, J., Rieping, W., Wandersman, C., Nilges, M., Delepierre, M. and Lecroisey, A. (2008) Comparative analysis of structural and dynamic properties of the loaded and unloaded hemophore HasA: functional implications. J. Mol. Biol. **376**: 517–525.

Yang, L., Barken, K.B., Skindersoe, M.E., Christensen, A.B., Givskov, M. and Tolker-Nielsen, T. (2007) Effects of iron on DNA release and biofilm development by *Pseudomonas aeruginosa*. Microbiology **153**: 1318–1328.

Zuo, R. (2007) Biofilms: strategies for metal corrosion inhibition employing microorganisms. Appl. Microbiol. Biotechnol. **76**: 1245–1253.

PART 4:
MICROBIAL MATS IN EXTREME ENVIRONMENTS

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MATS OF FILAMENTOUS AND UNICELLULAR CYANOBACTERIA IN HYPERSALINE ENVIRONMENTS

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1. Introduction

Cyanobacteria are characteristic components of many types of microbial mat ecosystems: these include terrestrial and marine environments, hypersaline lakes, thermal springs, and many others. Because of their large metabolic flexibility and their ability to adapt to low as well as high temperatures, extremely high salinities, high pH, and low nutrient levels, they can be found in nearly any microbial mat system in which light is available for photosynthesis. As many cyanobacteria excrete massive amounts of polysaccharide as extracellular slime or sheaths, the cyanobacteria are often the organisms that are primarily responsible for the formation of a cohesive biofilm structure that is then colonized by other types of microorganisms. This, and the fact that their presence is so evident due to their pigmentation, has made the cyanobacteria very popular objects for microbial mat research (Stal, 1995, 2000).

The drawing, reproduced in Fig. 1, of a stratified microbial mat with colored layers of photosynthetic microorganisms, including the filamentous cyanobacterium *Microcoleus chthonoplastes*, is very characteristic of the appearance of cyanobacterial layers in microbial mat ecosystems worldwide. That drawing was published nearly 200 years ago in Volume 9 of the Flora Danica (Hornemann, 1813). We see a typical leathery-looking coherent cyanobacterial layer on top of the sediment and additional green layers below, alternating with other layers including pink material that probably contains purple sulfur bacteria. This pink layer is found where anaerobic conditions prevail and sulfide, derived from dissimilatory sulfate reduction in the lower layers, is present, as well as sufficient light that penetrates through the layers above. While surely originating from Scandinavia, the collection site of the material drawn by the artist was not stated, but we may safely assume that it was found in a marine or even hypersaline system. *Microcoleus* mats are typically found in shallow marine environments in which the salinity has increased by evaporation. Somewhat similar but less well-developed microbial mats with multiple colored layers are found extensively in shallow coastal areas of Germany, the Netherlands, and Scandinavia, a phenomenon known as "Farbstreifensandwatt" (Hoffmann, 1942).

Figure 1. *Microcoleus* (*Coleofasciculus*) pictured as Conferva Chthonoplastes Mert. in the 1813 edition of the Flora Danica – probably the first illustration of a microbial mat with cyanobacteria in the scientific literature (Hornemann, 1813 (from http://www.kb.dk/GUIDResolver/template/single?src=online_master_arkiv/non-archival/DUP/floradanica/h25/floradanica_1485.tif) (*upper panel, left*), photomicrographs of *Microcoleus* bundles within their polysaccharide sheath from the "petola" mat in the saltern ponds of Sečovlje, Slovenia (Gunde-Cimerman et al., 2005; Oren and Gunde-Cimerman, 2005; Schneider, 1995; Schneider and Herrmann, 1979; Žagar, 1995), Nomarski interference contrast (*right panels*), and a gypsum crust from the salterns in Eilat, Israel, showing colored layers containing unicellular cyanobacteria (*orange*), filamentous cyanobacteria (*green*), and photosynthetic sulfur bacteria (*purple*) (Oren, 2000a; Oren et al., 1995, 2009) (*lower panel, left*) (photograph: Dr. Rhena Schumann, University of Rostock).

Even more conspicuous are cyanobacterial mats in extreme environments, especially at high temperatures and high salinities. In "normal" environments, the development and accretion of coherent mats is often limited by predation by protozoa as well as by higher organisms, and such organisms are not active at the environmental extremes that still support cyanobacterial growth. Some types of protozoa – naked amoebae and amoeboflagellates, ciliates as well as heterotrophic flagellates, have indeed been found up to the highest salinities: many grow over 20% salt and some over 30% and up to saturation (Hauer and Rogerson, 2005; Oren, 2005), but their activity does not appear to greatly affect the development of the phototrophic communities. In thermal environments, cyanobacteria can grow up to 73–74°C, while no predatory eukaryotes are known to live above 56°C (Brock, 1978).

This chapter provides an overview of the properties of two types of cyanobacteria-dominated mats found in hypersaline environments formed by partial evaporation of seawater. First, the properties of the filamentous cyanobacterium *Microcoleus* (*Coleofasciculus*) are presented with its distribution and activity in saline and hypersaline microbial mats worldwide. The second part deals with the often very thick multilayered microbial mats embedded within gypsum crusts that are formed in natural and man-made environments where seawater gets concentrated and the solubility of calcium sulfate is exceeded. Both the *Microcoleus* mats and the cyanobacterial communities in gypsum crusts have become popular model systems for the study of microbial mats and of the adaptation of microbial communities to life at high salt concentrations. And last but not the least, the esthetic aspects of the colorful layers of the phototrophic microbial communities also have contributed to the attractiveness of these systems as study objects (Gunde-Cimerman et al., 2005; Oren, 2005; Oren and Gunde-Cimerman, 2005).

2. *Microcoleus chthonoplastes* as a Mat-Forming Microorganism

Microcoleus chthonoplastes ("the little soil-forming sack") Thuret (or *Microcoleus chthonoplastes* Mertens (Zanardini ex Gomont)) was first described as *Conferva chthonoplastes* Mertens in the Flora Danica in 1813 (Fig. 1). Geitler in his monograph on the cyanobacteria (Geitler, 1932) summarized the properties of the species as follows (translation A.O.):

Microcoleus chthonoplastes Thuret, Ann. sc. nat., 6 sér., Bot. 1, S. 378, 1875. .... Filaments single or forming dirty – to black-green extended laminated layers, flexuous, rarely branched. Sheath gelatinous, rough, of variable thickness, not staining violet with chlor-zinc-iodide, containing large numbers of densely woven trichomes. Cells constricted at the cross-walls, 2.5–6 μm wide, one to two times as long as wide, blue-green, 3.6–10 μm long, lacking granules at the cross-walls. Terminal cells not capitate, pointed-conical. At sea coasts, also inland in salty water, cosmopolitan. Often forms coherent so-called "meteor paper" at sites where its extensive layers dry out, becoming whitish after desiccation.

The marine mat-forming species of the genus *Microcoleus* were recently reclassified in a new genus, *Coleofasciculus* (Thur. ex Gomont) Siegesmund et al. comb. nov. (Siegesmund et al., 2008). For reasons of convenience the name *Microcoleus* is kept in the sections below. About the phenomenon of "meteor paper" formation, more information is found in a chapter dedicated to this topic (Oren, this volume).

As suggested by the above species description, *M. chthonoplastes* is abundant worldwide in marine and hypersaline environments, where it very often is the dominant mat-forming cyanobacterium. Some of the best documented sites of massive development of *Microcoleus* are the evaporation ponds of salterns for the production of solar salt. Good examples are the ponds in the lagoons of Guerrero Negro (Baja California Sur, Mexico) (D'Antoni D'Amelio et al., 1989; Nübel et al., 2000), the Spanish salterns in the Ebro delta (Clavero et al., 1994), the salrerns on the Mediterranean coast of Santa Pola (Alicante, Spain) (Thomas, 1984) and the Salin de Giraud, France (Thomas and Geisler, 1982), the hypersaline Solar Lake (Sinai, Egypt) (D'Antoni D'Amelio et al., 1989), the hypersaline (47 g/l) and alkaline (pH 8.5) and Lake Khilganta (Buryatiya, Russia) (Gerasimenko et al., 2003); there are many more. The characteristic occurrence of dense bundles of *Microcoleus* within their common polysaccharide sheaths can be easily recognized in thin sections in the electron microscope, and there are numerous ultrastructure studies of marine and hypersaline microbial mats that show the spatial arrangement of the *Microcoleus* filaments in relation to the other components of the microbial mats (Casillas-Martinez et al., 2005; Clavero et al., 1994; D'Antoni D'Amelio et al., 1989; Gerasimenko et al., 2003; Thomas, 1984).

The cosmopolitan nature of *M. chthonoplastes* is not only apparent from the morphological appearance of the filament bundles, but also from molecular studies. A comparison was made of isolates collected from a number of geographically distant sites, including intertidal marine areas (Wadden Sea, Germany), and hypersaline environments (Solar Lake, Egypt; Guerrero Negro – Baja California Sur, Mexico) (Prufert-Bebout and Garcia-Pichel, 1994). On the basis of 16S rRNA gene comparison using denaturing gradient gel electrophoresis and gene sequencing, the species proved to be phylogenetically extremely coherent (Garcia-Pichel et al., 1996). A chemosystematic study of these strains based on their content of carotenoid pigments and UV-absorbing mycosporine-like amino acids confirmed the coherence of the species (Karsten and Garcia-Pichel, 1996).

Concerning its relation to salt, *M. chthonoplastes* can be classified as a mesohaline (with a moderately high salt optimum) and euryhaline species (able to grow in a wide range of salt concentrations), based on the definitions given by Golubic (1980). A strain from Solar Lake grew at up to 120 g/l salt (Karsten, 1996). In the salterns of Santa Pola (Spain), *Microcoleus* was found in the bottom sediments of evaporation ponds between 65 and 70 and 140 g/l total dissolved salts (Thomas, 1984), and in salterns in Puerto Rico, *Microcoleus* mats were reported at salinities between 40 and 150 g/l in the wet season and 150–265 g/l in the dry season (Casillas-Martinez et al., 2005). In the lagoons of Guerrero Negro, *Microcoleus*

dominated up to a salinity of 110 g/l, as shown also by molecular 16S rRNA-based studies (Nübel et al., 2000).

Only seldom are *Microcoleus* mats encountered at salinities above 150 g/l. A notable exception is the "petola" mat in the salterns of Sečovlje, Slovenia, which is discussed later in more details. Only few systematic studies of the growth responses of *M. chthonoplastes* appear to have been performed to test its responses to salt concentration and other environmental factors. Growth tests of strains isolated from intertidal as well as hypersaline environments (Solar Lake, Sinai; Guerrero Negro, Mexico) showed optimal growth at salt concentrations between 15 and 60 g/l, optimal rates being between 0.20 and 0.54/day (Karsten, 1996). A (bacteria-free) isolate from the Arabatskaya Strelka lagoon grew optimally at 150 g/l salt, with hardly any growth at 200 g/l. However, some photoincorporation of $^{14}CO_2$ still proceeded at 260 g/l salt. The pH for growth was relatively narrow, from 7 to 8.5, with an optimum at 7.5 (Dubinin et al., 1992).

To provide osmotic balance with its external medium with its high and often variable salt concentrations, *Microcoleus* accumulates two organic osmotic solutes: glycosylglycerol and trehalose. Glycosylglycerol accumulation is especially important at the higher salinities (Karsten, 1996). Its intracellular concentration is regulated according to the salt concentration of the environment. Accumulation of glycosylglycerol up to 40% of the cell dry weight has been measured at the highest salt concentrations supporting growth (Kevbrin et al., 1992).

In addition to its oxygenic phototrophic metabolism in the light and aerobic dark respiration, *M. chthonoplastes* has a limited potential of anaerobic metabolism in the dark: intracellular storage carbohydrates accumulated in the light can be fermented to acetate. In addition, elemental sulfur can be used as an electron acceptor in anaerobic respiration (Dubinin and Gerasimenko, 1994). These options of energy generation in the absence of oxygen may be important to the survival of the cyanobacteria within the layered benthic microbial mats, where availability of oxygen at the different depths of the sediment is subject to sharp diel changes, from oxygen supersaturation during daytime to anaerobiosis during the night.

An especially interesting case of massive occurrence of *Microcoleus* mats is found on the bottom of the saltern ponds of Sečovlje, on the Adriatic coast near the border between Slovenia and Croatia. In these salterns, operated today in the same traditional way that has been used since the Middle Ages when the ponds were established, a dense microbial mat, the so-called "petola," covers the bottom of the production ponds in which the salt crystallizes. The salt-makers promote the establishment of this mat by lining the pond bottom with a mixture of gypsum, carbonates, and pieces of older mats. During the operation of the ponds, they walk on this mat and carefully collect the salt from the top without mixing of sediment and salt (Žagar, 1995). In the lower salinity evaporation ponds at Sečovlje, green algae (*Entophysalis*) and other cyanobactera (*Lyngbya aestuarii*) are also present, but in the crystallizer ponds, *Microcoleus* is the only phototroph left (Schneider, 1995; Schneider and Herrmann, 1979). This is of special interest, as the salt concentration in such ponds increases to values above 300 g/l during

the salt production season. This is far above both the optimum and the maximum salt concentrations that support growth of *M. chthonoplastes* in the laboratory, and also much above the salt concentrations at which *Microcoleus* is generally found at other locations. As the right panels of Fig. 1 show, the bundles of *Microcoleus* filaments in the Sečovlje crystallizer ponds look perfectly healthy. No further studies of the in situ activities and possible special properties of the cyanobacterial "petola" mats appear to have been performed yet.

3. Mats of Unicellular and Filamentous Cyanobacteria Embedded in Gypusm Crusts

When seawater is evaporated in saltern ponds for the production of NaCl, the first salt to precipitate is calcium carbonate. When the salt concentration has increased above 140–150 g/l, gypsum ($CaSO_4.2H_2O$) starts to sink to the bottom of the ponds, and NaCl precipitates as halite at salinities above 300 g/l (Javor, 1989; Oren, 2002).

The gypsum crusts that accumulate on the bottom of saltern ponds of intermediate salinity often contain multicolored stratified microbial communities. The gradients of light intensity, oxygen, and sulfide concentrations govern the position of different types of unicellular and filamentous cyanobacteria, as well as purple sulfur bacteria. Such layered microbial mats within gypsum crusts have been described from salterns in Spain (Thomas, 1984), France (Caumette et al., 1994), Italy (Margheri et al., 1987), and from evaporite systems elsewhere (e.g., Rothschild et al., 1994).

In the saltern ponds in Eilat, Israel, a particularly well-developed gypsum crust with brightly colored layers of microorganisms is found at salt concentrations between 190 and 240 g/l (Fig. 1, lower left panel). The upper 0.5–2 cm is densely populated by orange-brown unicellular *Halothece*/*Euhalothece*-type cyanobacteria (Garcia-Pichel et al., 1998). Below, green *Phormidium*-type filamentous cyanobacteria are found, sometimes together with *Halospirulina* spp. (Nübel et al., 2000a). Both cyanobacterial layers, but especially the brown-orange one, contain large amounts of slimy extracellular polysaccharides excreted by the cells. The brown and the green zones are sometimes separated by a white zone devoid of phototrophs. Below the green layer, we find a bright purple layer with anoxygenic phototrophs (*Halochromatium* and *Halorhodospira* types), and the lower layer is black-gray (Oren, 2000, 2006; Sørensen et al., 2005).

In the past 15 years, we have made interdisciplinary in-depth studies of the properties of the biota of the microbial mats in this gypsum crust, with special emphasis on the cyanobacteria. These cyanobacteria are most conspicuous due to their bright pigmentation, but this does not imply that they are numerically the dominant microorganisms in the mats. Enumeration of cells stained with DAPI (4',6-diamidino-2-phenylindole) by epifluorescence microscopy showed oxygenic phototrophs to be greatly outnumbered by other types of microorganisms: only 12% and 27% of the cells consisted of cyanobacteria in the brown and in the

green layer, respectively (Sørensen et al., 2005). When molecular techniques based on clone-libraries of PCR-amplified 16S rRNA genes were applied to the study of the Eilat gypsum crust, cyanobacteria were even less represented among the phylotypes in the clone libraries compared. Three cyanobacterial phylotypes were found (Sørensen et al., 2005). Two were closely affiliated (97–99% identity) with the unicellular *Halothece*-cluster (Garcia-Pichel et al., 1998). The third was distantly related (93%) to *Microcoleus chthonoplastes*. Similar sequences have been detected, e.g. in the hypersaline microbial mats in the salterns of Guerrero Negro, Mexico (Nübel et al., 2000b).

The brown *Euhalothece* layer, exposed to high light intensities, contains only little chlorophyll, but has a high content of carotenoids – mainly myxoxanthophyll and echinenone. By the use of light microsensors, we showed that the top of the green layer received only about 1–2% of the intensity of the 620–675 nm light, compared to that at the surface of the crust. Most of the wavelengths below 550 nm were absorbed by the carotenoid pigments in the orange layer (Oren et al., 1995). The upper layer also strongly absorbed ultraviolet light (Oren, 1997). Two UV-absorbing pigments belonging to the group of mycosporine-like amino acids have been identified in the *Euhalothece* cells. The first shows maximum absorbance at 331 nm and has been identified as mycosporine-2-glycine (Kedar et al., 2002). The second, with an absorption peak at 365, was recently identified as a novel compound and its structure was elucidated (Volkmann et al., 2006). These mycosporine-like amino acids may have a double function in the cyanobacteria in the hypersaline microbial mat: they may absorb harmful UV-B radiation, but – in view of the very high concentrations at which these compounds are present within the cells – they also may have an osmotic function as organic "compatible solutes" (Oren, 1997, 2000).

To estimate photosynthesis rates within the different layers of the gypsum crust at different times of the day, we used oxygen microelectrodes. During daytime, oxygen penetrated to a depth of 2 cm – the bottom of the green layer in the sample analyzed. During the light hours, two separate peaks in oxygen concentration were observed at the depths of the two cyanobacterial populations. Up to fourfold oxygen supersaturation was reached in the brown-orange layer around noon. In the green layer, some oxygen was produced early in the morning, but rates were low. Elevated oxygen concentrations did not persist through the day in the green layer, but instead, anaerobic conditions prevailed there for part of the day and all of the night (Canfield et al., 2004).

In view of the different lifestyles of the cyanobacteria in the upper orange layer (aerobic) and the lower green layer (mainly exposed to anoxic conditions), we analyzed the fatty acid composition of the biomass in the layers. Although cyanobacteria are numerically not the most abundant type of microorganisms in the crust, their contribution to biovolume and lipid content is much greater than expected on the basis of cell numbers only, this in view of the large size of their cells and the existence of intracellular photosynthetic membrane systems. The lipids extracted from the brown-orange layer had a high content of polyunsaturated fatty acids (16:2 cis 7,10 and 18:2 cis 9,12 being 21% and 7%, respectively, of the

total fatty acids) (Ionescu et al., 2007; Oren et al., 2005). The Cyanobacteria represent one of the very rare groups of prokaryotes that contain polyunsaturated fatty acids. Among the unicellular types, some contain saturated and monounsaturated fatty acids only, others also have di-unsaturated acids, and some possess tri-unsaturated fatty acids as well. Molecular oxygen is required for the biosynthesis of polyunsaturated fatty acids, and therefore the presence of polyunsaturated fatty acids points an aerobic lifestyle.

In contrast to the upper orange layer, no polyunsaturated fatty acids were encountered in the green layer. This was somewhat unexpected, as most types of filamentous cyanobacteria have large amounts of di-, tri-, and even tetra-unsaturated fatty acids, and use oxygen-dependent pathways for the synthesis of these unsaturated fatty acids. This is also true for cyanobacteria in many hypersaline microbial mats. For example, the fatty acids 18:2 and some 16:2 was present in hypersaline (70–140 g/l salts) cyanobacterial mats from Spain where *Phormidium* or *Microcoleus* were the main primary producers (Grimalt et al., 1992). Moreover, the mono-unsaturated fatty acids present in the green layer of the Eilat gypsum crust were different from those in the upper brown-orange layer: 16:1 cis 7 and 18:1 cis 9 dominated in the green layer, while in the upper brown layer the dominant positional isomer of 16:1 was 16:1 cis 9 (Ionescu et al., 2007; Oren, 2005). Presence of this isomer may indicate use of an "anaerobic," oxygen-independent pathway of fatty acid biosynthesis. The key reaction that leads to the formation of the double bond here is the dehydration of an intermediate β-hydroxydecanoyl moiety bound to the acyl carrier protein involved in fatty acid biosynthesis. There are a few reports in the literature on filamentous cyanobacteria that lack polyunsaturated fatty acids and use an oxygen-independent ("anaerobic") pathway for the biosynthesis of their mono-unsaturated fatty acids. All these cases relate to organisms from environments low in oxygen and often rich in sulfide. Such cyanobacteria often can use sulfide as an alternative electron donor in photosynthesis (Jahnke et al., 1989; Oren et al., 1985). This potential is also realized by the cyanobacterial community in the green layer of the Eilat gypsum crust: in the presence of sulfide, CO_2 photoassimilation proceeds in the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) also, an inhibitor of photosynthetic electron flow between photosystem II and photosystem I in oxygenic photosynthesis (Oren, 2005). The filamentous cyanobacteria in the green layer of the crust thus appear to be well adapted to life under partially anaerobic conditions, as shown by their ability to use sulfide as electron donor in an anoxygenic type of photosynthesis, their lack of polyunsaturated fatty acids, and the possession of an oxygen-independent pathway for the biosynthesis of monounsaturated fatty acids.

To determine the effect of the salinity of the brine on the photosynthetic activity of the cyanobacteria in the orange-brown and in the green cyanobacterial layer, we assessed photosynthesis rates both based on oxygen production (microelectrode measurements) and on $^{14}CO_2$ photoassimilation. Both the unicellular and the filamentous cyanobacteria had their optimal activity near the in situ

salinity (between 180 and 220 g/l). However, in culture experiments, the unicellular cyanobacteria isolated from the brown layer grew optimally at far lower salt concentrations (30–50 g/l). Salinities above 230 g/l strongly inhibited photosynthesis in the green layer (Sørensen et al., 2004).

In addition to the above-summarized studies on the cyanobacterial communities in the gypsum crust, we have investigated many aspects of the other microorganisms inhabiting the microbial mats and the activities they display. These include the nature and activity of the anoxygenic phototrophic bacteria in the purple later, the possible presence of green filamentous anoxygenic phototrophs, the nature of different types of heterotrophic prokaryotes in the different layers, activities of aerobic chemoautotrophic sulfur bacteria, the presence and activity of the anaerobic bacteria responsible for dissimilatory sulfate reduction with the production of sulfide, and the production of methane from different substrates in the anaerobic layers of the crust. Details on all these topics are found in Oren et al. (2009).

4. Final Comments

The stratified microbial mats that develop on the bottom of hypersaline saltern evaporation ponds worldwide are excellent environments for the study of the behavior of cyanobacteria in stratified microbial communities. The types of cyanobacteria that develop in such systems worldwide vary very little. *Microcoleus chthonoplastes* is a cosmopolitan organism whose properties appear to vary very little; it is abundant in ponds of the lower salinity range. When salinity increases and gypsum starts to precipitate, *Microcoleus* is generally no longer found. Within the gypsum crust, unicellular cyanobacteria, adapted to life at high light intensities, colonize the upper layer, and a green layer of *Phormidium*-like filamentous cyanobacteria is found deeper in the crust where the light intensity is around 1–2% of that at the surface.

Both the *Microcoleus* mats and the cyanobacterial communities in gypsum crusts have become popular objects for research. As shown above, the different cyanobacteria show many interesting adaptations to life in the hypersaline environments with their sharp dynamic gradients of oxygen, sulfide, and light intensity. The stable vertical stratification of the microorganisms embedded between the gypsum crystals allows analyses of the different layers with a high spatial resolution. In addition, much information has been obtained on the microbial diversity at high salinities and the way the cyanobacteria and other microorganisms in these hypersaline microbial mats cope with life at high salt concentrations.

Some interesting challenges remain for future studies. The properties of the "petola" in the Slovenian salterns, where *Microcoleus* forms stable mats at salinities far above its optimum salt concentration for growth and photosynthetic activity, have never yet been elucidated. Such studies will undoubtedly show many more unexpected properties of these metabolically flexible cyanobacteria.

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6. References

Brock, T.D. (1978) *Thermophilic Microorganisms and Life at High Temperatures.* Springer, New York.

Casillas-Martinez, L., Gonzalez, M.L, Fuentes-Figueroa, Z., Castro, C.M., Nieves-Mendez, D., Hernandez, C., Ramirez, W., Sytsma, R.E., Perez-Jimenez, J. and Visscher, P.T. (2005) Community structure, geochemical characteristics and mineralogy of a hypersaline microbial mat, Cabo Rojo, PR. Geomicrobiol. J. **22**: 269–281.

Caumette, P., Matheron, R., Raymond, N. and Relexans, J.-C. (1994) Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). FEMS Microbiol. Ecol. **13:** 273–286.

Clavero, E., Grimalt, J.O., Merino, V. and Hernández-Mariné, M. (1994) Structure of the sediment at depositional saline environments, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats. Structure, Development and Environmental Significance*. Springer, Berlin, pp. 91–96.

D'Antoni D'Amelio, E., Cohen, Y. and Des Marais, D.J. (1989) Comparative functional ultrastructure of two hypersaline submerged cyanobacterial mats: Guerrero Negro, Baja California Sur, Mexico, and Solar Lake, Sinai, Egypt, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats; Physiological Ecology of Benthic Microbial Communities.* American Society for Microbiology, Washington, DC, pp. 97–113.

Des Marais, D.J. (1995) The biogeochemistry of hypersaline microbial mats, In: J.G. Jones (ed.) *Advances in Microbial Ecology,* Vol. 14. Plenum, New York, pp. 251–274.

Dubinin, A.V. and Gerasimenko, L.M. (1994) Dark anaerobic metabolism of halophilic cyanobacterium *Microcoleus chthonoplastes*. Microbiology (translation of Mikrobiologiya) **62**: 391–394.

Dubinin, A.V., Gerasimenko, L.M. and Gusev, M.V. (1992) Physiological features of a strain of *Microcoleus chthonoplastes* from a hypersaline reservoir. Microbiology (translation of Mikrobiologiya) **61**: 47–51.

Garcia-Pichel, F., Prufert-Bebout, L. and Muyzer, G. (1996) Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. Appl. Environ. Microbiol. **62**: 3284–3291.

Garcia-Pichel, F., Nübel, U. and Muyzer, G. (1998) The phylogeny of unicellular, extremely halotolerant cyanobacteria. Arch. Microbiol. **169**: 469–482.

Geitler, L. (1932) *Cyanophyceae*, In: Kolkwitz R. (ed.) *Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Vierzehnter Band – Die Algen.* Reprinted 1985, Koeltz Scientific Books, Koenigstein.

Gerasimenko, L.M., Mityushina, L.L. and Namsaraev, B.B. (2003) *Microcoleus* mats from alkaliphilic and halophilic communities. Microbiology (translation of Mikrobiologiya) **72**: 71–79.

Golubic, S. (1980) Halophily and halotolerance in cyanophytes. Origin of Life **10**: 169–183.

Grimalt, J.O., de Wit, R., Teixidor, P. and Albaiges, J. (1992) Lipid biogeochemistry of *Phormidium* and *Microcoleus* mats. Org. Geochem. **19**: 509–530.

Gunde-Cimerman, N., Oren, A. and Plemenitaš, A. (2005) *Mikrosafari – Čudoviti Svet Mikroorganizmov Solin – The Beautiful World of Microorganisms in the Salterns.* Bilingual Slovenian/English publication. Državna Založba Slovenije, Ljubljana.

Hauer, G. and Rogerson, A. (2005) Heterotrophic protozoa from hypersaline environments, In: N. Gunde-Cimerman, A. Oren and A. Plemenitaš (eds.) *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya.* Springer, Dordrecht, pp. 521–539.

Hoffmann, C. (1942) Beiträge zur Vegetation des Farbstreifen-Sandwattes. Kieler Meeresforsch. **4**: 85–108.

Hornemann, J.W. (ed.) (1813) *Flora Danica. Icones Plantarum sponte nascentium in regnis Daniae et Norvegiae, in ducatibus Slesvici et Holsatiae et in comitatibus Oldenburgi et Delmenhorstiae: Ad illustrandum opus de iisdem Plantis, Regio jussu exarandum Florae Danicae nomine inscriptum,* Vol. 9. Hafniae, Copenhagen.

Ionescu, D., Lipski, A., Altendorf, K. and Oren, A. (2007) Characterization of the endoevaporitic microbial communities in a hypersaline gypsum crust by fatty acid analysis. Hydrobiologia **576**: 15–26.

Jahnke, LL., Lee, B., Sweeney, M.J. and Klein, H.P. (1989) Anaerobic biochemistry of unsaturated fatty acids in the cyanobacterium, *Oscillatoria limetica*: Ann. Microbiol. **152**: 215–217.

Javor, B. (1989) *Hypersaline Environments. Microbiology and Biogeochemistry*. Springer, Berlin.

Karsten, U. (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (Cyanobacteria) from benthic microbial mats: response to salinity change. J. Phycol. **32**: 501–506.

Karsten, U. and Garcia-Pichel, F. (1996) Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* (Cyanobacteria): a chemosystematic study. System. Appl. Microbiol. **19**: 285–294.

Kevbrin, V.V., Dubinin, A.V. and Osipov, G.A. (1992) Osmoregulation in the marine cyanobacterium *Microcoleus chthonoplastes*. Microbiology (translation of Mikrobiologiya) **60**: 407–410.

Kedar, L., Kashman, Y. and Oren, A. (2002) Mycosporine-2-glycine is the major mycosporine-like amino acid in a unicellular cyanobacterium (*Euhalothece* sp.) isolated from a gypsum crust in a hypersaline saltern pond. FEMS Microbiol. Lett. **208**: 233–237.

Margheri, M.C., Tredici, M.R., Barsanti, L. and Balloni, W. (1987) The photosynthetic community of the Trapani saline lagoons: an alternative option for the exploitation of an extreme environment: Ann. Microbiol. **37**: 203–215.

Nübel, U., Garcia-Pichel, F., Clavero, E. and Muyzer, G. (2000) Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ. Microbiol. **2**: 217–226.

Oren, A. (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. Geomicrobiol. J. **14**: 233–242.

Oren, A. (2000) Salts and brines, In: B.A. Whitton and M. Potts (eds.) *Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer, Dordrecht, The Netherlands, pp. 281–306.

Oren, A. (2001) The bioenergetic basis for the decrease in metabolic diversity in increasing salt concentrations: implications for the functioning of salt lake ecosystems. Hydrobiologia **466**: 61–72.

Oren, A. (2002) *Halophilic Microorganisms and Their Environments*. Kluwer, Dordrecht, The Netherlands.

Oren, A. (2005) Microscopic examination of microbial communities along a salinity gradient in saltern evaporation ponds: a 'Halophilic Safari', In: N. Gunde-Cimerman, A. Oren and A. Plemenitaš (eds.) *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya.* Springer, Dordrecht, The Netherlands, pp. 41–57.

Oren, A. (2006) Life at high salt concentrations, In: M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer and E. Stackebrandt (eds.) *The Prokaryotes. A Handbook on the Biology of Bacteria,* Vol. 2. Springer, New York, pp. 263–282.

Oren, A. (2010) Paper from outer space – on 'meteorpapier' and microbial mats, In: J. Seckbach and A. Oren (eds.) *Microbial Mats.* Springer, Dordrecht, This volume.

Oren, A. and Gunde-Cimerman, N. (2005) The variety of microbial life forms in the salterns – a microsafari from seawater to saturated brines, In: N. Gunde-Cimerman, A. Oren and

A. Plemenitaš (eds.) *Mikrosafari – Čudoviti Svet Mikroorganizmov Solin (The Beautiful World of Microorganisms in the Salterns)*. Bilingual Slovenian/English publication. Državna Založba Slovenije, Ljubljana, pp. 24–57.

Oren, A., Fattom, A., Padan, E. and Tietz, A. (1985) Unsaturated fatty acid composition and biosynthesis in Oscillatoria limnetica and other cyanobacteria. Arch. Microbiol. **141**: 138–142.

Oren, A., Kühl, M. and Karsten, U. (1995) An endoevaporitic microbial mat within a gypsum crust: zonation of phototrophs, photopigments, and light penetration. Mar. Ecol. Prog. Ser. **128:** 151–159.

Oren, A., Ionescu, D., Lipski, A. and Altendorf, K. (2005) Fatty acid analysis of a layered community of cyanobacteria developing in a hypersaline gypsum crust. Algol. Stud. **117**: 339–347.

Oren, A., Sørensen, K.B., Canfield, D.E., Teske, A.P., Ionescu, D., Lipski, A. and Altendorf, K. (2009) Microbial communities and processes within a hypersaline gypsum crust in a saltern evaporation pond (Eilat, Israel). Hydrobiologia **626**: 15–26.

Prufert-Bebout, L. and Garcia-Pichel, F. (1994) Field and cultivated *Microcoleus chthonoplastes*: the search for clues to its prevalence in marine microbial mats, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats. Structure, Development and Environmental Significance.* Springer, Berlin, pp. 111–116.

Rothschild, L.J., Giver, L.J., White, M.R. and Mancinelli, R.L. (1994) Metabolic activity of microorganisms in evaporites. J. Phycol. **30:** 431–438.

Schneider, J. (1995) Eindunstung von Meerwasser und Salzbildung in Salinen. Kali und Steinsalz **11**: 325–330.

Schneider, J. and Herrmann, A.G. (1979) Saltworks – natural laboratories for microbiological and geochemical investigations during the evaporation of seawater, In: A.H. Coogan and L. Hauder (eds.) *5th Symposium on Salt.* Northern Ohio Geological Society, Cleveland, pp. 371–381.

Siegesmund, M.A., Johansen, J.R., Karsten, U. and Friedl, T. (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J. Phycol. **44**: 1572–1585.

Sørensen, K.B., Canfield, D.E. and Oren, A. (2004) Salt responses of benthic microbial communities in a solar saltern (Eilat, Israel). Appl. Environ. Microbiol. **70:** 1608–1616.

Sørensen, K.B., Canfield, D.E., Teske, A.P. and Oren, A. (2005) Community composition of a hypersaline endoevaporitic microbial mat. Appl. Environ. Microbiol. **71**: 7352–7365.

Stal, L.J. (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol. **131**: 1–32.

Stal, L.J. (2000) Cyanobacterial mats and stromatolites. In: B.A. Whitton and M. Potts (eds.) *Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer, Dordrecht, The Netherlands, pp. 61–120.

Thomas, J.-C. (1984) Formations benthiques à Cyanobactéries des salins de Santa Pola (Espagne): composition spécifique, morphologie et charactéristiques biologiques des principaux peuplements. Rev. Inv. Geol. **38/39**: 139–158.

Thomas, J.-C. and Geisler, D. (1982) Peuplements benthiques à Cyanophycées des marais salants de Salin-de-Giraud (sud de la France). Geol. Médit. **9:** 391–411.

Volkmann, M., Gorbushina, A.A., Kedar, L. and Oren, A. (2006) The structure of euhalothece-362, a novel red-shifted mycosporine-like amino acid, from a halophilic cyanobacterium (*Euhalothece* sp.). FEMS Microbiol. Lett. **258**: 50–54.

Žagar, Z. (1995) Salt-making on the northeastern coast of the Adriatic Sea, In: *Museum of Salt-Making – Salzgartenmuseum. Maritime Museum "Sergej Mašera"*, Piran, Slovenia. Catalogue no. 7, pp. 24–41.

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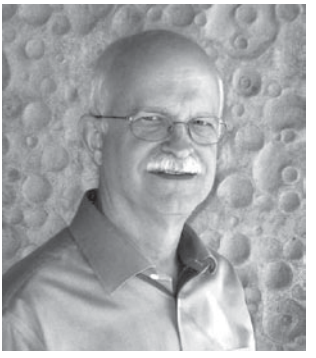

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MARINE HYPERSALINE *MICROCOLEUS*-DOMINATED CYANOBACTERIAL MATS IN THE SALTERN AT GUERRERO NEGRO, BAJA CALIFORNIA SUR, MEXICO: A SYSTEM-LEVEL PERSPECTIVE

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1. Introduction

Cyanobacterial mats in extensive seawater evaporation ponds at Guerrero Negro, Baja California, Mexico have been excellent subjects for microbial ecology research. Exportadora de Sal, S.A. (ESSA) has maintained this solar saltern for more than 50 years. The studies reviewed here have documented the steep and rapidly changing environmental gradients experienced by mat microorganisms and the very high rates of biogeochemical processes that they maintained. Recent genetic studies have revealed an enormous diversity of bacteria as well as the spatial distribution of Bacteria, Archaea, and Eucarya. These findings, together with emerging insights into the intimate interactions between these diverse populations, have contributed substantially to our understanding of the origins, environmental impacts, and biosignatures of photosynthetic microbial mats.

Light and nutrients sustain high rates of oxygenic and anoxygenic photosynthesis in the mats. In turn, photosynthesis provides energy, organic substrates, and oxygen to the community (Fig. 1). Although photosynthetic bacteria might dominate the biomass and productivity of the mat, many key properties of this ecosystem ultimately reflect the activity of the associated nonphotosynthetic biota, including anaerobes and Eucarya. Such activity also applies a "biological filter" on chemical biomarkers (e.g., porphyrins, hopanes, isoprenoids, and other biogenic hydrocarbons) and on isotopic and geologic biosignatures that subsequently enter the fossil record. Gases emitted from microbial mats might have substantially influenced the evolution of our atmosphere.

The diversity of biota, the functional complexity of the mats, and the highly proximal and ordered spatial arrangement of microorganisms offer the potential for a staggering number of interactions. The products of each group can affect the responses of other groups in both positive and negative ways. For example, cyanobacteria generate not only organic matter (a potential growth and energy substrate for other organisms) but also oxygen (a toxin for many anaerobic processes). Anaerobic activity not only recycles nutrients to the phototrophic community, but also generates potentially toxic sulfide (van Gemerden, 1993).

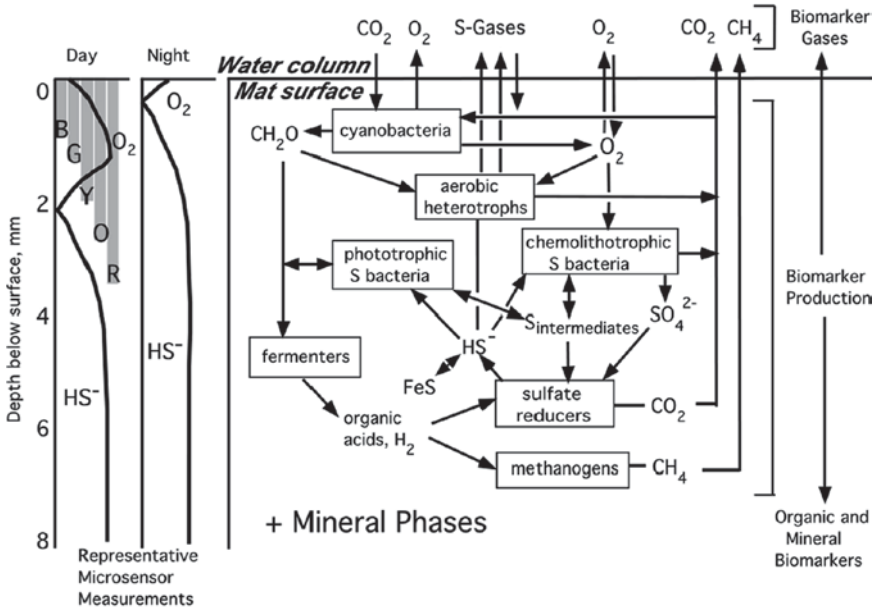

Figure 1. Scheme of a cyanobacterial microbial mat with associated depth-related light and chemical gradients. The flow diagram at the center is modeled after Fenchel and Finlay (1995). Boxes denote functional groups of microorganisms, and arrows denote flows of chemical species into or out of microorganisms. Sintermediate indicates sulfur in intermediate oxidation states. The diagram at the left depicts vertical gradients of O_2 and sulfide during the day and at night. Oxygen concentrations are shown decreasing to zero at a depth of 2 mm during the day, and just below the mat surface at night. The vertical bars at upper left represent the relative depths of penetration of blue (B), green (G), yellow (Y), orange (O), and red (R) light.

Accordingly, microorganisms have developed strategies to cope with the daily oscillation between extremes of eutrophy and toxicity.

2. Seawater Evaporation Ponds and Photosynthetic Biota along the Salinity Gradient in the Exportadora de Sal, S. A. (ESSA), Guerrero Negro Saltern

The ESSA evaporation ponds are situated adjacent to Laguna Ojo de Liebre (Scammon's Lagoon) on the Pacific coast (~27°40′N, ~113°55′W), about 700 km south of the Mexico–USA border. Annual precipitation ranges between 15 and 120 mm $year^{-1}$ and exceeds 300 mm $year^{-1}$ typically less than once per decade. Winds are W to W-NW; they average 5 m s^{-1} but vary seasonally, attaining their lowest activity from August through December. Daily air (pond water) summer temperatures range from 20°C to 32°C (22–29°C); winter temperatures vary between 8°C and 24°C (14°C and 22°C). Seawater is pumped from the lagoon into Pond 1 (Fig. 2), then flows to Ponds 2, 3, 4, 5, 6, etc. These ponds receive

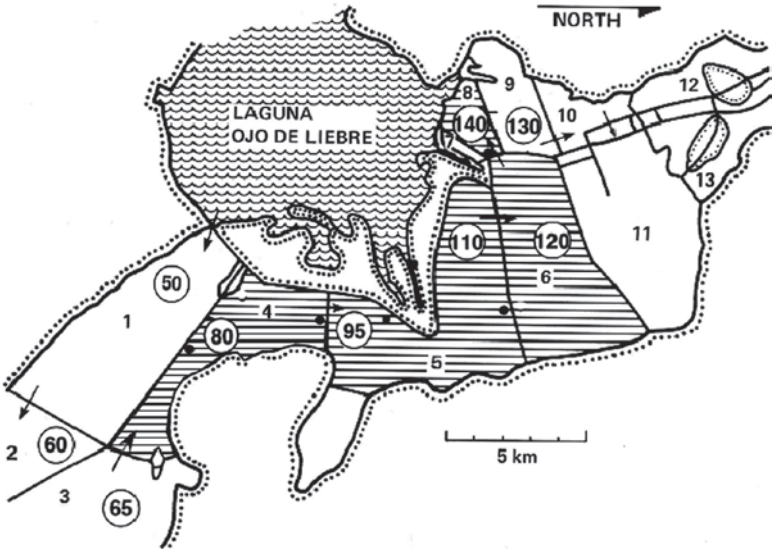

Figure 2. Seawater evaporation ponds of the company Exportadora de Sal, S.A., at Guerrero Negro, Baja California Sur, Mexico. Arrows indicate direction of flow of evaporating seawater. Circled numbers give average salinities (‰) for each pond. Ponds 4, 5, and 6 (*horizontal lines*) host permanently submerged mats. Filled circles indicate sites where *Microcoleus*-dominated mats were investigated.

no groundwater because they lie above the local hydrologic gradient. Runoff has minimal effect on the brines except during very infrequent wet years. This saltern produces an annual harvest of NaCl exceeding 6 million metric tons, placing it among the world's largest producers.

Along the salinity gradient, the evaporating brines display distinctive chemical trends (Des Marais et al., 1989). For each of the concentrating ponds, long-term average salinities (expressed as parts per thousand (‰): g salts/kg brine; ESSA company records) are shown in Fig. 2. Although highly productive microbial mats occur in the salinity range 65–125‰, a strong net uptake of dissolved inorganic carbon (DIC) by these mats is not indicated (Des Marais et al., 1989). No strong trends were observed in the stable carbon isotopic composition of either DIC or mat organic matter (Des Marais et al., 1989). Levels of phosphate, nitrate, and ammonia are low (Javor, 1983), due perhaps in part to negligible runoff. At these relatively low nutrient levels, benthic communities are favored over plankton, perhaps because they retain nutrients efficiently (Javor, 1983).

In the salinity range 40–65‰, the ponds were dominated by a seagrass, *Ruppia* sp., and a green alga, *Enteromorpha* (Javor, 1983). Also present were diatoms (*Navicula* sp., *Grammatophora* sp., *Striatella* sp., and *Licmophora* sp.) and cyanobacteria (*Entophysalis*). *Dunaliella* sp., a halophilic green alga, is typically abundant in other hypersaline environments but was uncommon here (Javor, 1983).

Well-developed permanently submerged cyanobacterial mats developed in the salinity range 65–120‰, which currently occurs in Ponds 4 through 6. Nübel et al. (2000) investigated the phylogenetic diversity of oxygenic photosynthetic microorganisms with respect to ambient salinity (Fig. 3). Most of the organisms they identified were related to cultivated strains whose responses to a range of salinities had been characterized. In most cases, the salinities in the laboratory where

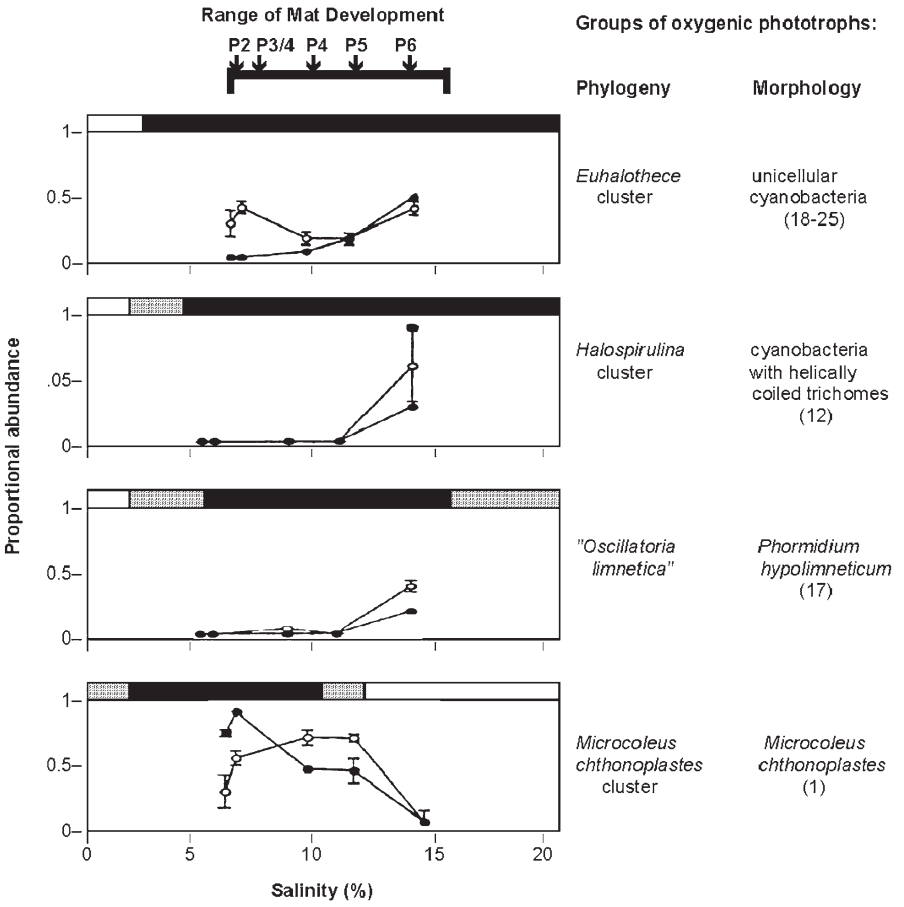

Figure 3. The proportional abundances of cyanobacteria in the mats vary in relation to ambient salinity (Modified from Nübel et al., 2000). The ordinate axes differ between panels. Cell counts (*open circles*) and amounts of DNA in DGGE bands (*closed circles*) are shown as means and standard errors based on triplicate analyses. Horizontal bars above each panel show salinity tolerances for the growth of cultivated strains of the phylogenetic groups investigated. Black bars indicate growth with at least half maximal rates (optimal salinities); hatched bars indicate growth at lower rates (suboptimal salinities). The two columns at right give definitions of the groups, based on phylogeny and morphology (see Nübel et al., 1999). The saltern pond numbers and range of salinities at which microbial mats developed are indicated at the top of the Figure.

the growth rates of a microorganism were optimal corresponded to the salinities in the field where the organism was most abundant. The cyanobacterium *Microcoleus chthonoplastes* and its close relatives dominated the mat communities up to salinities of 110‰. The phylogenetic clusters *Euhalothece* and *Halospirulina* were the most abundant cyanobacteria in the salinity range 110–140‰. Although *Euhalothece* and *Halospirulina* exhibited optimal growth rates over a wide range of salinities in the laboratory, their occurrences in the field were restricted to the highest salinities investigated by Nübel et al. (2000). Diatoms accounted for a small percentage of cells throughout the salinity gradient.

The thickest, most coherent filamentous cyanobacterial mats developed in Ponds 4 and 5. They were permanently submerged at 0.5–1 m water depth, where the salinity was maintained typically in the range 75–110‰ (about two-and-a-half to three times seawater salinity). This discussion focuses on *Microcoleus*-dominated mats because they have been most thoroughly studied and because they express most prominently the key components of robust photosynthetic mat ecosystems. The salinities of these ponds have varied somewhat during the 24-year period spanned by these studies. In response to these changes, field sites in Ponds 4 and 5 were moved occasionally to restrict the salinities at the study sites between 75‰ and 100‰. These values lie within the optimal range observed for *Microcoleus*-dominated mats in the ESSA saltern (Nübel et al., 2000). Salinity variations of this magnitude are not expected to alter this mat community substantially (Green et al., 2008).

3. Physical Properties of the *Microcoleus*-Dominated Mats

The mats in Ponds 4 and 5 had a cohesive, rubbery texture and ranged from 1 to 10 cm thick but were usually 4–7 cm thick (Des Marais et al., 1992). The mat surface was typically smooth and olive-tan in color, although orange-tan-colored blister-shaped communities of unicellular cyanobacteria and diatoms became more abundant at higher salinities. Internal laminations were frequently 0.3–3 mm thick and varied in color from dark green to tan to orange-brown. These laminations represented variations over time in the relative contributions to the mat by filamentous and unicellular cyanobacteria and by organic detritus derived from disrupted mats. The top 1–2 mm of the mat was by far the most active zone metabolically (e.g., Canfield and Des Marais, 1993). At depths typically exceeding 50–70 mm, the mat color darkened and, at its base, the mat graded into a fine-grained black sulfidic gypsum mud.

Jørgensen and Des Marais (1990) investigated the effects of the diffusion boundary layer (DBL) on transport across the mat–water interface in Pond 5, near the dike between Pond 5 and the lagoon (Fig. 1). The thickness of the DBL varied (from 0.2 to 0.8 mm) inversely versus water velocity above the mat (from 7.7 to 0.3 cm s^{-1}, respectively). Therefore, higher flow rates favored more rapid transport of solutes across the mat/water interface. If the vertical relief of the mat

surface (or surface roughness) was smaller than the DBL thickness, then this relief had a negligible effect on both the fluid flows along the surface and the transport of solutes across the mat–water interface (Boudreau and Guinasso, 1982). However, the surface roughness of these mats was approximately 1 mm or greater and thus exceeded the DBL thickness, which was typically 0.5 mm. Therefore, the upper surface of the DBL closely followed the topography of the underlying mat, although the DBL was thinner over elevated areas and thicker over depressions. The DBL was thinner on the upstream sides of small mounds than on their downstream sides. Overall, the greater surface areas of rougher mats increased the rates of O_2 transport across the mat–water interface, even though the upper surface of the DBL was smoother than the mat surface.

The light flux into the mat was measured both as downward irradiance (the total down-welling light that passes through a horizontal plane) and as scalar irradiance (the sum of all light that converges on a given point within the mat) (Jørgensen and Des Marais, 1988). Because of the high density of photosynthetic organisms, bacterial mucilage, and mineral particles in mats, the light-harvesting pigments of the phototrophic bacteria dominate the light absorption and light is also strongly scattered. Because absorption and scattering of light within the mat were quite substantial, scalar irradiance differed substantially from downward irradiance. Because scalar irradiance measures the total light actually available at a given location, it constitutes the most meaningful description of the environment of a microorganism.

Measurements of scalar irradiance were obtained both for a microbial mat that was dominated by *Microcoleus chthonoplastes* (Pond 5, near the dike between Pond 5 and the lagoon; Fig. 1) and for a mat that grew at higher salinity (Pond 6, near the dike between Ponds 5 and 6) and was dominated by unicellular cyanobacteria (Jørgensen and Des Marais, 1988). In the dark olive mat dominated by *Microcoleus* cyanobacteria, the light exhibited both a strong decline in intensity and a marked change in its spectral composition with depth. Minima in the spectra corresponded to the absorption maxima of the photosynthetic pigments of cyanobacteria. Chlorophyll *a* (Chl *a*) absorbs at wavelengths of about 430 and 670 nm, phycocyanin at about 620 nm, and various carotenoids in the range of 450–500 nm. In contrast, the mat that was dominated by unicellular cyanobacteria had a lower density of cells, a more gelatinous texture, and a light orange-tan color. Light penetrated more deeply into the unicellular cyanobacterial mat although blue light was strongly attenuated. The carotenoids in this mat absorbed most of the light. Longer-wavelength light, particularly light longer than 900 nm, penetrated farthest into both the *Microcoleus* and unicellular mats. These and other studies have illustrated how the mat matrix has affected the penetration of light and the physiology of the biota. For example, mat cyanobacteria that utilized light that has been filtered by overlying diatoms exhibited greatest photosynthetic activity at wavelengths between 550 and 650 nm (Jørgensen et al., 1987), a region that lies between the absorption maxima of Chl *a.* In contrast, planktonic cyanobacteria exposed to a broader spectrum of light in their natural environment

displayed significant activity at wavelengths corresponding to the absorption maxima of Chl *a* (Jørgensen and Des Marais, 1988).

4. Biogeochemistry

Very high rates of oxygenic photosynthesis in the mat's shallow photic zone (Fig. 4) create steep and variable gradients in pH and in concentrations of dissolved inorganic carbon (DIC) and O_2 (DO) (Canfield and Des Marais, 1993). The oxygenated zone reflects a dynamic balance between photosynthetic O_2 production and O_2 consumption by a host of sulfide-oxidizing and heterotrophic bacteria. Jørgensen and Des Marais (1986) performed the first measurements of the depth distribution of DO, sulfides, and pH in these mat porewaters. Extremely high rates of oxygenic photosynthesis sustained DO concentrations that were nearly five times the value of air-saturated brine, yet this O_2 had a residence time of only 2 min. Oxygen production became negligible at a depth of only 0.5 mm due to light limitation. However, O_2 diffused farther down to a depth where it overlapped with sulfide diffusing up from below. Abundant phototrophic bacteria (e.g., *Chloroflexus*) and *Beggiatoa* typically inhabited this interval. As sunset approached, the oxic zone collapsed quickly and the oxic–anoxic boundary approached the mat surface (Canfield and Des Marais, 1993). Accordingly, conditions alternated between O_2 supersaturation and anoxia with millimolar concentrations of sulfide. Diverse microbiota have adapted well to this highly variable environment.

The relative abundances of phototrophic bacteria (e.g., purple sulfur bacteria and green nonsulfur bacteria) versus chemolithotrophic sulfide-oxidizing bacteria were affected by the amount of light that reached the chemocline (Jørgensen and

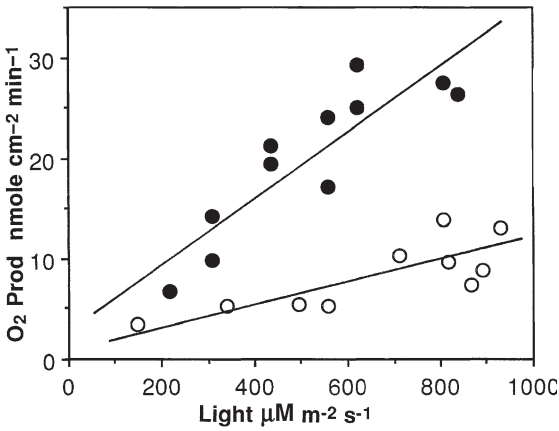

Figure 4. Rates of O_2 production as a function of light intensity at the mat surface at 20°C (*open circles*) and 30°C (*filled circles*). Mats were sampled from Pond 5, near the dike between Ponds 5 and 6. (Modified after Canfield and Des Marais, 1993.)

Des Marais, 1986). A light level as low as 1% of the incident near-infrared radiation (800–900 nm) was sufficient for *Chromatium*, a phototrophic purple sulfur bacterium, to dominate the chemocline. Thus, the balance between the depth of O_2 penetration into the mat and the light intensity in the mat's sulfide-rich zone has determined the relative abundances of sulfur bacteria.

Ley et al. (2006) estimated biomass (via ATP) and analyzed microbial pigments. ATP concentrations were more than four times greater in the oxic zone (defined as the topmost ~2 mm mat layer that becomes oxygenated during the day) than in the underlying anoxic zone (~2–~6 mm depth). The cyanobacterial pigment Chl *a* was by far the most abundant photosynthetic pigment in the mat, indicating that cyanobacteria fix far more DIC than the anoxygenic phototrophs and therefore provide the main sustenance of the mat. Bacteriochlorophylls were most abundant immediately beneath the oxic zone, consistent with earlier measurements of light spectra versus depth (Jørgensen and Des Marais, 1988).

Observations of the cycling of carbon, oxygen, and sulfur illustrated the intimate interactions between mat processes (Canfield and Des Marais, 1993; Des Marais et al., 2002). During the day, most of the O_2 produced was recycled within the mat by O_2 respiration and, to a lesser degree, by sulfide oxidation. At night, O_2 was consumed principally by sulfide oxidation near the mat–water interface. The principal source of DIC at night was microbial sulfate reduction. Although abundant *Chloroflexus*-type (anoxic phototroph) filaments were visible microscopically at the O_2–sulfide interface, anoxygenic photosynthesis accounted for less than 10% of the total carbon fixation rate. A careful comparison of the relative fluxes of O_2 and DIC across the mat–water interface revealed that, during the day, more DIC diffused into the mat than O_2 diffused out (Canfield and Des Marais, 1993; Des Marais et al., 2002). At night, more DIC diffused out of the mat than O_2 diffused into the mat. However, both the net O_2 and the net DIC fluxes were balanced over the 24-h cycle. Therefore, during the day, carbon having an oxidation state greater than zero accumulated in the mat. At night, carbon having a similarly high oxidation state was released into the water column. These relatively oxidized carbon compounds have not yet been identified; however, glycolate, a well-known product of photorespiration, is one candidate. The rates of the key processes that cycled carbon, sulfur, and oxygen all increased strongly with temperature by approximately the same amount (Canfield and Des Marais, 1993; Des Marais et al., 2003). Over a 24-h period, the net accumulation of carbon in the sediment was relatively low. High rates of photosynthetic carbon fixation fueled high rates of carbon oxidation in this closely coupled system. The efficient oxidation of organic components regenerated nutrients that, in turn, maintained high rates of primary production.

Studies of carbon isotopic discrimination in these mats have contributed additional key insights about the biogeochemical cycling of carbon in these mats (Des Marais and Canfield, 1994; Kelley et al., 2006).

Several key attributes of nitrogen cycling became apparent during observations conducted over the diel cycle (Bebout et al., 1994). Rates of N_2 fixation were

highly variable, with highest rates occurring at night. Because O_2 inhibits nitrogenase, mat microorganisms fixed nitrogen fixation at night when no O_2 was produced within the mat (Paerl et al., 1994). However, the observed rates of N_2 fixation rates were substantially less than required in order to sustain the measured rates of primary production. Bebout et al. (1994) observed some uptake from the overlying brine of dissolved inorganic nitrogen, principally as NH_4^+, but the net incorporation of such nitrogen was low. However, they also found that NH_4^+-rich porewaters beneath the photic zone could have supplied most of the fixed nitrogen required for primary production. Recycled fixed nitrogen apparently provided most of the nitrogen required by these *Microcoleus*-dominated mats. These mats also can retain fixed nitrogen efficiently. Canfield and Des Marais (1994) observed that no NH_4^+ escaped from the mat at any time during the diel cycle.

At night, O_2 was consumed by sulfide oxidation at the mat surface and lowermost water column (Canfield and Des Marais, 1993), thus the entire mat became anoxic (Fig. 1). Accordingly, mat cyanobacteria employed fermentation reactions to obtain energy at night and thereby produced an array of low-molecular-weight compounds.

Fermentations of cyanobacterial carbon compounds provided substrates that sustain other key processes, including the redox cycling of sulfur and the production of methane and other reduced gases. Effluxes in the overlying water column of short-chain fatty acids (Albert et al., 2000) and of H_2, CH_4 (Hoehler et al., 2001) were much greater at night in the absence of O_2, emphasizing the importance of fermentation. Des Marais et al. (2003) observed production rates of low-molecular-weight fatty acids under anaerobic conditions that amounted to several percent of the rate of gross primary production. Hoehler et al. (2001) observed that these mats also generated CO, CH_4, and significant amounts of H_2. Rates of CO emission correlated with rates of photosynthesis, indicating that cyanobacteria, diatoms, or both, produced this CO. The H_2 emission rates were greatest at night under anoxic conditions, consistent with its production by fermentation. Nitrogenase activity in these mats was only a minor source of this H_2 (Hoehler et al., 2004). Rates of methane emission were unchanged during the diel cycle, indicating that the CH_4 source was located beneath the zone in the mats that was oxygenated during the day.

Visscher et al. (2003) examined the production and consumption of methanethiol (MT) and dimethylsulfide (DMS) in *Microcoleus*-dominated mats from Pond 4, near the dike between Ponds 4 and 5 (Fig. 1). Although dimethlysulfoniopropionate (DMSP) is commonly a chemical precursor of MT and DMS in other environments, it was not observed in these mats. Instead, mat phototrophs produced low-molecular-weight organic compounds that reacted with H_2S produced by microbial sulfate reduction. DMS was readily formed when sulfide or MT was added to filter-sterilized mat extracts in the absence of DMSP (Visscher et al., 2003). Monooxygenase-utilizing microorganisms were the major consumers of DMS in oxygenated mat porewaters. Under anoxic conditions, sulfate-reducing bacteria and methanogens were the major consumers of DMS and MT, respectively.

Some of the MT and DMS escaped into the overlying water column. These volatile organosulfur compounds can indicate high rates of microbial carbon fixation and sulfate reduction in marine hypersaline environments and therefore can be biosignatures of the microbial mat community (Visscher et al., 2003).

Paerl et al. (1993) characterized the production and uptake of dissolved organic matter (DOM) in *Microcoleus*-dominated mats from Pond 5, near the dike between Ponds 5 and 6 (Fig. 1). Whereas D-glucose, acetate, and an L-amino acid mixture were consumed in dark-incubated mats, their consumption rates were significantly enhanced under natural illumination. Rates of light-stimulated DOM uptake were not reduced by the photosystem II inhibitor 3-(3,4 dichlorophenyl)-1,1-dimethyl urea (DCMU), indicating that anoxygenic phototrophs were important consumers. Microautoradiographs revealed that a variety of pigmented and nonpigmented bacteria consumed DOM. Some of these consumers occupied the sheaths and mucilage surrounding the filamentous cyanobacteria, and therefore were associated closely with oxygenic photoautotrophs.

Sulfate-reducing bacteria were also quantitatively important consumers of dissolved organic matter in these mats (Canfield and Des Marais, 1993). In addition, the sulfide they produced sustained diverse phototrophs and chemotrophs. The highest rates of sulfate reduction occurred in the shallowest part of the subtidal *Microcoleus* mat (Fig. 5), close to the photosynthetic source of fresh organic matter (Canfield and Des Marais, 1993).

Although O_2 is an effective inhibitor of bacterial sulfate reduction, the highest sulfate reduction rates were observed within the mat's aerobic zone during the daytime (Canfield and Des Marais, 1991). The oxygenated zone was searched

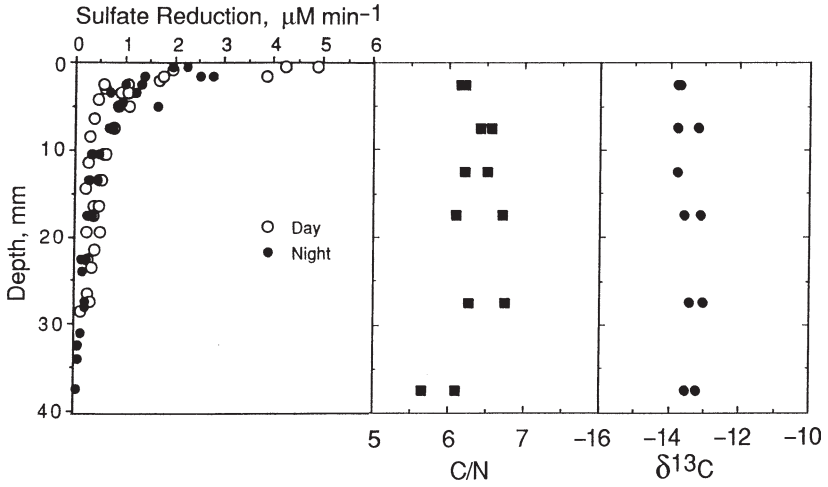

Figure 5. Trends with depth in a *Microcoleus*-dominated mat (Modified from Des Marais et al., 1992): (**a**) Sulfate reduction rates (Canfield and Des Marais, 1993); (**b**) Elemental C to N ratios of total organic carbon; (**c**) Isotopic composition of total organic carbon, $\delta^{13}C = (^{13}C/^{12}Csample)/(^{13}C/^{12}C_{PDB}) - 1)*1000$.

thoroughly with O_2 microelectrodes for anaerobic microenvironments that might have provided refugia for sulfate-reducing bacteria, yet no such microenvironments were found. The factors that attenuated this O_2 inhibition of sulfate reduction are not known. Perhaps, chemically reduced fermentation products adjacent to the cyanobacteria helped to neutralize the toxic effects of oxidants such as O_2.

The composition of organic matter preserved at depth reflected the composition of the fraction of the organics from the surface photosynthetic community that had survived diagenesis, plus those organics that were synthesized at depth, either by surviving photosynthesizers (e.g., Jørgensen and Cohen, 1987) or by anaerobic bacteria. Many photosynthetic carotenoid pigments survived well below the photic zone of these subtidal mats (Palmisano et al., 1989; Ley et al., 2006), attaining depths corresponding to a sediment age greater than 8 years. However, the carotenoid/chlorophyll abundances increased with depth, indicating that Chl *a* had decomposed substantially over a timescale of months. The relative concentrations of the carotenoids generally reflect the relative abundances of their source organisms. For example, the carotenoid myxoxanthophyll is relatively abundant and thus reflects the dominance of cyanobacteria.

Elemental C/N and $^{13}C/^{12}C$ values of total organic matter changed minimally with depth in the mat (Fig. 5; Des Marais et al., 1992). The relatively low C/N values indicated that the organic matter persisting at depth retained abundant nitrogen. These trends are consistent with light microscopic observations indicating that at least some remnants of components (e.g., cyanobacteria and sheath material) that had been abundant in the surface layer of the mat had apparently retained their original morphological and chemical attributes at depths of several centimeters.

5. Genetic and Biomarker Assays of Microbial Populations

The application of modern nucleic acid based molecular methods has revealed the enormous diversity of microorganisms in these *Microcoleus*-dominated mats. In their pioneering study at Guerrero Negro, Ley et al. (2006) characterized small-subunit rRNA genes from community genomic DNA extracted from a mat in Pond 5, near the dike between Ponds 4 and 5 (Fig. 1). When they combined the universal clone libraries from all mat layers, they observed that the Bacteria/Archaea/Eucarya ratio was 57:7:1. They generated 1,586 bacterial 16S rRNA gene sequences from the entire mat; these consisted of 1,336 unique sequences. When Ley et al. created collector's curves for taxa with >90% sequence identity, they found that the coverage of their libraries did not provide a comprehensive estimate of the actual number of unique sequences in the mat. The actual number of sequences was apparently much larger. When Ley et al. employed two quantitative estimators of richness to analyze the distribution of observed sequences, they estimated that >10,000 unique bacterial sequences were present. These assessments indicated that diversity of these mats was very high and most of it had not yet been described.

5.1. BACTERIA

Both Ley et al. (2006) and Green et al. (2008) assessed the diversity and distribution of the bacterial taxa (Table 1) of a *Microcoleus*-dominated mat from Pond 4, near the dike between Ponds 4 and 5 (Fig. 1). Ley et al. (2006) noted several trends in the small-subunit rRNA genes extracted from millimeter-thick layers as a function of depth. The cyanobacterial genes attained their greatest relative abundance in the oxic zone, whereas *Chloroflexi*, *Proteobacteria*, and *Bacteroidetes* maintained high relative abundances throughout the topmost 60 mm of mat. Ley et al. concluded that although cyanobacteria dominate the biomass in the topmost 2 mm of mat, the dominance of *Chloroflexi* beneath 2 mm made them the most abundant bacteria overall. Extending the earlier observations by Nübel et al. (2001) of *Chloroflexi* in these mats, Ley et al. (2006) reported a remarkably high level of diversity of this phylum. Only one fourth of the *Chloroflexi* sequences closely resembled known photosynthetic organisms; other sequences with no known cultured relatives occurred only in surface layers, indicating that additional unrecognized photosynthetic *Chloroflexi* species were present. In addition to cyanobacteria and *Chloroflexi*, Ley et al. identified 28 other previously

Table 1. Comparison of general bacterial 16S rRNA gene clone libraries generated from *Microcoleus*-dominated hypersaline microbial mats. (Adapted from Green et al., 2008.)

	Green et al. (2008)[a, b]		Ley et al. (2006)[b]	
	96-Well plate[c]		Full library (0–5 mm)	
Taxon	**Clones**	**Library (%)**	**Clones**	**Library (%)**
Cyanobacteria + plastids	17	20.2	38	4.0
Proteobacteria	13	15.5 (19.4)	211	22.2 (23.2)
Spirochetes	13	15.5 (19.4)	53	5.6 (5.8)
Bacteroidetes	13	15.5 (19.4)	168	17.7 (18.4)
Chloroflexi	8	9.5 (11.9)	210	22.1 (23.1)
Planctomycetes	6	7.1 (9)	35	3.7 (3.8)
Verrucomicrobia	3	3.6 (4.5)	39	4.1 (4.3)
GN14	3	3.6 (4.5)	2	0.2 (0.2)
Chlorobi	2	2.4 (3)	1	0.1 (0.1)
Deinococcus-Thermus	1	1.2 (1.5)	5	0.5 (0.5)
OD1	1	1.2 (1.5)	5	0.5 (0.5)
Actinobacteria	1	1.2 (1.5)	1	0.1 (0.1)
Other	3	3.6 (4.5)	181	19.1 (19.9)
Total	84		949	

[a]Screening of the full clone library revealed that 94 of 466 clones contained cyanobacterial sequences (20.2%).
[b]Percent abundance values in parentheses represent the abundance in the clone library with cyanobacterial sequences removed.
[c]Of 96 positions on the plate sent for screening, six positions were left empty and six clones did not contain inserts.

described phyla; some of these identifications expanded the known habitats and diversity of several candidate phyla.

Ley et al. (2006) also reported 15 novel candidate phyla, that is, phyla that are known only from their constituent 16S rRNA gene sequences. The distributions of these novel phyla versus depth in the mat provided clues about their physiologies. Ley et al. found nine of these 15 novel phyla at 2–6 mm depth, a zone where both oxygen and H_2S levels were low. One novel (photosynthetic?) taxon was found only near the mat surface and two others were restricted to deeper, permanently anoxic zones.

Ley et al. (2006) compared communities within distinct biogeochemical zones delineated by microelectrode measurements of O_2 and H_2S, using the UniFrac metric analysis (Lozupone and Knight, 2005). They found that three distinct zones delineated by O_2 and H_2S concentration gradients harbored distinct bacterial communities. They proposed that phylogenetic groups apparently share physiological attributes that are manifested as biogeochemical niche preferences at particular depths in the mat. For example, close relatives of known photosynthetic *Chloroflexi* species were abundant in the shallowest mat layers. Close relatives of the sulfate-reducing members of the delta group of proteobacteria were most abundant where rates of sulfate reduction were highest. Members of the phylum *Bacteriodetes*, known to degrade polysaccharides anaerobically (Xu et al., 2003), were abundant throughout the anoxic zone of the mat.

Green et al. (2008) observed that the sequences for *Microcoleus chthonoplastes* (24 of 54 cyanobacterial sequences) and *Oscillatoriales* (22 of 54 sequences) were by far the most diverse and abundant cyanobacteria in mats from Pond 4, near the dike between Ponds 4 and 5 (Fig. 1). They detected *Microcoleus* sequences from the mat surface to a depth of 25 mm. These findings are consistent with earlier observations of Guerrero Negro mats (D'Amelio et al., 1989; Nübel et al., 1999) and with the ability of diverse *Microcoleus* to thrive over a broad range of salinities as evidenced by its global prominence in hypersaline environments (Green et al., 2008). In contrast, Ley et al. (2006) found only one *Microcoleus* sequence. This discrepancy in the reported diversity of *Microcoleus* is still unexplained. However, the preferential degradation of *Microcoleus chthonoplastes* DNA has been observed on multiple freezing and thawings of mat samples prior to DNA extraction (Bebout et al., 2002) and as a consequence of long-term cold storage of microbial mat DNA extracts (Green et al., 2008).

Regarding other cyanobacteria, Green et al. (2008) found only one sequence from the *Halothece*/*Cyanothece*/*Euhalothece* clade and no sequences from the *Lyngbya*/*Phormidium*/*Plectonema* clade.

Observations of other bacterial phyla by Green et al. (2008) and Ley et al. (2006) are broadly consistent (Table 1). *Proteobacteria*, *Spirochaetes*, *Bacteroidetes*, *Chloroflexi*, and Planctomycetes were the most abundant sequences recovered, comprising 63% (Green et al., 2008) and 71% (Ley et al., 2006) of the bacterial sequences.

5.2. SULFATE-REDUCING BACTERIA

Populations of sulfate-reducing bacteria (SRB) varied with depth in *Microcoleus*-dominated mats from Pond 5 (Risatti et al., 1994). *Desulfococcus* was most abundant in the shallowest mat layers, including the photic zone. *Desulfococcus* is apparently a nutritional generalist, consistent with its proximity to diverse organic substrates produced within the photic zone. *Desulfovibrio* spp. became relatively more prominent beneath the photic zone, where sulfate reduction rates declined (Fig. 5). *Desulfovibrio* spp. incompletely oxidizes a relatively limited array of organic substrates, typically lactate and ethanol, to produce acetate. The acetate-oxidizing *Desulfobacter* and *Desulfobacterium* SRB become more dominant at even greater depths. This depth-related trend for SRB is consistent with the fact that acetate is situated further downstream along the anaerobic food chain relative to the substrates utilized by *Desulfovibrio*. The spatial distributions of the SRB appear to be consistent with depth-related trends in the availability of various key organic substrates.

Fike et al. (2008) mapped the distribution of SRB, sulfide abundances, and stable sulfur isotopic compositions at the micron scale. They observed heterogeneities in all three of these parameters at spatial scales ranging from 1 to 400 μm. *Desulfobacteraceae* occurred abundantly in both oxic and anoxic zones as distinct layers, heterogeneously dispersed large aggregates, and single cells. Fike et al. observed a large range in the sulfur isotopic composition of sulfides at the microscale. This range probably reflects variations in factors such as sulfate reduction rates, type of electron donor and carbon source, extent of oxidation of organic substrates, temperature, and species-specific effects (Fike et al., 2008). Future research will extend observations such as these to include other sulfate reducers, sulfur disproportionators, and phototrophic and chemolithotropic sulfide and sulfur oxidizers. All these groups probably occur in these *Microcoleus*-dominated mats.

5.3. ARCHAEA

Jahnke et al. (2008) employed lipid biomarker analyses and molecular phylogenetic methods to characterize the diversity and distribution of Archaea in a *Microcoleus*-dominated mat from Pond 4 near the dike between Ponds 1 and 4 (see Fig. 1). Archaea constituted about 1–4% of the uppermost 100 mm of mat. The relative abundance of Archaea increased with depth. Below 100 mm, archaeal lipids were twice as abundant as bacterial lipids. Archaeol was the most abundant archaeal lipid throughout the mat. Additional archaeal lipids included caldarchaeol, phytane, biphytane, a novel C_{30} isoprenoid (squalane), pentamethylicosane, and crocetane. *Thermoplasmatales* of marine benthic group D dominated the clone libraries of 16S rRNA genes from most depth intervals. Crenarchaeota from marine benthic group B were generally present below 17 mm.

Halobacteriaceae dominated the clone library from the topmost 2 mm of mat and consisted primarily of sequences closely related to *Natronomonas pharaonis*.

Orphan et al. (2008) combined measurements of lipids with analyses of genes for archaeal 16S rRNA and methyl coenzyme M reductase to identify methanogens and determine their spatial distribution within the same mat examined by Jahnke et al. (2008). Laboratory incubations of mat samples with trimethylamine (TMA) yielded CH_4 concentrations that were three orders of magnitude greater than those observed from incubations with H_2/CO_2. These TMA-stimulated enrichments revealed diagnostic archaeal biomarkers and diverse methanogens belonging to *Methanosarcinales*. Key biomarkers included archaeol, sn-2-hydroxyarchaeol polar lipids, and the acyclic isoprenoids 2,6,10,15, 19-pentamethylicosene (PMI), and 2,6,11,15-tetramethylhexa-decane (crocetane). Incubations of samples from near the mat photic zone were dominated by *Methanolobus* spp. and PMI, whereas sediments from beneath the mat (>100 mm depth) yielded principally *Methanococcoides* and hydroxyarcheol.

The factors that caused the populations of methylotrophic methanogens to change with increasing depth in the mat have not yet been determined. The mat microenvironment near the surface experienced substantial biogeochemical fluctuations over the diel cycle. Methylated amines are produced by the breakdown of glycine betaine (Oren, 1990). Methylated sulfides arose from reactions between low molecular weight organic compounds and sulfides in mat porewaters (Visscher et al., 2003; see Section 3). Cultured representatives of *Methanolobus* utilized a broad repertoire of substrates that include methylated sulfur compounds. Orphan et al. (2008) proposed that this versatility might afford *Methanolobus* a competitive advantage over *Methanococcoides*, which has not yet been reported to utilize methylated sulfides (Kendall and Boone, 2006). The deeper sedimentary horizon where *Methanococcoides* became more abundant had relatively lower nutrients and carbon than the shallower zones of the mat. The relative ratio of archaeal to bacterial lipids increased markedly below a depth of 100 mm.

5.4. EUCARYA

Feazel et al. (2008) determined the eukaryotic diversity of these mats by constructing clone libraries of 18S rRNA genes from DNA extracted from each of three depth intervals. They found only 15 species among 890 clones analyzed, indicating a remarkably low diversity. With the exception of one cluster, all the sequences were closely related either to sequences of organisms described previously or to sequences previously encountered in the environment. One cluster had no kingdom-level affiliation with known sequences. This finding could not be attributed to "fast clock" artifacts; therefore, it revealed a deeply branching eukaryotic lineage. Nematodes constituted more than half of the total sequences and were dominated by members of the *Monhysteridae* and *Rhabdolaimidae* families.

Other sequences represented an insect, a crustacean, and a stramenopile. Feazel et al. (2008) proposed that the diversity of eukaryotes was low because their metabolic repertoire was limited to fermentation in the absence of bacterial symbionts, and they had adapted to survive under persistently anaerobic, sulfidic conditions. Still, these eukarya probably played important roles that included physical disruption, transport of nutrients, and selective grazing.

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7. References

Albert, D.B., Hoehler, T.M., Des Marais, D.J. and Bebout, B.M. (2000) Fluxes of organic acids from hypersaline microbial mats (Abstract). *Astrobiology Science Conference 2000:* 317. NASA Ames Research Center, Moffett Field, CA.

Bebout, B.M., Paerl, H.W., Bauer, J.E., Canfield, D.E. and Des Marais, D.J. (1994) Nitrogen cycling in microbial mat communities: the quantitative importance of N-fixation and other sources of N for primary productivity, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 265–271.

Bebout, B.M., Carpenter, S.P., Des Marais, D.J., Discipulo, M., Embaye, T., Garcia-Pichel, F., Hoehler, T.M., Hogan, M., Jahnke, L.L., Keller, R.M., Miller, S.R., Prufert-Bebout, L.E., Raleigh, C., Rothrock, M. and Turk, K. (2002) Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating Earth's present and past field environments. Astrobiology **2**: 383–402.

Boudreau, B.P. and Guinasso, N.L. (1982) The influence of a diffusive sublayer on accretion, dissolution, and diagenesis at the seafloor, In: K.A. Fanning and F.T. Manheim (eds.) *The Dynamic Environment of the Ocean Floor*. D.C. Heath & Co., Lexington, pp. 115–145.

Canfield, D.E. and Des Marais, D.J. (1991) Aerobic sulfate reduction in microbial mats. Science **251**: 1471–1473.

Canfield, D.E. and Des Marais, D.J. (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim. Cosmochim. Acta **57**: 3971–3984.

Canfield, D.E. and Des Marais, D.J. (1994) Cycling of carbon, oxygen, sulfur and nutrients in a microbial mat, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 255–263.

D'Amelio, E.D., Cohen, Y. and Des Marais, D.J. (1989) Comparative functional ultrastructure of two hypersaline submerged cyanobacterial mats: Guerrero Negro, Baja California Sur, Mexico, and Solar Lake, Sinai, Egypt, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats, Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, pp. 97–113.

Des Marais, D.J. and Canfield, D.E. (1994) The carbon isotope biogeochemistry of microbial mats, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 289–298.

Des Marais, D.J., Cohen, Y., Nguyen, H., Cheatham, M., Cheatham, T. and Munoz, E. (1989) Carbon isotopic trends in the hypersaline ponds and microbial mats at Guerrero Negro, Baja California Sur, Mexico: implications for Precambrian stromatolites, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats, Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, pp. 191–205.

Des Marais, D.J., D'Amelio, E., Farmer, J.D., Jørgensen, B.B., Palmisano, A.C. and Pierson, B.K. (1992) Case study of a modern microbial mat-building community: the submerged cyanobacterial mats of Guerrero Negro, Baja California Sur, Mexico, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere: A Multidisciplinary Study.* Cambridge University Press, New York, pp. 325–334.

Des Marais, D.J., Albert, D., Bebout, B., Discipulo, M., Hoehler, T. and Turk, K. (2002) Biogeochemical contrasts between subtidal and intertidal-to-supratidal hypersaline microbial mats (Abstract). *Astrobiology Science Conference 2002*: 188. NASA Ames Research Center, Moffett Field, CA.

Des Marais, D.J., Bebout, B.M., Carpenter, S., Discipulo, M. and Turk, K. (2003) Carbon and oxygen budgets of hypersaline cyanobacterial mats: effects of diel cycle and temperature (Abstract). American Society of Limnology and Oceanography, 2003 Annual Meeting, Salt Lake City, UT.

Feazel, L.M., Spear, J.R., Berger, A.B., Kirk Harris, J., Frank, D.N., Ley, R.E. and Pace, N.R. (2008) Eucaryotic diversity in a hypersaline microbial mat. Appl. Environ. Microbiol. **74**: 329–332.

Fenchel, T. and Finlay, B.J. (1995) *Ecology and Evolution in Anoxic Worlds.* Oxford University Press, Oxford.

Fike, D.A., Gammon, C.L., Ziebis, W. and Orphan, V.J. (2008) Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. ISME J. **2**: 749–759.

Green, S.J., Blackford, C., Bucki, P., Jahnke, L.L. and Prufert-Bebout, L. (2008) A salinity and sulfate manipulation of hypersaline microbial mats reveals stasis in the cyanobacterial community structure. ISME J. **2**: 1–14.

Hoehler, T.M., Bebout, B.M. and Des Marais, D.J. (2001) The role of microbial mats in the production of reduced gases on the early Earth. Nature **412**: 324–327.

Hoehler, T.M., Albert, D.B., Bebout, B.M., Turk, K.A. and Des Marais, D.J. (2004) Quantitative relationships between photosynthetic, nitrogen-fixing and fermentative H_2 metabolism in a photosynthetic microbial mat (Abstract). Int. J. Astrobiol., Suppl. **1**: 31.

Jahnke, L.L., Orphan, V.J., Embaye, T., Turk, K.A., Kubo, M., Summons, R.E. and Des Marais, D.J. (2008) Lipid biomarkers and phylogenetic analyses to reveal archaeal biodiversity and distribution in hypersaline microbial mat and underlying sediment. Geobiology **6**: 394–410.

Javor, B.J. (1983) Planktonic standing crop and nutrients in a saltern ecosystem. Limnol. Oceanogr. **28**: 153–159.

Jørgensen, B.B. and Cohen, Y. (1987) Photosynthetic potential and light-dependent oxygen consumption in a benthic cyanobacterial mat. Appl. Environ. Microbiol. **54**: 176–182.

Jørgensen, B.B. and Des Marais, D.J. (1986) Competition for sulfide among colorless and purple sulfur bacteria in cyanobacterial mats. FEMS Microbiol. Ecol. **38**: 179–186.

Jørgensen, B.B. and Des Marais, D.J. (1988) Optical properties of benthic photosynthetic communities: fiber optic studies of cyanobacterial mats. Limnol. Oceanogr. **33**: 99–113.

Jørgensen, B.B. and Des Marais, D.J. (1990) The diffusive boundary layer of sediments: oxygen microgradients over a microbial mat. Limnol. Oceanogr. **35**: 1343–1355.

Jørgensen, B.B., Cohen, Y. and Des Marais, D.J. (1987) Photosynthetic action spectra and adaptation to spectral light distribution in a benthic cyanobacterial mat. Appl. Environ. Microbiol. **53**: 879–886.

Kelley, C.A., Prufert-Bebout, L.E. and Bebout, B.M. (2006) Changes in carbon cycling ascertained by stable isotopic analyses in a hypersaline microbial mat. J. Geophys. Res. **111**: G04012, doi: 10.1029/2006JG000212.

Kendall, M.M. and Boone, D.R. (2006) The order methanosarcinales, In: M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt (eds.) *The Prokaryotes. Vol. 3: Archaea, Bacteria: Firmicutes, Actinomyces.* Springer, New York, pp. 244–256.

Ley, R.E., Kirk Harris, J., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

Lozupone, C. and Knight, R. (2005) Unifrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. **71**: 8228–8235.

Nübel U., Garcia-Pichel, F., Kühl, M. and Muyzer, G. (1999). Quantifying microbial diversity: morphotypes, 15S rRNA genes, and carotenoids of oxygenic photographs in microbial mats. Appl. Environ. Microbiol. **65**: 422–430.

Nübel, U., Garcia-Pichel, F., Clavero, E. and Muyzer, G. (2000) Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ. Microbiol. **2**: 217–226.

Nübel, U., Bateson, M.M., Madigan, M.T., Kühl, M. and Ward, D.M. (2001) Diversity and distribution in hypersaline microbial mats of bacteria related to *Chloroflexus* spp. Appl. Environ. Microbiol. **67**: 4365–4371.

Oren, A. (1990) Formation and breakdown of glycine betaine and trimethylamine in hypersaline environments. Antonie van Leeuwenhoek **58**: 291–298.

Orphan, V.J., Jahnke, L.L., Embaye, T., Turk, K.A., Pernthaler, A., Summons, R.E. and Des Marais, D.J. (2008) Characterization and spatial distribution of methanogens and methanogenic biosignatures in hypersaline microbial mats of Baja California. Geobiology **6**: 376–393.

Palmisano, A.C., Cronin, S.E., D'Amelio, E.D., Munoz, E. and Des Marais, D.J. (1989) Distribution and survival of lipophilic pigments in a laminated microbial mat community near Guerrero Negro, Mexico, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats, Physiological Ecology of Benthic Microbial Communities*. American Society for Microbiology, Washington, DC, pp. 138–152.

Paerl, H.W., Bebout, B.M., Joye, S.B. and Des Marais, D.J. (1993) Microscale characterization of dissolved organic matter production and uptake in marine microbial mat communities. Limnol. Oceanogr. **38**: 1150–1161.

Paerl, H.W., Bebout, B.M., Currin, C.A., Fitzpatrick, M.W. and Pinckney, J.L. (1994) Nitrogen fixation dynamics in microbial mats, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 325–337.

Risatti, J.B., Capman, W.C. and Stahl, D.A. (1994) Community structure of a microbial mat: the phylogenetic dimension. Proc. Natl. Acad. Sci. USA **91**: 10173–10177.

van Gemerden, H. (1993). Microbial mats: a joint venture. Mar. Geol. **113**: 3–25.

Visscher, P.T., Baumgartner, L.K., Buckley, D.H., Rogers, D.R., Hogan, M., Raleigh, C.R., Turk, K. and Des Marais, D.J. (2003) Dimethyl sulfide and methanethiol formation in microbial mats: potential pathways for biogenic signatures. Environ. Microbiol. **5**: 296–308.

Xu, J., Bjursell, M.K., Himrod, J., Deng, S., Carmichael, L.K., Chiang, H.C., Hooper, L.V. and Gordon, J.I. (2003) A genomic view of the human-*Bacteriodes thetaiotaomicron* symbiosis. Science **299**: 2074–2076.

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ENVIRONMENTAL DYNAMICS, COMMUNITY STRUCTURE AND FUNCTION IN A HYPERSALINE MICROBIAL MAT

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1. Introduction

Microbial communities are frequently organized as aggregates, and laminated layers, including biofilms or mats (Paerl and Kuparinen, 2002; Simon et al., 2002; Stal, 2000; Stal and Caumette, 1994). These structures provide a three-dimensional habitat in which metabolically diverse microbial populations closely coexist and interact. Their activities create steep biogeochemical (O_2, pH, redox) gradients and provide a suite of microhabitats in which individual populations can thrive. This allows for beneficial exchange of metabolites and nutrients with other populations that may have different ambient environmental growth requirements and tolerances. By coexisting as consortia of mutually beneficial populations, where "one organism's trash is another's treasure," microbial communities are ensured survival and reproductive success even when facing extreme conditions, such as nutrient deprivation, temperature and irradiance extremes, and periodic desiccation (Paerl and Pinckney, 1996; Paerl et al., 2000). If the latter condition persists, it may result in hypersalinity, where concentrations of salts and other chemical compounds can exceed those found in normal seawater by several-fold.

Hypersaline environments can be found in marine and inland waters, springs, and soils; they are most common in regions where seasonal and annual evaporation rates exceed freshwater inputs. In both the marine and inland situation, the process of desiccation leads to the concentration of major and minor ions in the process of salinization (Oren, 2000). In some habitats (i.e., tropical lagoons, Arctic and Antarctic lakes and streams), this process is controlled by natural events such as droughts, storms, and climate change, including global warming, but in other habitats, salinization can result from anthropogenic changes such as channelization and draining of soils, use of water bodies for irrigation, drinking water, and industrial purposes (Kaushal et al., 2005; Nielsen et al., 2003).

Under hypersaline conditions, microbial metabolic activities, including photosynthesis, respiration, nutrient transformations, and growth are affected and most often reduced, relative to lower salinity conditions (Javor, 1989; Oren, 1993; Pinckney et al., 1995a; Yannarell and Paerl, 2007). Extremely high salinity

(hypersalinity: >45 parts per thousand or percentage) can reduce and halt activity and growth, although the microorganisms affected do not necessarily die, but may enter an inactive state or stasis (Oren, 2000; Potts, 1999, 2001). Microbial communities often form aggregates, biofilm, or mat communities in response to environmentally stressful conditions such as hypersalinity. The "glue" that holds aggregates, biofilms, and mats together is mucoid material excreted by participating microbial populations (Decho et al., 2005; Paerl and Kuparinen, 2002). The production of extracellular polymeric materials (e.g., extracellular polysaccharides [EPS], transparent exopolymer particles [TEP]) is a common microbial response to environmental changes and stress (Caumette et al., 1994; Engel et al., 2004; Oren, 1993, 2000). These extracellular mucilageous materials typically retard diffusion of gases (e.g., O_2, H_2S), other dissolved substances, including diverse ions and even water (Decho et al., 2005). Microbial populations are also capable of producing osmotically active compounds that may be instrumental in protecting participating populations against hypersaline conditions (Margesin and Schinner, 2001; Oren, 2000; Potts, 1999). When situated in mats, microbial populations are protected from hypersalinity by manipulation of diffusion through excretion and accumulation of mucilageous materials and by osmolite production (Caumette et al., 1994; Paerl and Kuparinen, 2002; Paerl et al., 2000).

In this chapter, we will explore the structural and functional organization of marine microbial mats with special emphasis on their adaptation to hypersalinity and associated climatic extremes. Although marine mats also form in deep-sea habitats such as methane seeps, we will limit our discussion here to mats where photoautotrophy is the dominant source of fixed carbon. We will examine the strategies and benefits embodied in maintaining high microbial diversity that enables mats to survive and thrive as metabolically independent microecosystems in a biosphere experiencing both episodic and longer-term chronic environmental changes. Relevant questions include: How can mats serve as model ecosystems that help us elucidate "life on the edge" and potentially, extraterrestrial lifestyles? What can mats teach us with regard to fundamental aspects of microbial – and ultimately higher – levels of biotic organization?

2. Microbial Mats in Hypersaline Systems: Their Structure and Function

A common characteristic of hypersaline systems, such as lagoons, evaporative lakes, and salterns, is the presence and persistence of benthic microbial mats, dominated by cyanobacteria and diatoms as phototrophs and including a wide range of heterotrophic and chemoautotrophic bacteria (Fig. 1). In these shallow hypersaline ecosystems, a large proportion of primary and secondary production is confined to mats. Despite the extreme conditions in these systems, mats exhibit remarkable microbial diversity. They also contain a range of protistan, metazoan, and invertebrate consumers (Stal, 2000; Stal and Caumette, 1994). In effect, they function as self-contained microecosystems, in which carbon, nitrogen, phosphorus,

Figure 1. Hypersaline lake systems on San Salvador Island, Bahamas. *Upper left*: view of Salt Pond, showing the microbial mats on the lake sediments. *Upper middle*: view of Storrs Lake, showing microbial mats on lake sediments. *Upper right*: view of southern arm of Storrs Lake, which at the time of this photograph was nearly dried up. *Lower left*: mound-like microbial mats in Storrs Lake. *Lower middle*: view of laminated microbial mats in Salt Pond. The mats have been dissected to show the laminations, including layers of carbonate sand that were interspersed with cyanobacterial and photosynthetic bacterial layers. *Lower right*: microscopic view of the Salt Pond mat matrix, showing filamentous cyanobacteria, photosynthetic purple bacteria, and mucilaginous extracellular polysaccharide material (EPS).

sulfur, and metals may be completely cycled between oxidized and reduced forms (Paerl et al., 2000; Stal, 2000). Complete nutrient cycling, in addition to ensuring biologically available forms of nutrients, also provides readily available sources of reductants and oxidants, and thus support the energy needs of a wide variety of microbes. Microbial mats evolved at least 2 billion years ago, during the evolution of the Earth's atmosphere and early biosphere (Knoll, 2003), marked by the transition from anoxic to oxic conditions.

There are numerous remnants and reminders of Precambian life in mat microbial communities, including the presence of anoxic phototrophs, oxygen-sensitive chemolithotrophs and heterotrophs, which tend to make up the subsurface anoxic layers of mats. In many respects, mats have created conditions for these environmentally restricted microorganisms to maintain a presence in protective microzones in the modern-day oxic biosphere (Paerl et al., 2000).

Figure 2. Examples of stromatolitic mat systems in hypersaline waters. *Left frame*; Hamelin Pool, Shark Bay, Western Australia. *Right frame*; Highbourne Key, Exumas, Bahamas.

Perhaps, the most striking example of ancient and contemporary microbial mat life forms in extreme environments are lithifying laminated mat systems, or stromatolites, that are preserved in the geological record and can still be found in intertidal and subtidal tropical and subtropical coastal marine environments (Reid et al., 2000; Riding et al., 1991) (Fig. 2). Stromatolitic mat communities are most common in full salinity to hypersaline waters, including beaches, lagoons, and intertidal pools (Bahamas, Shark Bay, etc.). The "working end" of these microecosystems operates very similar to non-lithifying mats, that is, primary production is dominated by cyanobacteria and diatoms, strong vertical biogeochemical gradients exist along which metabolically diverse and interactive prokaryotes, eukaryotic grazers, and invertebrates are oriented in their respective desirable microenvironments (Stal, 2000). Molecular phylogenetic studies indicate remarkable microbial diversity in both lithifying and non-lithifying mats (Olson et al., 1999; Papineau et al., 2005; Steppe and Paerl, 2002; Steppe et al., 2001; Taton et al., 2003; Yannarell et al., 2006). Collectively, microbial mats are vast repositories of genetic diversity (Ley et al., 2006), which is not surprising, given their long evolutionary history. From geochemical and climatic perspectives, these communities "have seen it all," and as such they should be well adapted to environmental extremes, including periodic hypersalinity.

3. Who Are the Players? Microbial Community Structure and Function in Production and Nutrient Cycling

All three domains of life are represented in hypersaline mats. Bacteria are the most diverse and the most important in terms of biomass. Over 42 bacterial phyla have been identified from 16S rRNA clone libraries from a single mat (Ley et al., 2006). Eukaryote diversity in mats is considerably lower, with nematodes and diatoms among the most commonly documented groups (Feazel et al., 2008; Nübel et al., 1999). Archaeal diversity and cell numbers are also low in comparison to bacteria (Sørensen et al., 2005), with haloalkaliphilic groups such as *Natronomonas pharaonis*

and various marine benthic Crenarchaeota documented (Jahnke et al., 2008). In the face of this diversity, it is convenient to consider mat microorganisms in terms of a few key functional groups, whose activities structure the important gradients in mats and form the bases for microbial interactions. There are broad similarities in the composition of these functional groups in saline and hypersaline mat systems from around the globe.

Photoautotrophs play a fundamental role as suppliers of oxygen and organic carbon, and cyanobacteria are generally the most important phototrophs in terms of biomass (Nübel et al., 2000; Sørensen et al., 2005). The phylogenetic similarity of cyanobacteria from geographically distant hypersaline mats (Green et al., 2008; Yannarell et al., 2006) points to the existence of widely distributed clades of mat-adapted organisms. The most conspicuous mat cyanobacteria are non-heterocystous filamentous forms, although a number of coccoid forms have also been documented (Figs. 3 and 4). Commonly reported taxa include: *Microcoleus chthonoplastes*, *Oscillatoria*, *Leptolyngbya*, *Lyngbya*, *Halospirulina*, *Calothrix*, *Microcystis*, *Chroococcus*, *Euhalothece*, *Gloeocapsa*, *Synechocystis*, and *Pleurocapsa* (Fourçans et al., 2004; Nübel et al., 1999, 2000; Vincent et al., 1993). While eukaryotes generally constitute a low proportion of photosynthetic cells in mats at salinities higher than seawater (Nübel et al., 2000), diatoms of the genera *Nitzschia*, *Brachysira*, *Navicula*, *Amphora*, *Mastogloia*, *Entomoneis*, and *Gyrosigma* have been reported in mats, as have green algae of the genus *Dunaliella* (Nübel et al., 1999). The balance between diatoms and cyanobacteria can be influenced by the availability of nitrogen, which favors diatoms, and phosphorus, which favors cyanobacteria (Camacho and de Wit, 2003). However, cyanobacteria are by far the dominant oxygenic phototrophs in hypersaline mats.

The maximum biomass of cyanobacteria occurs in the upper few millimeters of mats (Ley et al., 2006; Sørensen et al., 2005), and this layer is therefore the center of production of oxygen and new organic matter. This position at the top of the mat can sometimes expose cyanobacteria to excessive levels of UV radia-

Figure 3. Cyanobacterial mats in hypersaline systems. Shown are lithifying (*left*) and soft, non-lithifying (*right*) mat types found in hypersaline lakes of San Salvador Island, the Bahamas.

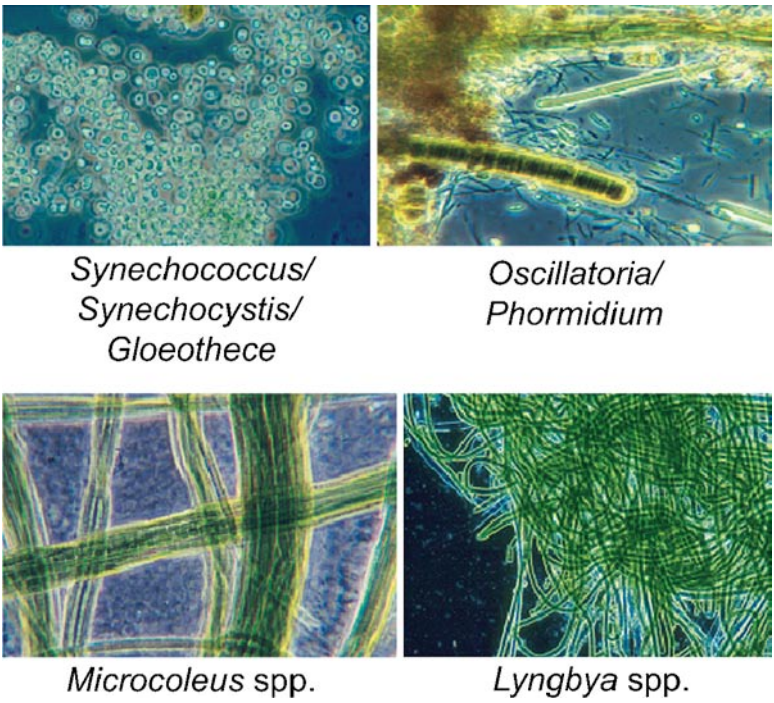

Figure 4. Photomicrographs of dominant cyanobacterial mat-building genera.

tion and visible light, and mat cyanobacteria employ a range of strategies to avoid photoinhibition. These include the production of protective compounds, such as accessory pigments or extracellular polymeric secretions (Decho et al., 2005; Hill et al., 1994). Some cyanobacteria, such as *Oscillatoria* cf. *laetivirens* and *Spriulina* cf. *subsalsa*, have been shown to migrate downward in response to UV and visible intensities that cause photoinhibition (Garcia-Pichel et al., 1994; Kruschel and Castenholz, 1998; Nadeau et al., 1999). These wavelengths can penetrate for some distance into the mat, and they can serve as positioning cues that allow migrating cyanobacteria to optimize their orientation in the light field. Antarctic *Oscillatoria* have been shown to be more sensitive to these light intensities than their relatives from middle latitudes (Nadeau et al., 1999), and some Antarctic mats have been observed to show the "upside-down" structure, with cyanobacterial chlorophyll located at depth, beneath mat layers with high concentrations of light-screening pigments (Vincent et al., 1993). The importance of cyanobacteria for oxygen and organic matter production means that their vertical distribution in mats has important consequences for members of other functional groups.

Anoxygenic phototrophs also play a role in carbon sequestration in mats, and they may be responsible for as much as 25% of the fixed carbon in some systems (Pinckney and Paerl, 1997). The purple sulfur bacteria (*Gammaproteobacteria* division) of the family *Chromatiaceae*, such as *Chromatium* sp., *Thiocapsa*

pfennigii, and *Thiocapsa roseopersicina* are commonly reported from mats (Caumette et al., 1994; Jonkers et al., 1998; Visscher et al., 1992). These organisms have been reported to form distinctive red layers in mats (Caumette et al., 1994; Visscher et al., 1992), often just below the cyanobacteria-dominated upper layer. Mats also contain green sulfur bacteria (phylum *Chloroflexi*) of the family *Chloroflexaceae*, who can perform anoxygenic photosynthesis when stimulated with near-infrared light (Bachar et al., 2007, 2008; Pierson et al., 1994). *Chloroflexus*-like organisms dominate the biomass of many microbial mats (Ley et al., 2006; Pierson et al., 1994). These organisms can also grow aerobically on organic substrates, and they may be responsible for the bulk of oxygen consumption in the lower portions of mat oxic zones (Bachar et al., 2008).

Mat anoxygenic phototrophs produce sulfate through the oxidation of sulfide. The complementary part of the sulfur cycle is the generation of sulfide through the oxidation of organic carbon coupled to sulfate reduction. The diverse organisms responsible for this process are collectively known as "sulfate reducing bacteria" (SRB). Members of the families *Desulfovibrionaceae* and *Desulfobacteraceae* (*Deltaproteobacteria* division) are the most commonly documented SRB in mats (Fike et al., 2008; Fourçans et al., 2008; Minz et al., 1999a). Although sulfate reduction is an anaerobic process, the presence of SRB in the oxic portions of mats is well documented (Fike et al., 2008; Krekeler et al., 1997; Minz et al., 1999b; Teske et al., 1998), and sulfate reduction rates in the oxygenated parts of mats can sometimes equal or exceed those in the anaerobic zones (Teske et al., 1998). The distributions of SRB and *Chloroflexaceae* in the oxic parts of a mat have been observed to be related (Bachar et al., 2008), suggesting that these organisms associate because of their complementary metabolisms. However, different SRB populations are differentially distributed in mats with regard to the oxycline (Fike et al., 2008; Minz et al., 1999a), and different populations have been observed to migrate up or down with respect to advancing oxygen fronts (Fourçans et al., 2008). The variety of distributions and responses to oxygen exhibited by mat SRB suggests that mats harbor a diversity of different SRB adapted to a number of different niches. For example, the species *Desulfovibrio oxyclinae*, first described in the oxic part of a hypersaline mat, can grow aerobically on a variety of substrates (Krekeler et al., 1997). In addition to their importance for the sulfur cycle, SRB have also been associated with several other important mat processes. Their activities may account for 7–8% of CO_2 for photosynthetic demand (Teske et al., 1998). In calcifying mats, their activities may be important for the incorporation of sulfur and metals into carbonates (Braissant et al., 2007). Finally, they have been implicated in the fixation of atmospheric nitrogen (Steppe and Paerl, 2002; Yannarell et al., 2006), and they may be responsible for significant portions of nighttime nitrogen fixation (Steppe and Paerl, 2002).

Many marine and hypersaline mats are nitrogen-depleted, and photosynthesis in these systems relies on inputs of nitrogen through the activity of diazotrophs, who fix atmospheric nitrogen into biologically available ammonium. Although cyanobacteria in mats are generally non-heterocystous, many of them can fix nitrogen, and they are generally regarded as the most numerous nitrogen fixers in mats (Omoregie et al., 2004a; Yannarell et al., 2006). Surveys of the *nifH* gene, which is necessary for nitrogen fixation, indicate that the most common nitrogen-fixing

cyanobacteria in mats are the filamentous *Oscillatoriales*, *Plectonema*, and *Phormidium*, and the unicellular *Halothece*, *Myxosarcina*, and *Synechocystis* (Omoregie et al., 2004a; Yannarell et al., 2006). The incompatibility of oxygen-producing photosynthesis and oxygen-inhibited nitrogen fixation generally leads to a temporal separation of these activities, with photosynthesis occurring during the day, and maximal nitrogen fixation rates occurring at night (Bebout et al., 1993; Omoregie et al., 2004a, b; Paerl et al., 1993a, 1996; Steppe and Paerl, 2005; Steppe et al., 2001). However, heterotrophic bacteria and methanogenic Archaea have also been found to fix nitrogen in mats, and sometimes these organisms appear to be the most active diazotrophs (Bauer et al., 2008; de Wit et al., 2005; Olson et al., 1999; Steppe and Paerl, 2002). In addition to SRB such as *Desulfovibrio*, these organisms include *Spirochaetes* and *Proteobacteria* related to *Klebsiella*, *Azotobacter*, and *Azospirillum* (Omoregie et al., 2004a, b; Steppe et al., 1996; Yannarell et al., 2006). Systems where non-cyanobacterial diazotrophs are dominant can exhibit the reverse of the diel pattern described above; that is, nitrogen fixation rates are highest during the day and/or in the light (Bauer et al., 2008; de Wit et al., 2005). In this case, diazotrophs may be directly utilizing photosynthate produced by cyanobacteria to fuel the energy-demanding nitrogen-fixing reaction. This can lead to intimate relationships between members of these functional groups. For example, the filamentous mat cyanobacterium, *Microcoleus* spp., which lacks the ability to fix nitrogen, has been shown to harbor epiphytic bacteria that express the *nifH* gene, which encodes for a nitrogenase protein component (Steppe et al., 1996).

4. Mats as Self-Sustaining Microbial Habitats/Ecosystems

The activities of the various mat functional groups, and the interactions between these groups, establish the important gradients in microbial mats. These gradients, in turn, create the niches available to mat organisms and structure the different populations that live in the mat. Oxygenic photosynthesis plays a central role in structuring mats, as the vertical distribution of organisms in the mat is largely controlled by the supply of oxygen and the availability of oxidizable substrates (Epping et al., 1999). In general, the concentrations of total carbon, organic carbon, total nitrogen, hydrolysable amino acids, carbohydrates, extracellular polymeric substances, and cyanobacteria-derived hydrocarbons are highest at the top of mats (Wieland et al., 2008), where cyanobacteria are concentrated and oxygen production occurs. Cyanobacteria-derived hydrocarbons decrease at depths below 1 mm, where amino acids and carbohydrates are the largest carbon pools (Wieland et al., 2008). This highlights the importance of cyanobacterial primary production in supporting the activities of other mat organisms. The high production of organic carbon at the surface of mats may alleviate competition between aerobic hetetrotrophs and anaerobic sulfate-reducing bacteria (Jonkers and Abed, 2003), and this may be one of the factors allowing populations of SRB to persist in mat oxic zones. The activities of these organisms are linked by the exchange

of substrates, and the populations in different parts of the mat have adapted to utilize the carbon sources most readily available in the neighborhood of the mat. For example, sulfate reduction in the top of mats responds to light stimulation of photosynthesis, and it can be stimulated by the addition of photosynthate glycolate; however, sulfate reduction in the anoxic layers of mats is stimulated by fermentation products (Fründ and Cohen, 1992). Similarly, respiration in the oxic and anoxic zones is stimulated by glycolate or acetate, respectively (Grötzschel et al., 2002). Respiration and oxygen production are closely linked in mats (Wieland and Kühl, 2000b), and experimental additions of a variety of substrates have been shown to rapidly stimulate both oxygen consumption and gross photosynthesis (Grötzschel et al., 2002; Ludwig et al., 2006). High respiration rates produce excess inorganic carbon at rates faster than or equal to the rate of CO_2 diffusion into the mat (Canfield and Des Marais, 1993), and this can stimulate photosynthesis (Grötzschel et al., 2002). Light stimulation of photosynthesis leads to higher uptake rates of dissolved organic matter (Yannarell and Paerl, 2007), and some of the organisms responsible for light-stimulated organic matter uptake are localized to the sheaths and extracellular mucilage of filamentous cyanobacteria (Paerl et al., 1993b). The proximity of organisms with complementary metabolisms, and the exchange of substrates across microscale gradients can facilitate the establishment and maintenance of tightly coupled, "consortial" interactions (Paerl and Pinckney, 1996; Paerl et al., 2000).

5. Where Do We Find Mats in Hypersaline Environments?

Because mat microorganisms can sequester inorganic carbon and nitrogen, and because of the presence of complementary metabolisms that allow for efficient elemental cycling, mats have the potential to establish almost anywhere there is sufficient light and minimal nutrient availability. The fossil record indicates that mats were once widespread and globally distributed (Schopf, 2000), and today they can be found on all seven continents, from the tropics to the poles. Apart from water availability, a major factor limiting the geographic distribution of modern microbial mats is the presence of multicellular organisms, which can outcompete mats for light (macroalgae and submersed rooted plants) or disturb their structure through bioturbation (invertebrates). For this reason, modern microbial mats tend to be located in "extreme" environments that exclude the presence of most plants and animals. Thus, while mats can be found all over the world, they are particularly conspicuous in environments that show extremes in salinity, temperature, and/or pressure. Many of the most well-studied cyanobacterial mats are located in hypersaline and tidal lagoons, mud and salt flats, salterns, or deserts. A partial list includes:

- Guerrero Negro, Baha California (Ley et al., 2006; Omoregie et al., 2004a, b; Orphan et al., 2008)
- Tomales Bay, California (Olson et al., 1999; Steppe et al., 1996)

- North Carolina (Paerl et al., 1991, 1993a, 1996; Steppe and Paerl, 2002)
- Salins-de-Giraud, Camargue, SE France (Caumette et al., 1994; Fourçans et al., 2008; Wieland and Kühl, 2006)
- Lake Chiprana, NE Spain (Bachar et al., 2007; Camacho and de Wit, 2003; Jonkers et al., 2005, 2003; Polerecky et al., 2007)
- Bahamas (Paerl et al., 2001, 2003; Pinckney et al., 1995a, b; Riding et al., 1991; Steppe et al., 2001; Yannarell and Paerl, 2007; Yannarell et al., 2006, 2007)
- Solar Lake, Sinai Penninsula (Conrad et al., 1995; Sørensen et al., 2004; 2005; Teske et al., 1998; Wieland and Kühl, 2000a, b)
- Australia (Riding et al., 1991; Wood et al., 1991) also Shark Bay (Papineau et al., 2005; Riding et al., 1991)
- Antarctica (Jungblut et al., 2005; Nadeau et al., 1999; Vincent et al., 1993)

The adaptations allowing mat microorganisms to thrive under saline and hypersaline conditions are generally useful in many different arid environments, regardless of latitude. The organisms and structures found in cyanobacterial mats appear to have much in common with endolithic communities and those found in desert soil crusts, and organisms from hypersaline mats around the world appear to be more closely related to each other than to organisms from less arid environments (Green et al., 2008; Yannarell et al., 2006). Some of these mat organisms may have widespread or even global distribution.

6. Liquid Water: The Ultimate Limiting Factor

Adaptations to saline and hypersaline conditions are principally adaptations to water stress. While these adaptations allow mats to persist in a number of arid environments, from the tropics to the polar regions, the availability of water is still the ultimate limiting factor that controls the viability and activity of mat microorganisms. This is not to say that it is the only factor controlling microbial activity. For example, temperature and irradiance can be important drivers of seasonal (Joye and Paerl, 1994; Steppe and Paerl, 2005; Steppe et al., 2001), and diel (Epping and Kühl, 2000; Kühl et al., 1996; Wieland and Kühl, 2000a, b) activities. However, microbial activity is related to these drivers only within a range of permissive salinities, above which activity rates are strongly (negatively) correlated with salinity (Wieland and Kühl, 2006; Yannarell et al., 2007). The precise point at which salinity becomes inhibiting depends upon the prevailing conditions to which any particular mat community is exposed, with mats from higher salinity environments tending to respond to higher salinity values (Abed et al., 2007; Pinckney et al., 1995a; Pinckney and Paerl, 1997; Sokolov and Trotsenko, 1995). However, many mats from arid environments are exposed periodically or chronically to inhibiting salinities over the course of the year, making water stress a primary challenge for mat microorganisms.

Prolonged exposure or an increasing frequency of hypersaline conditions can exclude sensitive organisms from the mat system, and thereby influence the community composition of important functional groups. While hypersaline conditions

reduce the species diversity of all major mat groups (Casillas-Martinez et al., 2005), there is evidence that this trend is weaker for cyanobacteria (Green et al., 2008; Rothrock and Garcia-Pichel, 2005; Yannarell et al., 2006); although, see Abed et al. (2007) for a counter-example. Cyanobacteria have a variety of physiological adaptations that allow them to persist for a prolonged period under water-stressed conditions (Potts, 1996). They are particularly resistant to desiccation, when high temperature, irradiation, and oxidative stress are superimposed upon that of salinity. Among the known responses of cyanobacteria to desiccation are the production of compatible solutes (e.g., trehalose, sucrose, glycine betaine, and dimethylsulfoniopropionate), production of UV-absorbing pigments, excretion, and modification of extracellular polymeric substances, expression of superoxide dismutase, and strict gene regulation of cellular and metabolic machinery (Breanne et al., 2000; Hill et al., 1994; Potts, 1994, 1996; Scherer and Potts, 1989).

Anhydrophilic cyanobacteria, diatoms, photosynthetic bacteria, and non-photosynthetic mat microorganisms often produce copious amounts of extracellular polymeric substances (EPS). These substances provide much of the "new" organic matter supporting metabolic activities and structural integrity of mats. EPS is mainly comprised of polysaccharides, but it also includes non-carbohydrate compounds such as pyruvate and succinate, as well as inorganic moieties such as sulfate or phosphate (Decho et al., 2005). In microbial mats, EPS plays an important role as the "glue" for microbial attachment, providing a cohesive matrix in which diverse biogeochemical reactions take place. In lithifying microbial mats EPS is involved in the precipitation of calcium carbonate minerals, generating the calcified laminated layers characteristic of stromatolites (Reid et al., 2000). EPS also mediates diffusive properties of mats (Decho et al., 2005). This, in addition to its important role in mediating mat carbon metabolism and energy flux, appears to be central in protecting the mat from water loss under hypersaline conditions. Combined with the ability of mat microbes to produce a variety of osmotically active compounds, EPS provides a protective matrix, in which resident microbes can conduct key processes supporting growth and maintenance.

Many natural hypersaline systems experience fluctuations in salinity over the course of the day (e.g., tidal systems) or year (e.g., seasonally arid systems), and mats in these areas are good places to study the short- and long-term responses of mat microorganisms to hypersalinity. Salt Pond, a shallow ~3.5 ha lake on the eastern coast of San Salvador Island (the Bahamas; 24° 05' N, 74° 30' W), contains a benthic cyanobacterial mat that has been studied for over a decade (Paerl et al., 2003; Pinckney and Paerl, 1997; Yannarell and Paerl, 2007; Yannarell et al., 2006, 2007) (Fig. 1). The salinity of the water in Salt Pond fluctuates seasonally, with fluctuations from ~60‰ to over 300‰ possible within a single year (Yannarell et al., 2006). Low salinity conditions are typically confined to a brief wet season occurring from September to November (Shaklee, 1996), and during these months rainfall frequently comes in the form of tropical storms, including two major hurricanes since 1999 (Paerl et al., 2003; Yannarell et al., 2007). The seasonal cycling of salinity between wet and dry seasons in Salt Pond

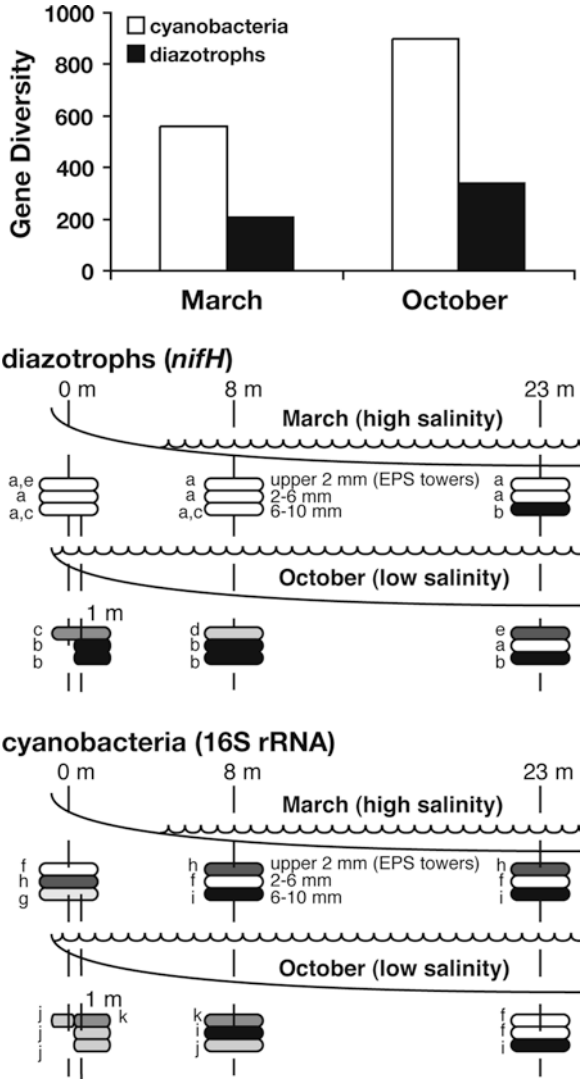

Figure 5. Seasonal variation in Salt Pond mat communities. Data were obtained during 2003, with salinities of 104% in March and 87% in October. *Upper*: minimal diversity of unique gene sequences (95% lower confidence bound of Chao 1 nonparametric estimates) for cyanobacteria (16S rRNA gene) and diazotrophs (*nifH* gene). *Middle and lower*: vertical and horizontal variation of functional group diversity for diazotrophs (*nifH*, *middle*) and cyanobacteria (16S, *lower*). Letters and colors indicate layers that contained significantly different assemblages, based on F_{ST} tests ($\alpha < 0.05$).

is a major determinant of species diversity and spatial positioning of cyanobacteria and diazotrophs. Both groups show reduced diversity during dry, high salinity seasons (Yannarell et al., 2006) (Fig. 5). The reduction in diversity during dry

months is particularly pronounced for non-cyanobacterial diazotrophs. Many of these organisms can be found only in the lower layers of the mat in dry months, and wet months show an increased diversity of non-cyanobacterial diazotrophs in the upper 2 mm of the mat (Yannarell et al., 2006). Only one clade of cyanobacteria, often referred to as the *Lyngbya*/*Phormidium*/ *Plectonema* group of non-heterocystous diazotrophic cyanobacteria, displays similar layer-specific diversity changes with wet and dry seasons (Yannarell et al., 2006). The implication is that many diazotrophs preferentially accumulate near the top of the mat when salinity is low, possibly to capitalize on the photosynthetic potential at the mat surface. High salinity excludes them from the mat altogether, or it causes them to accumulate near the bottom of the mat, where they may be protected from the hypersalinity by intervening layers of biomass or EPS. During the dry months, diazotrophs appear to be uniformly distributed along a desiccation gradient, but there is noticeable horizontal heterogeneity in their distribution in the upper 2 mm layer during the wet months (Yannarell et al., 2006) (Fig. 6). Cyanobacteria always display horizontal heterogeneity in their distribution: communities above the water line are strikingly different from submerged communities in the dry months,

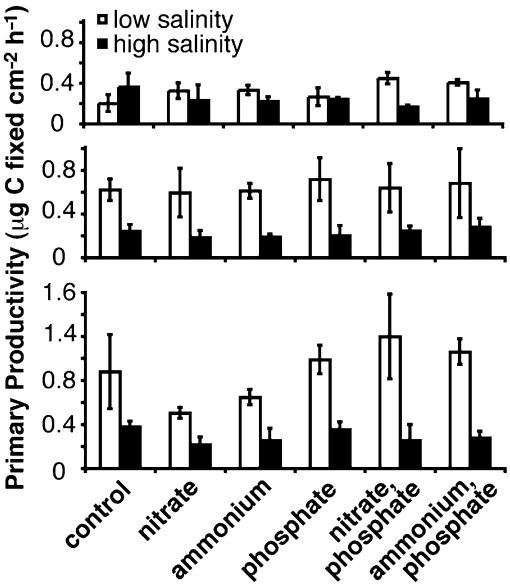

Figure 6. Effect of salinity reduction on primary productivity of Salt Pond mat. Sections of mat were incubated at low (37‰) or high (178‰) salinity, and with the addition of various inorganic substrates. Water replacements were performed every 2 days to maintain the treatment. Error bars are standard deviation. *Top*: 2 days of incubation. *Middle*: 4 days. *Bottom*: 6 days. Only after water stress is relieved in low salinity treatments is it apparent that primary production is also phosphorus limited.

and deep, offshore communities are different from those of shallower sites in the wet months (Yannarell et al., 2006). Taken together, these patterns indicate that high and low salinity conditions control the diversity and the horizontal and vertical location of key microbial players in mat carbon and nitrogen cycling.

The activity rates of phototrophy and diazotrophy in Salt Pond are also affected by salinity, with both being higher in low salinity months (Paerl et al., 2003; Yannarell et al., 2007). Over the long term, these processes display a negative exponential relationship with salinity (Yannarell et al., 2007), and salinities over 100‰ reduce activity rates dramatically. Experimentally lowering the salinity below this inhibiting level consistently increases the rate of photosynthesis (sometimes with a lag of a few days) showing that cyanobacteria are "primed" to respond rapidly to salinity-reducing events such as tropical storms (Fig. 5). Salt Pond diazotrophy can sometimes be stimulated in this manner, although sometimes nitrogen fixation appears to be additionally inhibited by ammonia accumulating in the mat (i.e., end product suppression) (Paerl et al., 2003), or by limited phosphorus availability. It has recently been demonstrated that the uptake of dissolved organic carbon and nitrogen compounds can rapidly be stimulated by reductions in salinity (Yannarell and Paerl, 2007). Cyanobacteria were responsible for some of this organic matter uptake, as were bacteria associated with the sheaths and mucilage of cyanobacteria (Yannarell and Paerl, 2007). The long-term dynamics of this are not well understood. However, in the short term, the ability to rapidly utilize residual organic matter when favorable growth conditions are encountered can help fuel the revival of dormant mat populations that will eventually fix new carbon and nitrogen.

While rainfall to the Salt Pond mat can stimulate microbial activity and allow the re-emergence of sensitive populations, it can influence the mat system in other ways. Tropical storms during the wet season often deliver several centimeters of rain at once, meaning that salinity reduction can occur in pulses. For a basin the size of Salt Pond, even 3 cm of rainfall can advance the waterline by several meters (Fig. 7), providing water cover to sediments that may have been dry for months. These newly covered sediments may be "hotspots" for colonization and interactions between colonizing organisms and reviving organisms that persisted during the exposed period. Larger storms and hurricanes can provide new surfaces for colonization because their activity can deposit fresh layers of sand and sediment on top of existing mat biomass (Paerl et al., 2003; Yannarell et al., 2007). A study of Salt Pond conducted shortly after the passage of Hurricane Frances (2004), showed that mat community composition of newly colonized sediments was distinct from that of the older, established mat (Yannarell et al., 2007). The previously dominant *Microcoleus* had been replaced by *Chroococcus* and *Lyngybal/Phormidium/Plectonema* group organisms. However, despite this community shift, photosynthesis and nitrogen fixation rates of newly established mats were equal to those expected in established mats (Yannarell et al., 2007). Newly established mats in other systems also exhibit distinct composition and high activity rates (Omoregie et al., 2004b; Villbrandt et al., 1991), suggesting that successional changes can play an important role in mat responses in dynamic environments.

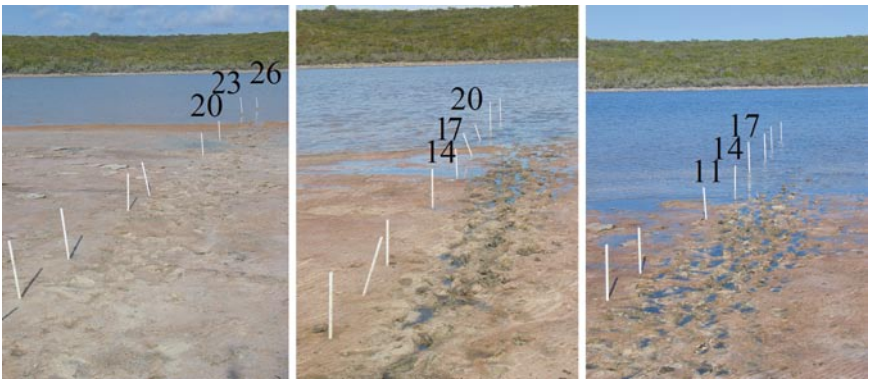

Figure 7. Advance of Salt Pond waterline by rain. Photographs were taken on consecutive days in March, 2005. The numbers indicate the marker distance (m) from the start of a sampling transect perpendicular to the waterline. *Left*: prior to rain. *Middle*: following 4.3 cm rainfall. *Right*: following additional 3.4 cm rainfall.

7. Future Perspectives: Mats and Genomics

The dwindling cost of DNA sequencing promises to revolutionize the field of biology. The number of fully sequenced microbial genomes is currently increasing at an exponential rate, opening up tremendous opportunities for comparative genomics. Anhydrophilic mats present several fruitful lines for genomic research. Certain clades of microorganisms appear to be restricted to mat habitats, yet still have widespread geographic distributions (Green et al., 2008; Yannarell et al., 2006). Comparative genomic studies focused on these clades may yield insights into the evolutionary history and biogeography of these organisms, and genomic comparisons with close relatives who are not mat residents can shed light on the adaptations involved in the mat lifestyle. Genomic investigations of anhydrophilic microorganisms may reveal novel mechanisms of salt and desiccation resistance, with implications for fields of biotechnology, microbial ecology, and astrobiology. The genomes of mat-dwelling microorganisms, especially EPS-producers, may constitute an untapped source of information on natural products and their underlying metabolic processes with novel applications. Metagenomics, the study of genomic data from mixed assemblages of microorganisms, should be a useful way to explore coevolution in mats and to help unravel the mechanisms at work in the consortial relationships that are central to mat persistence and functioning.

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9. References

Abed, R.M.M., Kohls, K. and de Beer, D. (2007) Effect of salinity changes on the bacterial diversity, photosynthesis and oxygen consumption of cyanobacterial mats from an intertidal flat of the Arabian Gulf. Environ. Microbiol. **9**: 1384–1392.

Bachar, A., Omoregie, E., de Wit, R. and Jonkers, H.M. (2007) Diversity and function of *Chloroflexus*-like bacteria in a hypersaline microbial mat: phylogenetic characterization and impact on aerobic respiration. Appl. Environ. Microbiol. **73**: 3975–3983.

Bachar, A., Polerecky, L., Fischer, J.P., Vamvakopoulos, K., de Beer, D. and Jonkers, H.M. (2008) Two-dimensional mapping of photopigment distribution and activity of *Chloroflexus*-like bacteria in a hypersaline microbial mat. FEMS Microbiol. Ecol. **65**: 434–448.

Bauer, K., Diez, B., Lugomela, C., Seppala, S., Borg, A.J. and Bergman, B. (2008) Variability in benthic diazotrophy and cyanobacterial diversity in a tropical intertidal lagoon. FEMS Microbiol. Ecol. **63**: 205–221.

Bebout, B.M., Fitzpatrick, M.W. and Paerl, H.W. (1993) Identification of the sources of energy for nitrogen fixation and physiological characterization of nitrogen-fixing members of a marine microbial mat community. Appl. Environ. Microbiol. **59**: 1495–1503.

Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M. and Visscher, P.T. (2007) Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. Geobiology **5**: 401–411.

Breanne, S., Kovarcik, D.P., Wright, D.J., Wilmoth, G., Prickett, T.F., Helm, R.F., Gregory, E.M. and Potts, M. (2000) Active Fe-containing superoxide dismutase and abundant *sodF* mRNA in *Nostoc commune* (Cyanobacteria) after years of desiccation. J. Bacteriol. **182**: 189–197.

Camacho, A. and de Wit, R. (2003) Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat. Microb. Ecol. **32**: 261–273.

Canfield, D.E. and Des Marais, D.J. (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim. Cosmochim. Acta **57**: 3971–3984.

Casillas-Martinez, L., Gonzalez, M.L., Fuentes-Figueroa, Z., Castro, C.M., Nieves-Mendez, D., Hernandez, C., Ramirez, W., Sytsma, R.E., Perez-Jimenez, J. and Visscher, P.T. (2005) Community structure, geochemical characteristics and mineralogy of a hypersaline microbial mat, Cabo Rojo, PR. Geomicrobiol. J. **22**: 269–281.

Caumette, P., Matheron, R., Raymond, N. and Relexans, J.C. (1994) Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). FEMS Microbiol. Ecol. **13**: 273–286.

Conrad, R., Frenzel, P. and Cohen, Y. (1995) Methane emission from hypersaline microbial mats: lack of aerobic methane oxidation activity. FEMS Microbiol. Ecol. **16**: 297–305.

de Wit, R., Falcon, L.I. and Charpy-Roubaud, C. (2005) Heterotrophic dinitrogen fixation (acetylene reduction) in phosphate-fertilised *Microcoleus chthonoplastes* microbial mat from the hypersaline inland lake 'la Salada de Chiprana' (NE Spain). Hydrobiologia **534**: 245–253.

Decho, A.W., Visscher, P.T. and Reid, R.P. (2005) Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Palaeogeogr. Palaeocl. **219**: 71–86.

Engel, A., Delilled, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbruggen, A. and Zondervan, I. (2004) Transparent exopolymer particles and dissolved organic carbon production by *Emiliana huxleyi* exposed to different CO_2 concentrations: a mesocosm experiment. Aquat. Microb. Ecol. **34**: 93–104.

Epping, E. and Kühl, M. (2000) The responses of photosynthesis and oxygen consumption to short-term changes in temperature and irradiance in a cyanobacterial mat (Ebro Delta, Spain). Environ. Microbiol. **2**: 465–474.

Epping, E.H.G., Khalili, A. and Thar, R. (1999) Photosynthesis and the dynamics of oxygen consumption in a microbial mat as calculated from transient oxygen microprofiles. Limnol. Oceanogr. **44**: 1936–1948.

Feazel, L.M., Spear, J.R., Berger, A.B., Harris, J.K., Frank, D.N., Ley, R.E. and Pace, N.R. (2008) Eucaryotic diversity in a hypersaline microbial mat. Appl. Environ. Microbiol. **74**: 329–332.

Fike, D.A., Gammon, C.L., Ziebis, W. and Orphan, V.J. (2008) Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. ISME J. **2**: 749–759.

Fourçans, A., de Oteyza, T.G., Wieland, A., Sole, A., Diestra, E., van Bleijswijk, J., Grimalt, J.O., Kühl, M., Esteve, I., Muyzer, G., Caumette, P. and Duran, R. (2004) Characterization of functional bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue, France). FEMS Microbiol. Ecol. **51**: 55–70.

Fourçans, A., Ranchou-Peyruse, A., Caumette, P. and Duran, R. (2008) Molecular analysis of the spatio-temporal distribution of sulfate-reducing bacteria (SRB) in Camargue (France) hypersaline microbial mat. Microb. Ecol. **56**: 90–100.

Fründ, C. and Cohen, Y. (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl. Environ. Microbiol. **58**: 70–77.

Garcia-Pichel, F., Mechling, M. and Castenholz, R.W. (1994) Diel migrations of microorganisms within a benthic, hypersaline mat community. Appl. Environ. Microbiol. **60**: 1500–1511.

Green, S.J., Blackford, C., Bucki, P., Jahnke, L.L. and Prufert-Bebout, L. (2008) A salinity and sulfate manipulation of hypersaline microbial mats reveals stasis in the cyanobacterial community structure. ISME J. **2**: 457–470.

Grötzschel, S., Abed, R.M.M. and de Beer, D. (2002) Metabolic shifts in hypersaline microbial mats upon addition of organic substrates. Environ. Microbiol. **4**: 683–695.

Hill, D.R., Hladun, S.L., Scherer, S. and Potts, M. (1994) Water-stress proteins of *Nostoc commune* (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associate with 1,4-β-D-xylanxylanohydrolase activity. J. Biol. Chem. **269**: 7726–7734.

Jahnke, L.L., Orphan, V.J., Embaye, T., Turk, K.A., Kubo, M.D., Summons, R.E. and Des Marais, D.J. (2008) Lipid biomarker and phylogenetic analyses to reveal archaeal biodiversity and distribution in hypersaline microbial mat and underlying sediment. Geobiology **6**: 394–410.

Javor, B. (1989) *Hypersaline Environments. Microbiology and Biogeochemistry*. Springer, Berlin.

Jonkers, H.M. and Abed, R.M.M. (2003) Identification of aerobic heterotrophic bacteria from the photic zone of a hypersaline microbial mat. Aquat. Microb. Ecol. **30**: 127–133.

Jonkers, H.M., Koopmans, G.F. and van Gemerden, H. (1998) Dynamics of dimethyl sulfide in a marine microbial mat. Microb. Ecol. **36**: 93–100.

Jonkers, H.M., Ludwig, R., De Wit, R., Pringault, O., Muyzer, G., Niemann, H., Finke, N. and de Beer, D. (2003) Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol. Ecol. **44**: 175–189.

Jonkers, H.M., Koh, I.O., Behrend, P., Muyzer, G. and de Beer, D. (2005) Aerobic organic carbon mineralization by sulfate-reducing bacteria in the oxygen-saturated photic zone of a hypersaline microbial mat. Microb. Ecol. **49**: 291–300.

Joye, S.B. and Paerl, H.W. (1994) Nitrogen cycling in microbial mats: rates and patterns of denitrification and nitrogen fixation. Mar. Biol. **119**: 285–295.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ. Microbiol. **7**: 519–529.

Kaushal, S.S., Groffman, P.M., Likens, G.E., Belt, K.T., Stack, W.R., Kelly, V.R., Band, L.E. and Fisher, G.T. (2005) Increased salinization of fresh water in the northeastern United States. Proc. Natl. Acad. Sci. USA **102**: 13517–13520.

Knoll, A.H. (2003) *Life on a Young Planet*. Princeton University Press, Princeton, NJ.

Krekeler, D., Sigalevich, P., Teske, A., Cypionka, H. and Cohen, Y. (1997) A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai), *Desulfovibrio oxyclinae* sp. nov. Arch. Microbiol. **167**: 369–375.

Kruschel, C. and Castenholz, R.W. (1998) The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypersaline waters. FEMS Microbiol. Ecol. **27**: 53–72.

Kühl, M., Glud, R.N., Ploug, H. and Ramsing, N.B. (1996) Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. J. Phycol. **32**: 799–812.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

Ludwig, R., Pringault, O., de Wit, R., de Beer, D. and Jonkers, H.M. (2006) Limitation of oxygenic photosynthesis and oxygen consumption by phosphate and organic nitrogen in a hypersaline microbial mat: a microsensor study. FEMS Microbiol. Ecol. **57**: 9–17.

Margesin, R. and Schinner, F. (2001) Potential of halotolerant and halophilic microoragnisms for biotechnology. Extremophiles **5**: 73–83.

Minz, D., Fishbain, S., Green, S.J., Muyzer, G., Cohen, Y., Rittmann, B.E. and Stahl, D.A. (1999a) Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl. Environ. Microbiol. **65**: 4659–4665.

Minz, D., Flax, J.L., Green, S.J., Muyzer, G., Cohen, Y., Wagner, M., Rittmann, B.E. and Stahl, D.A. (1999b) Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl. Environ. Microbiol. **65**: 4666–4671.

Nadeau, T.L., Howard-Williams, C. and Castenholz, R.W. (1999) Effects of solar UV and visible irradiance on photosynthesis and vertical migration of *Oscillatoria* sp (cyanobacteria) in an Antarctic microbial mat. Aquat. Microb. Ecol. **20**: 231–243.

Nielsen, D.L., Brock, M.A., Rees, G.N. and Baldwin, D.S. (2003) Effects of increasing salinity on freshwater ecosystems in Australia. Aust. J. Biol. **51**: 655–665.

Nübel, U., Garcia-Pichel, F., Kühl, M. and Muyzer, G. (1999) Quantifying microbial diversity: morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. Appl. Environ. Microbiol. **65**: 422–430.

Nübel, U., Garcia-Pichel, F., Clavero, E. and Muyzer, G. (2000) Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ. Microbiol. **2**: 217–226.

Olson, J.B., Litaker, R.W. and Paerl, H.W. (1999) Ubiquity of heterotrophic diazotrophs in marine microbial mats. Aquat. Microb. Ecol. **19**: 29–36.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004a) Determination of nitrogen-fixing phylotypes in *Lyngbya* sp. and *Microcoleus chthonoplastes* cyanobacterial mats from Guerrero Negro, Baja California, Mexico. Appl. Environ. Microbiol. **70**: 2119–2128.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004b) Comparison of diazotroph community structure in *Lyngbya* sp. and *Microcoleus chthonoplastes* dominated microbial mats from Guerrero Negro, Baja, Mexico. FEMS Microbiol. Ecol. **47**: 305–318.

Oren, A. (1993) Ecology of extremely halophilic microorganisms, In: R.H. Vreeland and L.I. Hochstein (eds.) *The Biology of Halophilic Bacteria.* CRC Press, Boca Raton, FL, pp. 25–53.

Oren, A. (2000) Salts and brines, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria.* Kluwer, Dordrecht, pp. 281–305.

Orphan, V.J., Jahnke, L.L., Embaye, T., Turk, K.A., Pernthaler, A., Summons, R.E. and Des Marais, D.J. (2008) Characterization and spatial distribution of methanogens and methanogenic biosignatures in hypersaline microbial mats of Baja California. Geobiology **6**: 376–393.

Paerl, H.W. and Kuparinen, J. (2002) Microbial aggregates and consortia, In: G. Bitton (ed.) *Encyclopedia of Environmental Microbiology.* Wiley, New York, pp. 160–181.

Paerl, H.W. and Pinckney, J.L. (1996) A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. Microb. Ecol. **31**: 225–247.

Paerl, H.W., Prufert, L.E. and Ambrose, W.W. (1991) Contemporaneous N_2 fixation and oxygenic photosynthesis in the nonheterocystous mat-forming cyanobacterium *Lyngbya aestuarii*. Appl. Environ. Microbiol. **57**: 3086–3092.

Paerl, H.W., Joye, S.B. and Fitzpatrick, M. (1993a) Evaluation of nutrient limitation of CO_2 and N_2 fixation in marine microbial mats. Mar. Ecol. Prog. Ser. **101**: 297–306.

Paerl, H.W., Bebout, B.M., Joye, S.B. and Des Marais, D.J. (1993b) Microscale characterization of dissolved organic matter production and uptake in marine microbial mat communities. Limnol. Oceanogr. **38**: 1150–1161.

Paerl, H.W., Fitzpatrick, M. and Bebout, B.M. (1996) Seasonal nitrogen fixation dynamics in a marine microbial mat: potential roles of cyanobacteria and microheterotrophs. Limnol. Oceanogr. **41**: 419–427.

Paerl, H.W., Pinckney, J.L. and Steppe, T.F. (2000) Cyanobacterial–bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ. Microbiol. **2**: 11–26.

Paerl, H.W., Steppe, T.F. and Reid, R.P. (2001) Bacterially mediated precipitation in marine stromatolites. Environ. Microbiol. **3**: 123–130.

Paerl, H.W., Steppe, T.F., Buchan, K.C. and Potts, M. (2003) Hypersaline cyanobacterial mats as indicators of elevated tropical hurricane activity and associated climate change. Ambio **32**: 87–90.

Papineau, D., Walker, J., Mojzsis, S.J. and Pace, N.R. (2005) Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl. Environ. Microbiol. **71**: 4822–4832.

Pierson, B.K., Valdez, D., Larsen, M., Morgan, E. and Mack, E.E. (1994) *Chloroflexus*-like organisms from marine and hypersaline environments: distribution and diversity. Photosynth. Res. **41**: 35–52.

Pinckney, J.L. and Paerl, H.W. (1997) Anoxygenic photosynthesis and nitrogen fixation by a microbial mat community in a Bahamian hypersaline lagoon. Appl. Environ. Microbiol. **63**: 420–426.

Pinckney, J., Paerl, H.W. and Bebout, B.M. (1995a) Salinity control of benthic microbial mat community production in a Bahamian hypersaline lagoon. J. Exp. Mar. Biol. Ecol. **187**: 223–237.

Pinckney, J., Paerl, H.W., Reid, R.P. and Bebout, B. (1995b) Ecophysiology of stromatolitic microbial mats, Stocking Island, Exuma Cays, Bahamas. Microb. Ecol. **29**: 19–37.

Polerecky, L., Bachar, A., Schoon, R., Grinstein, M., Jørgensen, B.B., de Beer, D. and Jonkers, H.M. (2007) Contribution of *Chloroflexus* respiration to oxygen cycling in a hypersaline microbial mat from Lake Chiprana, Spain. Environ. Microbiol. **9**: 2007–2024.

Potts, M. (1994) Desiccation tolerance of prokaryotes. Microbiol. Rev. **58**: 755–805.

Potts, M. (1996) The anhydrobiotic cyanobacterial cell. Physiol. Plant. **97**: 788–794.

Potts, M. (1999) Mechanisms of desiccation tolerance in cyanobacteria. Eur. J. Phycol. **34**: 319–328.

Potts, M. (2001) Desiccation tolerance: a simple process? Trends Microbiol. **9**: 553–559.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J., Bebout, B., MacIntyre, I., Dupraz, C., Pinckney, J., Paerl, H.W., Prufert-Bebout, L., Steppe, T.F. and Des Marais, D.J. (2000) Microbial lithification in modern marine stromatolites. Nature **406**: 989–992.

Riding, R., Awramik, S.M., Winsborough, B.M., Griffin, K.M. and Dill, R.F. (1991) Bahamian giant stromatolites: microbial composition of surface mats. Geol. Mag. **128**: 227–234.

Rothrock, M.J. and Garcia-Pichel, F. (2005) Microbial diversity of benthic mats along a tidal desiccation gradient. Environ. Microbiol. **7**: 593–601.

Scherer, S. and Potts, M. (1989) Novel water-stress protein from a desiccation-tolerant cyanobacterium and partial characterization. J. Biol. Chem. **264**: 12546–12553.

Schopf, J.W. (2000) Solution to Darwin's dilemma: discovery of the missing Precambrian fossil record. Proc. Natl. Acad. Sci. USA **97**: 6947–6953.

Shaklee, R.V. (1996) *Weather and Climate of San Salvador Island, Bahamas*. Bahamian Field Station Publications, San Salvador, Bahamas.

Simon, M., Grossart, H.P., Schweitzer, B. and Ploug, H. (2002) Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. **28**: 175–211.

Sokolov, A.P. and Trotsenko, Y.A. (1995) Methane consumption in (hyper) saline habitats of Crimea (Ukraine). FEMS Microbiol. Ecol. **18**: 299–303.

Sørensen, K.B., Canfield, D.E. and Oren, A. (2004) Salinity responses of benthic microbial communities in a solar saltern (Eilat, Israel). Appl. Environ. Microbiol. **70**: 1608–1616.

Sørensen, K.B., Canfield, D.E., Teske, A.P. and Oren, A. (2005) Community composition of a hypersaline endoevaporitic microbial mat. Appl. Environ. Microbiol. **71**: 7352–7365.

Stal, L.J. (2000) Cyanobacterial mats and stromatolites, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria.* Kluwer, Dordrecht, pp. 61–120.

Stal, L.J. and Caumette, P. (eds.) (1994) *Microbial Mats. Structure, Development, and Environmental Significance*. Springer, Berlin.

Steppe, T.F. and Paerl, H.W. (2002) Potential N_2 fixation by sulfate-reducing bacteria in a marine intertidal microbial mat. Aquat. Microb. Ecol. **28**: 1–12.

Steppe, T.F. and Paerl, H.W. (2005) Nitrogenase activity and nifH expression in a marine intertidal microbial mat. Microb. Ecol. **49**: 315–324.

Steppe, T.F., Olson, J.B., Paerl, H.W., Litaker, R.W. and Belnap, J. (1996) Consortial N_2 fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol. Ecol. **21**: 149–156.

Steppe, T.F., Pinckney, J.L., Dyble, J. and Paerl, H.W. (2001) Diazotrophy in modern marine Bahamian stromatolites. Microb. Ecol. **41**: 36–44.

Taton, A., Grubisic, S., Brambilla, E., de Wit, R. and Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl. Environ. Microbiol. **69**: 5157–5169.

Teske, A., Ramsing, N.B., Habicht, K., Fukui, M., Kuver, J., Jørgensen, B.B. and Cohen, Y. (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. **64**: 2943–2951.

Villbrandt, M., Krumbein, W.E. and Stal, L.J. (1991) Diurnal and seasonal variations of nitrogen fixation and photosynthesis in cyanobacterial mats. Plant Soil **137**: 13–16.

Vincent, W.F., Downes, M.T., Castenholz, R.W. and Howard-Williams, C. (1993) Community structure and pigment organization of cyanobacteria-dominated microbial mats in Antarctica. Eur. J. Phycol. **28**: 213–221.

Visscher, P.T., van den Ende, F.P., Schaub, B.E.M. and van Gemerden, H. (1992) Competition between anoxygenic phototrophic bacteria and colorless sulfur bacteria in a microbial mat. FEMS Microbiol. Ecol. **101**: 51–58.

Wieland, A. and Kühl, M. (2000a) Short-term temperature effects on oxygen and sulfide cycling in a hypersaline cyanobacterial mat (Solar Lake, Egypt). Mar. Ecol. Prog. Ser. **196**: 87–102.

Wieland, A. and Kühl, M. (2000b) Irradiance and temperature regulation of oxygenic photosynthesis and O_2 consumption in a hypersaline cyanobacterial mat (Solar Lake, Egypt). Mar. Biol. **137**: 71–85.

Wieland, A. and Kühl, M. (2006a) Regulation of photosynthesis and oxygen consumption in a hypersaline cyanobacterial mat (Camargue, France) by irradiance, temperature and salinity. FEMS Microbiol. Ecol. **55**: 195–210.

Wieland, A., Pape, T., Mobius, J., Klock, J.H. and Michaelis, W. (2008) Carbon pools and isotopic trends in a hypersaline cyanobacterial mat. Geobiology **6**: 171–186.

Wood, A.P., Burke, C.M., Knott, B. and Kelly, D.P. (1991) Chemolithotrophic sulfur bacteria in sediments, mats, and stromatolites of western Australian saline lakes. Geomicrobiol. J. **9**: 41–49.

Yannarell, A.C. and Paerl, H.W. (2007) Effects of salinity and light on organic carbon and nitrogen uptake in a hypersaline microbial mat. FEMS Microbiol. Ecol. **62**: 345–353.

Yannarell, A.C., Steppe, T.F. and Paerl, H.W. (2006) Genetic variance in the composition of two functional groups (diazotrophs and cyanobacteria) from a hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 1207–1217.

Yannarell, A.C., Steppe, T.F. and Paerl, H.W. (2007) Disturbance and recovery of microbial community structure and function following Hurricane Frances. Environ. Microbiol. **9**: 576–583.

Biodata of **Pieter T. Visscher** and **Christophe Dupraz**, authors of ***"Biogeochemistry of Carbon Cycling in Hypersaline Mats: Linking the Present to the Past Through Biosignatures"***

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BIOGEOCHEMISTRY OF CARBON CYCLING IN HYPERSALINE MATS: LINKING THE PRESENT TO THE PAST THROUGH BIOSIGNATURES

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1. Introduction: The Microbial-Mediated Carbon Cycle

Prokaryotes are capable of using a wide variety of reduction–oxidation (redox) reactions to fulfill their biochemical energy needs (Nealson and Stahl, 1997; Visscher and Stolz, 2005). This metabolic flexibility enables microbes to thrive under a wide variety of planetary environmental conditions. The geochemical conditions of a given environment will determine which metabolic redox reactions may take place; for example, the presence of oxygen allows for aerobic respiration, and the absence of organic carbon favors metabolism supported by inorganic electron donors (H_2, S^{2-}, NH_3, etc.). The redox reactions that supply energy alter the geochemical environment in which the microbes live, creating conditions that may enable other metabolic reactions to take place. This environmental change can be short-term or long-term or cyclic or permanent. Some of these environmental changes can be preserved as biogenic signatures in the atmosphere and in the permanent rock record of planets. Many such signatures of life exist, ranging from biomolecules and bio-mediated mineral deposits, to biogenic gases.

As a model system for production of biosignatures, we studied microbial mats, which are laminated sedimentary biofilms. These early microbial ecosystems are ideal model systems to investigate both mineral and gaseous biosignatures for two major reasons: (1) microbial mat communities (including stromatolites, which are abundant in the Earth's rock record and the earliest evidence for life; Allwood et al., 2006) have played a crucial role in the evolution of our planet (e.g., production of O_2 to oxygenate Earth's atmosphere, production

and release of H_2, fixation of atmospheric N_2) (Des Marais, 1990; Awramik, 1992); (2) these early microbial ecosystems exhibit extremely high metabolic rates (Jørgensen, 2001), which display temporal (e.g., diel, seasonal, annual) and spatial variations (Dupraz and Visscher, 2005). These temporal and spatial fluctuations provide conditions under which parts of the carbon cycle (Fig. 1) are in disequilibrium leading to a net accumulation of biogenic products, which is a prerequisite for the production of biosignatures.

The high metabolic rates, characteristic of mats, result in a tight and complex coupling of element cycles: the carbon cycle fulfills the role of energy carrier, capturing

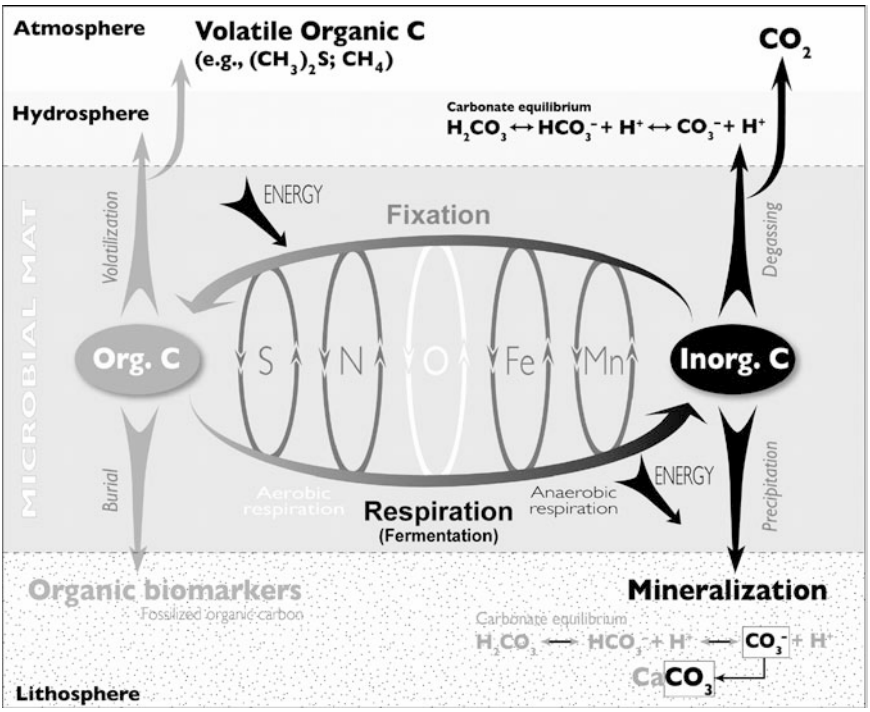

Figure 1. The microbially-mediated carbon cycle, as it is closely coupled to other major element cycles (S, N, Fe, O) at the interface between the lithosphere and hydrosphere or atmosphere, respectively. Microbial metabolism mediates the balance between the organic and the inorganic carbon. Organic molecules are formed during autotrophic CO_2 that requires energy (light or redox energy). The produced organic matter is then efficiently used as energy source by various groups of heterotrophic bacteria, which perform aerobic or anaerobic respiration using O_2 or a range of other compound (e.g., Fe(III)/Mn(IV), NO_3^-, SO_4^{2-}) as terminal electron acceptor, respectively. Alternatively to organic matter, inorganic compounds (e.g., H_2, Fe(II)/Mn(II), CO, HS^-, NH_4^+) can provide electrons to support redox reactions. The fraction of the organic carbon that is not (entirely) oxidized during microbial respiration can be preserved as organic biomarkers (following burial) or as biogenic gases (via volatilization). Transformations of CO_2 (during autotrophic uptake or respiratory production) can have a strong impact on the carbonate equilibrium, either through degassing of CO_2 (to the atmosphere) or precipitation as carbonate minerals (to the lithosphere). In this chapter, we focus on the microbial production of carbonates and organic C gases.

(predominantly light) energy into chemical bond energy when CO_2 is reduced into organic C during carbon fixation, releasing this when organic carbon is oxidized back to CO_2 (Fig. 1). Reduction of CO_2 requires an electron donor (e.g., H_2O, H_2S, Fe^{2+}, Mn^{2+}), while oxidation necessitates an electron acceptor (e.g., O_2, SO_4^{2-}, Fe^{3+}, Mn^{4+}), effectively coupling the carbon cycle to other cycles such as O, S, Fe, and Mn. In a very simplistic manner, when the conversion of CO_2 to organic C temporarily exceeds the reverse reaction, some of the organic C may volatilize (e.g., as methane or dimethyl sulfide (DMS), see below), or become buried as organic biomarkers (Fig. 1). In the opposite scenario, excess inorganic C is formed, which favors degassing of CO_2 and/or precipitation of carbonates. Since the fixation of inorganic C in mats is largely dependent on light energy, changes in light intensity may create an imbalance of both halves of the C cycle, potentially resulting in biosignature formation. Such conditions prevail at the beginning and end of each daylight period, as well as under overcast conditions. Short-term changes in the CO_2 concentration in the water or atmosphere over the mat can be pronounced but ephemeral (Canfield and Des Marais, 1993), whereas precipitation of carbonate minerals can document a long-term inorganic biosignature. Burial of organic C and volatilization to the atmosphere are both well-documented processes that create organic C biosignatures (Des Marais et al., 1989; Ward et al., 1989; Visscher et al., 1991b, 1996). Not surprisingly, research on biogenic gas production in microbial mats has focused primarily on methane, hydrogen, and methyl sulfides (Pilcher, 2003; Hoehler et al., 2001; Visscher et al., 1991b, 2003; Bebout et al., 2004), and research on biogenic mineral production has focused primarily on calcium carbonate precipitation (Cohen et al., 1994; Reid et al., 2000; Arp et al., 2001, 2003; Jonkers et al., 2003; Camoin et al., 2006; Vasconcelos et al., 2006; Bontognali et al., 2008).

Cyanobacterial photosynthesis plays a pivotal role in the biogeochemistry of mats, providing O_2 and fixed (organic) carbon, including copious amounts of exopolymeric substances (EPS). These EPS form the matrix of the sedimentary biofilm, protecting against UV damage, desiccation, and predation. The EPS matrix provides a three-dimensional structure which creates microenvironments with specific, ideal physicochemical conditions for certain microbes and their consortia, enhancing the survival of the complex community.

Contemporary mats are found in extreme environments where eukaryotic grazers and phototrophs are excluded: such as extreme temperatures and salinities, temporary complete desiccation, or excessive sulfide concentrations. Some microbial mat systems have been studied extensively including the Solar Lake, Egypt (e.g., Jørgensen and Cohen, 1977; Jørgensen et al., 1983), Guerrero Negro, Mexico (Des Marais, 1995; Ley et al., 2006), Octopus Springs and other mats in Yellowstone National Park (e.g., Ward et al., 1989; Castenholz, 1994; Spear et al., 2005), Sippewissett, Massachusetts (e.g., Pierson et al., 1987; Buckley et al., 2008), and Highborne Cay (Reid et al., 2000), while other mats that produce biosignatures are less well known (e.g., La Cirpiana, Spain (Jonkers et al., 2003), Salt Pan, Bahamas (Dupraz et al., 2004), Lagoa Vermelha, Brazil (Vasconcelos et al., 2006), Ebro Delta, Spain (Navarette et al., 2000), Cabo Rojo, Puerto Rico (Casillas-Martinez et al., 2005), Pyramid lake, USA (Benson, 1994; Arp et al., 1999), Storrs Lake,

Bahamas (Mann and Nelson, 1989) and Lake Thetis, Australia (Grey et al., 1990; Reitner et al., 1996)). Here, we present an overview of our research on the above-mentioned biosignatures through investigation of the production, consumption and fate of biogenic gases and carbonate minerals produced by prokaryotes.

2. Site Description and Approach

We investigated two tropical, hypersaline, slightly alkaline (pH = ca. 8) lakes that support microbial mat systems in the period from 2000 until 2008 in detail: Salt Pan, Bahamas, and Cabo Rojo, Puerto Rico (Fig. 2).

Salt Pan is a 0.5-km^2 hypersaline lake in northern Eleuthera, Bahamas (76°33'W, 25°24'N) the bottom of which is 90% covered with microbial mats. The lake has a salinity of 60–134 PSU and shows a gradient of microbial mat lithification from the edge toward the center (Fig. 3). In the shallower section, microbialites display various morphologies including millimeter-size clumps of Mg-calcite, thicker and continuous carbonate crusts with columnar morphologies, and isolated patches of carbonate crust (Dupraz et al., 2004). The deeper, central part of the lake bottom is colonized by a thick dark gelatinous, non-lithifying mat. Light microscopy indicates that cyanobacteria are important players in both lithifying and soft mats with the filamentous *Microcoleus* sp. and the coccoid *Entophysalis* sp. as the dominant forms (Fig. 3). The biogeochemistry of the Salt Pan mats was investigated from 2000 until 2008 with field visits annually, up to three times per year.

Cabo Rojo salterns (17°95.55'N, 67°19.71'W) are located in southwestern Puerto Rico (Fig. 2). These salterns consist of crystallizing ponds that are actively managed and several square kilometers of shallow, natural hypersaline lakes, including Fraternidad and Candelari (Casillas-Martinez et al., 2005). The area of Candelaria is located on the northwestern side of Cabo Rojo and consists of several abandoned crystallizer ponds surrounding a shallow hypersaline lake (ca. 1.25 km^2). The lake is replenished by precipitation during the rainy season (September–December and April–May) and through occasional introduction of seawater by the salt company management. The lake is separated from the ocean by a ca. 50-m wide range of sand dunes and is subject to minor water level changes (2–5 cm) due to tidal pumping. This pumping may slowly deliver seawater to the lake. However, the porewater salinity at ca. 30 cm depth never differed by more than 20% from surface values. The Candelaria mats undergo a salinity cycle twice per year, from about 30 PSU to about 350 PSU, when the mats are covered by a salt crust. The maximum water level of approximately 20 cm over the mats coincides with the lowest salinity (30–35 PSU). Changes in the composition of the cyanobacterial community during the pronounced salinity changes are described below. Seven salinity transition events in the Cabo Rojo salterns were followed during a 5-year period from 2003 until 2008.

We used similar approaches for both field sites. All mat samples were taken by hand coring. Light and phase contrast microscopy of mat samples were performed using an Olympus BX-52 microscope. Thin sections, both "dry" (mineral only) and "wet" (impregnated to preserve minerals and organic matter), were prepared and

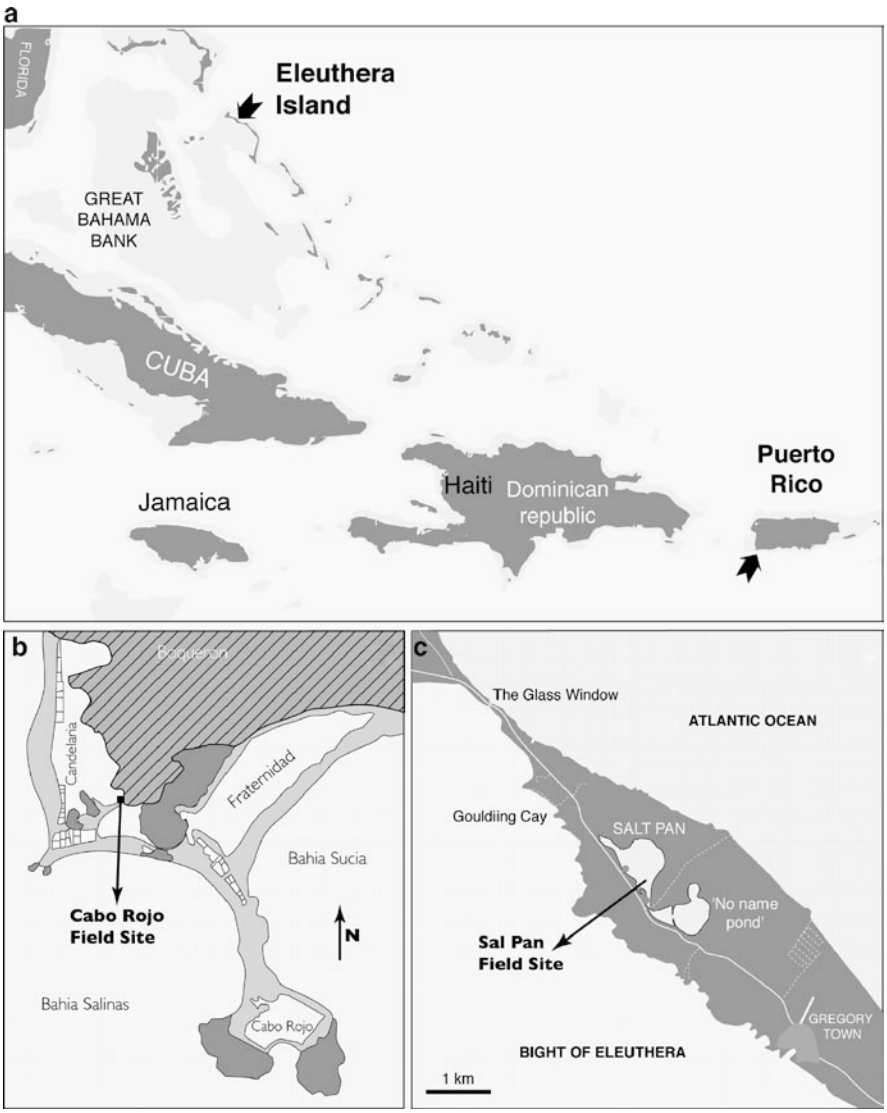

Figure 2. (**a**) Map of the hypersaline microbial mats of this study. (**b**) Candelaria (Cabo Rojo, Puerto Rico) in the south. (**c**) Salt Pan (Eleuthera, Bahamas; *lower right*) in the northern Caribbean. The shaded area in panel B represents Tertiary quartz deposits, the dark area is Ponce limestone and the light grey area is Holocene beach deposit.

studied using an Olympus petrographic BH-2 microscope. Further observations were made on samples frozen in liquid N_2 slush, using a Philips XL 30 field emission environmental scanning electron microscope (FE-ESEM) equipped with an Oxford high-resolution cryo-transfer system. This approach best preserves the three-dimensional organization of hydrated samples (e.g., Défarge et al., 1999;

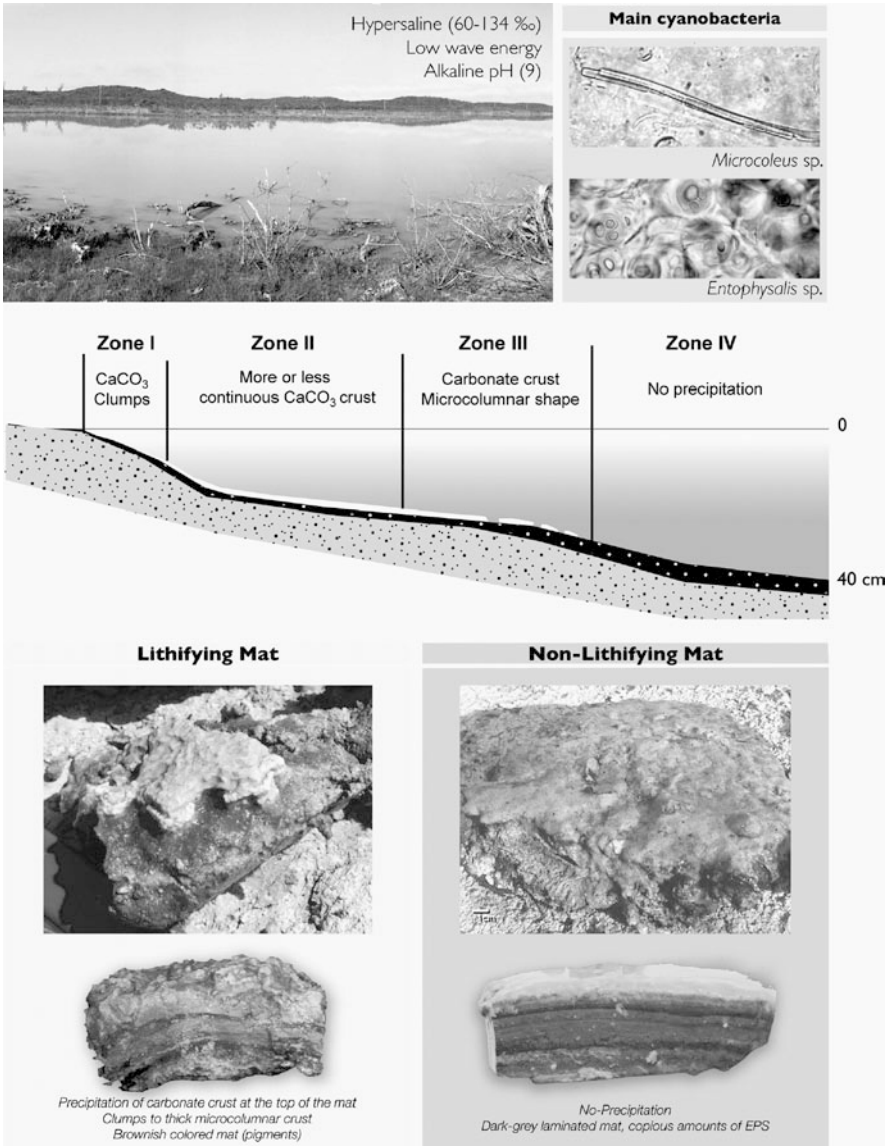

Figure 3. Overview of the lithifiying and non-lithifying hypersaline mats, Salt Pan, Bahama. View of the lake (*top left*) with physicochemical properties of the water column. Major cyanobacterial groups comprising the microbial mats (*top right*). Transect showing the zonation of different microbialites in the lake (*middle*). Hand samples and cross sections of lithifying (zone II–III) and non-lithifying (zone IV) mats (*bottom*).

Dupraz et al., 2004). The mineral composition was determined by x-ray diffraction (XRD) using a Scintag diffractometer. Isotopic composition of carbonate phases was performed with a VG Micromass 602 mass spectrometer at the Pierre et Marie

Curie University (Paris) using PDB standards. Depth profiles of [O_2] and [HS^-] were determined under *in situ* conditions using microelectrodes (Visscher et al., 1991, 2002). Oxygen production rates were estimated using the light–dark shift method (Epping et al., 1999; Visscher et al., 2002). Sulfate reduction was determined in two dimensions using silver foil coated with $^{35}SO_4^{2-}$ (Visscher et al., 2000). Sulfate-reducing bacteria (SRB) are potentially very important drivers of disequilibrium in both systems studied, and therefore, research on SRB and their EPS was undertaken. EPS chemical properties, distribution in mats, and potential for degradation were determined. SRB were cultured under anoxic conditions using a modified Bak and Widdel (1986) medium (Braissant et al., 2007). EPS from both natural mats and cultures were extracted and purified according to Braissant et al. (2007). Acid–base titrations of EPS were performed in a CO_2-free atmosphere to determine the proton-binding sites and the potential types and densities of functional groups (Braissant et al., 2007). The calcium-binding capacity of EPS was determined by $CaCl_2$ titration (Braissant et al., 2009). The depth distribution of EPS in mats was estimated using the phenol–sulfuric acid assay (which measures the amount of reducing sugar; Dubois et al., 1956) and the Alcian Blue assay (which quantifies the presence of anionic functional groups; Passow and Alldredge, 1995; Bober et al., 2005). The EPS degradation potential in mat samples was determined using the total reductase activity assay (triphenyltetrazolium chloride (TTC) assay; Relexans, 1996), and hydrolytic enzymes (α-glucosidase, β-glucosidase, and β-galactosidase) were assayed according to Hashimoto et al. (1998) and Nankai et al. (1999). Molecular diversity was investigated through 16S rRNA gene extraction; amplification and sequencing were performed according to Baumgartner et al. (2006), submitted. Sediment samples were sliced, and the [CH_4] concentration was measured using gas chromatography (Visscher et al., 1991a; Buckley et al., 2008). Gas fluxes were measured using transparent acrylic benthic flux chambers (6.7 cm internal diameter), which were deployed in triplicate across a 1-m^2 area and filled with site water to leave a 1-ml headspace. Each chamber was sampled in duplicate every 3–4 h over the course of 1–3 diel cycles (Visscher et al., 2003). Gases were analyzed using gas chromatography with flame ionization detection (for methane) or flame photometric detection (for methyl sulfides).

3. Mineral Biosignatures

Microbial mat ecosystems interact with minerals on various levels. They are able to trap and bind large amounts of sediments (particles or mud) and to precipitate minerals passively or actively. The interaction of microbial communities and detrital/chemical sediments can form benthic organosedimentary deposits called microbialites (Burne and Moore, 1987). The precipitation of minerals within a microbial organic matrix is referred to as organomineralization s.l. (Dupraz et al., 2009), the products of which are organominerals (Perry et al., 2007). Organominerals are not derived from the genetic control that is characteristic for eukaryotic biomineralization (e.g., bivalve shells, echinoids tests, human bones), and therefore need to

contain chemical and structural properties of their microbial origin to be considered as evidence of life, that is, biosignatures (Cady et al., 2003; Perry et al., 2007). These chemical and structural properties can be indicative of (1) microbial cells or extracellular substances and/or (2) processes associated with microbial activity (Cady et al., 2003). Very few fossil microbialites contain well-preserved microbial communities or individual organisms that may be at the origin of their formation (Grotzinger and Knoll, 1999). Therefore, it is critical to understand the precise role of modern microbial processes in the formation of mineral products, so that these potential biosignatures in the rock record can be unequivocally recognized.

In earlier work (Dupraz et al., 2004), it was hypothesized that differences in geochemical conditions account for the presence or absence of lithification in the microbial mats of Salt Pan. As outlined above, the differences in geochemical conditions within a mat are the result of the metabolism of the combined community. Therefore, we performed a study in which we assessed differences in community composition and geochemical properties of lithifying and non-lithifying mats (Fig. 4). Using 16S rRNA gene sequencing in clone libraries, approximately 2,000 sequences were obtained from both mat types as well as from the water column. Universal primers (515F and 1391R) as well as specific primers for cyanobacteria and sulfate-reducers (delta96F) were used (Baumgartner et al., 2006, submitted). Molecular sampling efforts covered two seasons (wet and dry) and two opposite sides of the lake. Major differences were observed between the two different mat types at each sampling time. Lithifying mats always revealed a lower diversity than the non-lithifying mats (Fig. 4c, d). The differences in diversity between mats were always greater than the variation between samples during one season within any single mat.

Confirming our earlier community composition observations, the biogeochemical properties also showed pronounced differences between mat types (Fig. 4). The lithifying mat displayed much steeper O_2 profiles with maxima of >200% O_2-saturation around 2 mm depth (Fig. 4e, f). At similar light levels, profiles measured in the non-lithifying mat had no clear maximum and much deeper O_2 penetration. Similarly, light–dark shift experiments with O_2 microelectrodes indicated strong differences in oxygen production (peaking at 69 and 12 μM O_2 min^{-1} in lithifying and in non-lithifying mats, respectively) and consumption (peaking at 33 and 10 μM O_2 min^{-1} in lithifying and in non-lithifying mats, respectively) (Fig. 4g, h). The overall microbial activity was consistently higher in the lithifying mats, thereby potentially modifying the carbonate alkalinity (CA) more than in the soft mats. Since the various types of aerobic and anaerobic metabolism promote either precipitation or dissolution of calcium carbonates (Visscher and Stolz, 2005), it was expected that combined metabolisms favoring precipitation outpaced dominated the types of metabolism favoring dissolution, particularly near the top of the mat where the $CaCO_3$ crust formed. For example, any type of autotrophy increases CA through CO_2 fixation (e.g., oxygenic photosynthesis). Anaerobic respiration (e.g., nitrate and sulfate reduction) that consumes organic acids, produces bases, and/or consumes an inhibitor of precipitation (i.e., SO_4^{2-}; Soetaert, et al., 2007) also creates alkalinity, as does methanogenesis. In contrast,

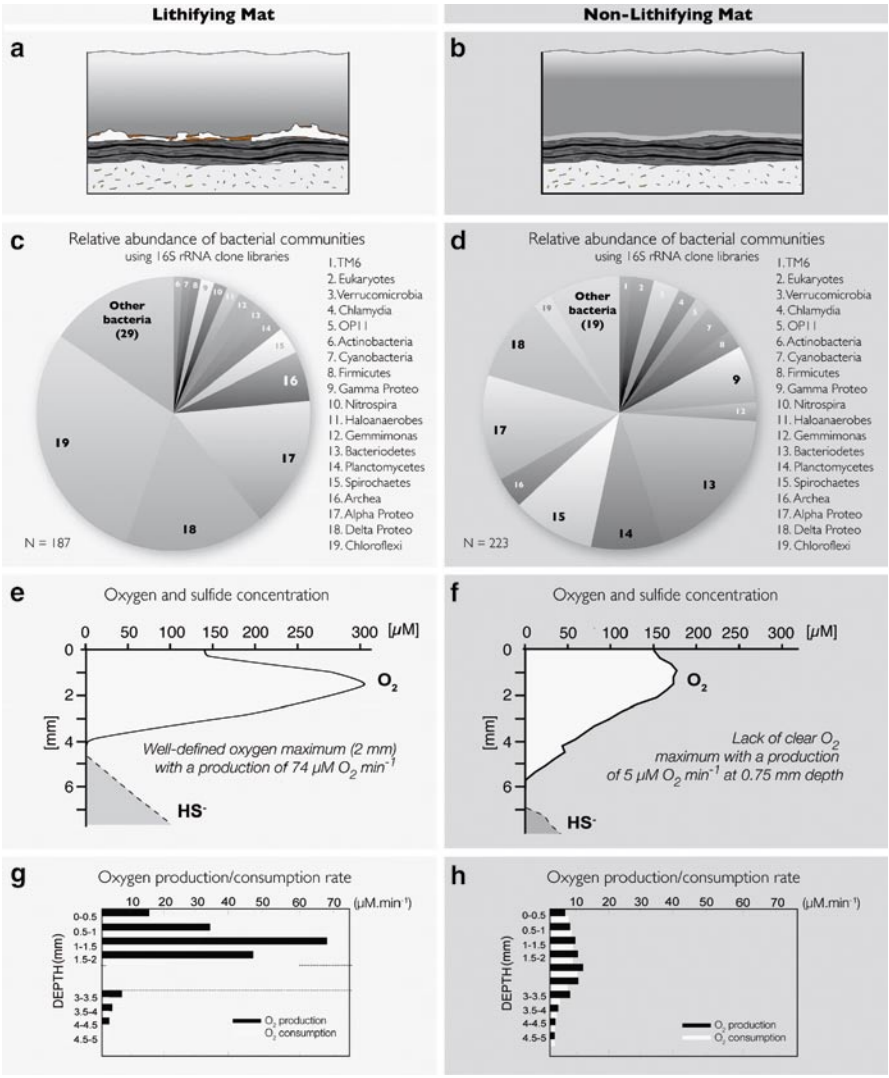

Figure 4. Summary of the major biogeochemical characteristics of the lithifying (*left*) and non-lythifying (*right*) mats in Salt Pan, Bahamas. (**a**, **b**) Cartoon of the mat composition. White crust shown at the surface (**a**). (**c**, **d**) 16S rRNA-base diversity of microbial mat communities showing major group distributions. Note the number of sequences (N = 187 and 223 for lithifying and non-lithifying mats, respectively). (**e**, **f**) Representative depth profiles of O_2 and HS^- determined with microeletrodes during peak photosynthesis (no cloud cover, light intensity 1,800–2,200 μE m^{-2} s^{-1}). (**g**, **h**) Net production photosynthesis and consumption aerobic and anaerobic respiration (the latter is accounted for through aerobic reoxidation of HS^- produced during sulfate reduction). Measurements were carried out using the light-dark method (see text).

aerobic respiration (increasing pCO_2) and sulfide oxidation most likely decrease CA (Visscher and Stolz, 2005; Dupraz and Visscher, 2005). During daytime, photosynthesis and aerobic respiration as well as sulfate reduction and sulfide oxidation are coupled. During the night, the microbial mat rapidly turns anoxic and sulfate reduction prevails; sulfide-oxidizing bacteria rely largely on oxygen and cease to be active under these conditions. Interestingly, the non-lithifying mat was characterized by low metabolic rates of all microbial groups (Fig. 4h). As a result, no steep gradients were formed to support the ideal spatial and temporal fluctuations of biogeochemical properties needed for disequilibrium processes leading to carbonate production (as measured in the lithifying mat).

In Salt Pan, two-dimensional mapping of sulfate reduction (Fig. 5a, b) and stable isotopic analyses of the mineral phase (Fig. 5d) revealed that the metabolism correlated with, and likely responsible for, carbonate precipitation in Salt Pan is sulfate reduction. Precipitation of $CaCO_3$ at the surface of the lithifying mat coincided with the maximum in sulfate-reducing activity (Fig. 5a). In contrast, the soft mats showed a diffuse activity pattern, without a maximum. The depth-integrated sulfate reduction rates confirmed much higher activity in the lithifying mats (37.2 μM sulfate h^{-1}) compared to the soft mats (7.7 μM sulfate h^{-1}). These sulfate reduction measurements strongly suggested that sulfate-reducing bacteria (SRB) were likely key players in the formation of the carbonate crust in this lake. This was further confirmed by the fractionation of stable carbon isotopes in the precipitated carbonates. The $\delta^{13}C$ values of the bulk crust are slightly negative (Fig. 5d), which could result from a combination of the following two processes: (1) heterotrophic degradation of organic carbon, which has a preference for lighter fraction results in a ^{13}C-depleted carbonate precipitate, and (2) an enriched ^{13}C signature originating from scattered trapped and bound sediments (ooids; Fig. 5d), together resulting in a heavier overall (bulk) signature. $\delta^{13}C$ values similar to these were reported for stromatolitic precipitates (Andres et al., 2006), which were believed to be of sulfate-reducing origin. Furthermore, the relatively low $\delta^{18}O$ values may rule out that evaporitic processes formed the carbonate deposits in Salt Pond. Lastly, carbonate precipitation was never observed either inside or on the surface of cyanobacterial sheaths (Fig. 5c) but rather in the EPS layer overlying the zone of active photosynthesis (Fig. 5e). This rules out photosynthetic activity as the prime source of $CaCO_3$ precipitation.

An increase in carbonate alkalinity is not the only prerequisite for precipitation of carbonate minerals. In addition, appropriate free cations (e.g., Ca^{2+} and Mg^{2+}) and nucleation sites are needed. The EPS matrix controls the presence of both. In microbialites of Salt Pan, the precipitate consisted of a micropeloidal structure, which was characterized by micritic micropeloids that were surrounded by microspar and spar cement (Fig. 5c). The nucleation of micrite started within the EPS matrix that contained embedded microbial communities (Fig. 5e). EPS were progressively replaced with high-Mg calcite. The microstructure of the initial precipitate consisted of 200–500 nm nanospheres (Fig. 5e). Patchy calcification of EPS produced a micropeloidal microfabric, possibly resulting from the presence of

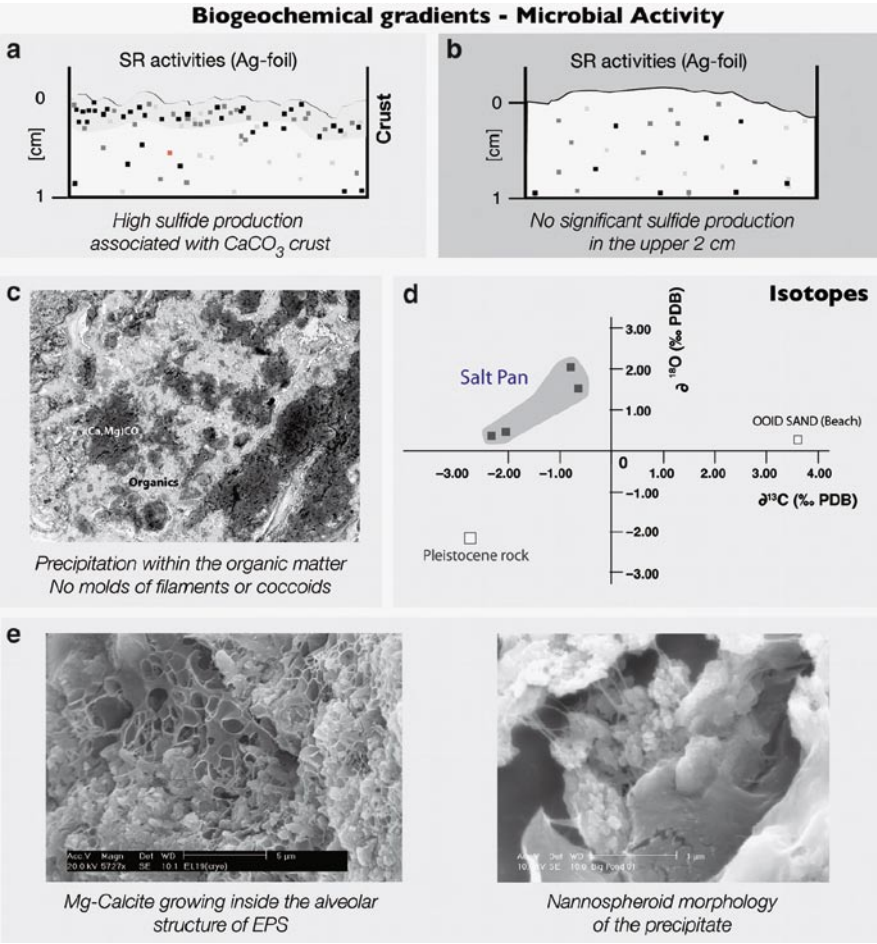

Figure 5. Microbial activity of Salt Pan mats. (**a**, **b**). Two-dimensional distribution of sulfate-reducing activity in lithified mat (**a**) and non-lithified (**b**) mats. High activity is clearly visible in the uppermost part of the lithified mat, which is associated with the carbonate crust. (**c**) Light microscopic observation showing the microstructure of the carbonate precipitates associated with the lithifying microbial mats. The carbonate crust develops within an organic matrix (*light grey*) and show micritic microstructure (*dark grey*). (**d**) Isotopic composition of the carbonate crust, Pleistocene rock and trapped ooid sand (see text for detail). (**e**) Carbonate precipitation (observed using FEG-ESEM using cryofixation) showing the progressive replacement of alveolar EPS with small Mg-calcite nanospheres (*right* image). The result is a full replacement of the EPS structure by carbonate precipitation (*left* image).

clusters of coccoid or remnants of filamentous cyanobacteria on which preferential nucleation could occur (Dupraz et al., 2004).

The pivotal role of SRB in $CaCO_3$ precipitation through increasing the CA was discussed above. We investigated the role of these heterotrophs further using a pure culture of an SRB obtained from Salt Pan mats, which produced copious amounts of EPS. This EPS was purified and characterized using various methods (Braissant et al., 2007, 2009). Acid–base titration (Fig. 6) revealed buffering capacities at three different pH ranges, corresponding to carboxyl groups (pK_a = 3), sulfhydryl groups, sulfonic and sulfinic acids ($pK_a \approx 7$), and amino groups (pK_a = 8.4–9.2), respectively, all of which are able to bind cations at higher pH. These negatively charged

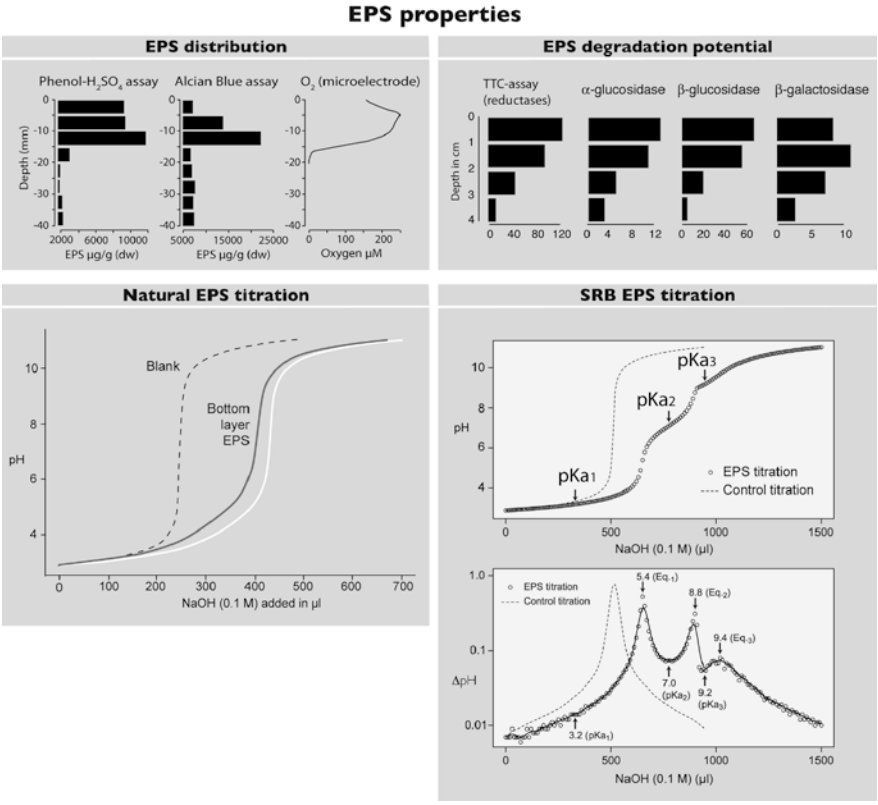

Figure 6. Properties of exopolymeric substance (EPS) from non-lithifying mats of Salt Pan. Depth distribution of EPS (*top left panel*) determined with the phenol–sulfuric acid assay and the Alcian Blue assay. Depth profiled of O_2 measured during peak photosynthesis (12:30 p.m.). Distribution of enzymes activities in the upper 40 mm of the microbial mat (*top right panel*): (1) Total reductase activities (TTC assay; n = 12), (2) α-glucosidase activity (n = 3), (3) β-glucosidase activity (n = 3), (4) β-galactosidate activity (n = 9). Acid–base titration curve (*bottom left panel*) of the EPS from the top and bottom layer, respectively. Acid–base titration of the EPS produced by *Desulfobacterium autotrophicum* (*bottom right*) showing three characteristic buffering zones at: (1) pH 3.2 attributed to carboxyl groups, (2) pH 7.0 attributed to thiol groups, and (3) pH 9.2 attributed to amino groups. (Redrawn from Braissant et al., 2007, 2009.)

functional groups were confirmed by Fourier Transform Infrared Spectroscopy (FT-IR; Braissant et al., 2007). This binding capacity, which is most pronounced in freshly produced EPS, can remove a large amount of cations from the surrounding micro-enviroment, inhibiting the formation of carbonate minerals (e.g., Dupraz and Visscher, 2005). This inhibition can be removed through EPS degradation or when the binding capacity is saturated, or through pH variations which are common in a diel cycle in an active microbial mat (Visscher et al., 1992, 2002; Dupraz et al., 2009).

Several lines of evidence support the relatively rapid degradation of the complex EPS molecules. Aerobic and anaerobic respiration in homogenized samples prepared from Salt Pan mats was stimulated after only a very brief lag phase (minutes) when EPS, sugar monomers or small molecular weight compounds (e.g., acetate, lactate) were added (Braissant et al., 2009). The same result was obtained in similar experiments with mats from other systems (Visscher et al., 1999, 2002; Decho et al., 2005). The presence and distribution of polysaccharide-cleaving hydrolytic enzymes was also determined in different layers of Salt Pan mats. α-glucosidase, β-glucosidase, β-galactosidase, and total reductases all decreased sharply below 20 mm (Fig. 6; top right panel). This suggested that capacity to (partially) degrade EPS was associated with the most active layer of the mat (the depth of which coincides with the depth of O_2 penetration; Fig. 6; top left panel). The change of hydrolytic enzymes with depth was also indicative of changing bioreactivity and thus composition of EPS: complex polymer hydrolyzing enzymes (α-glucosidase and β-glucosidase) decreased with depth, while disaccharide-cleaving activity of β-galactosidase peaked at 10–20 mm and persisted longer with depth. Lastly, the chemical properties of EPS in different layers also changed: the buffering (i.e., metal-binding capacity) decreased with depth and the total acidity of the EPS increased significantly, as indicated by the Alcian blue assay, while the total amount of EPS as determined by the phenol sulfuric acid assay stayed constant in the top 15 mm (Fig. 6). This suggests a change of functional group composition (and possibly less cation-binding sites) of the EPS with depth in the active part of the mat. This change of biogeochemical properties of EPS with depth may have important consequences for the availability of free Ca^{2+} and dissolved inorganic carbon (DIC) resulting from microbial degradation, locally increasing the saturation state with respect to $CaCO_3$, and also for the availability of mineral nucleation sites.

4. Volatile Biosignatures

As outlined above, the biogeochemistry of microbial mats is driven by organic matter produced by cyanobacteria in the uppermost layers of the mat during oxygenic photosynthesis (Stal et al., 1985; van Gemerden, 1993). During periods of transition in light conditions and during anoxia, these organosedimentary systems often release volatiles to the atmosphere (e.g., methane, methylsulfide; Visscher and van Gemerden, 1991; Visscher et al., 1991b, 1996, 2003) and characteristic diffusion

patterns of carbon dioxide and oxygen exist (Canfield and Des Marais, 1993, 1994). Cyanobacterial production of organic C in mats typically exceeds consumption by aerobic heterotrophs (Epping et al., 1999; Pinckney et al., 1995; Visscher et al., 1998; Casillas-Martinez et al., 2005; Megonigal et al., 2003). As a result, anaerobic respiration rates are high in mats: sulfate reduction rates as high as 5 μM min^{-1} have been observed in the upper mat layers during the day (Giani et al., 1984; Canfield and Des Marais, 1991; Des Marais, 1995). The abiotic reaction of sulfide, produced by SRB, with low molecular weight organic carbon (LMWOC; e.g., acetate, glycolate) yields a variety of organosulfur compounds (Visscher and van Gemerden, 1991; Visscher et al., 1991b; Luther et al., 1986), of which methanethiol (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$) are the most volatile compounds (Figs. 1 and 9). The production of methane is another important anaerobic process at the surface of contemporary microbial mats (Giani et al., 1984; Zhilina, 1986; King, 1988; Oremland and King, 1989; Visscher and van Gemerden, 1991; Sørensen et al., 2004; Bebout et al., 2004; Buckley et al., 2008). Methane is a biosignature with the potential for assisting in the search for life on extra-planetary bodies (Catling et al., 2001; Kasting et al., 2001; Chapelle et al., 2002; Des Marais et al., 2003; Pilcher, 2003). Although general trends relating methanogenesis to substrate availability and salinity have been reported (Giani et al., 1984; Oremland and King, 1989; Bebout et al., 2004), variability in methane production rates and salinity-related dynamics across sites can be pronounced (Zhilina, 1986; Slobodkin and Zavarzin, 1992; Sørensen et al., 2004). This section describes how changing salinity affects the composition and activity of a hypersaline microbial mat community, with a focus on the dynamics between sulfate reduction and methanogenesis, and the resulting flux of reduced gases (i.e., CH_4, $(CH_3)_2S$) that could be used as biosignatures (Pilcher, 2003; Caroff and Des Marais, 2005).

A marked change in biological properties of the Candelaria (Cabo Rojo) mat was observed with increasing salinity: microscopic observations revealed a shift from a mixed *Lyngbya-Microcoleus*-diatom community at 30–40 PSU to a *Microcoleus*-dominated community between 55 and 195 PSU, in which the presence of diatoms decreased and that of *Spirulina* increased. Above 210 PSU, the presence of *Microcoleus* sp. slowly declined, while *Spirulina* sp. prevailed and *Phormidium* sp. and *Gloecapsa* sp. became increasingly more abundant. At 350 PSU, approximately 60% of the cyanobacteria were coccoid forms (*Gloeocapsa*-like spp.), ca. 25% resembled *Phormidium* sp., 10% *Spirulina* and less than 5% *Microcoleus* sp. Purple-sulfur bacteria were present at all salinities and were located below the cyanobacteria. At lower salinities, both *Thiocapsa* sp. and *Chromatium* sp. were present, whereas at salinities above 210 PSU, abundant *Ectothiorhodospira* sp. and few *Thiocapsa* spp. were observed. The changes in community composition and activities were also documented in geochemical depth profiles and in activity measurements (Fig. 7). The maximum O_2 concentrations decreased with increasing salinity, in part because the amount of this gas that can dissolve decreases rapidly when the salinity increases, and in part due to a decrease in photosynthetic activity. When comparing the percentage of maximum O_2 saturation corrected for salinity, the depth profiles suggest

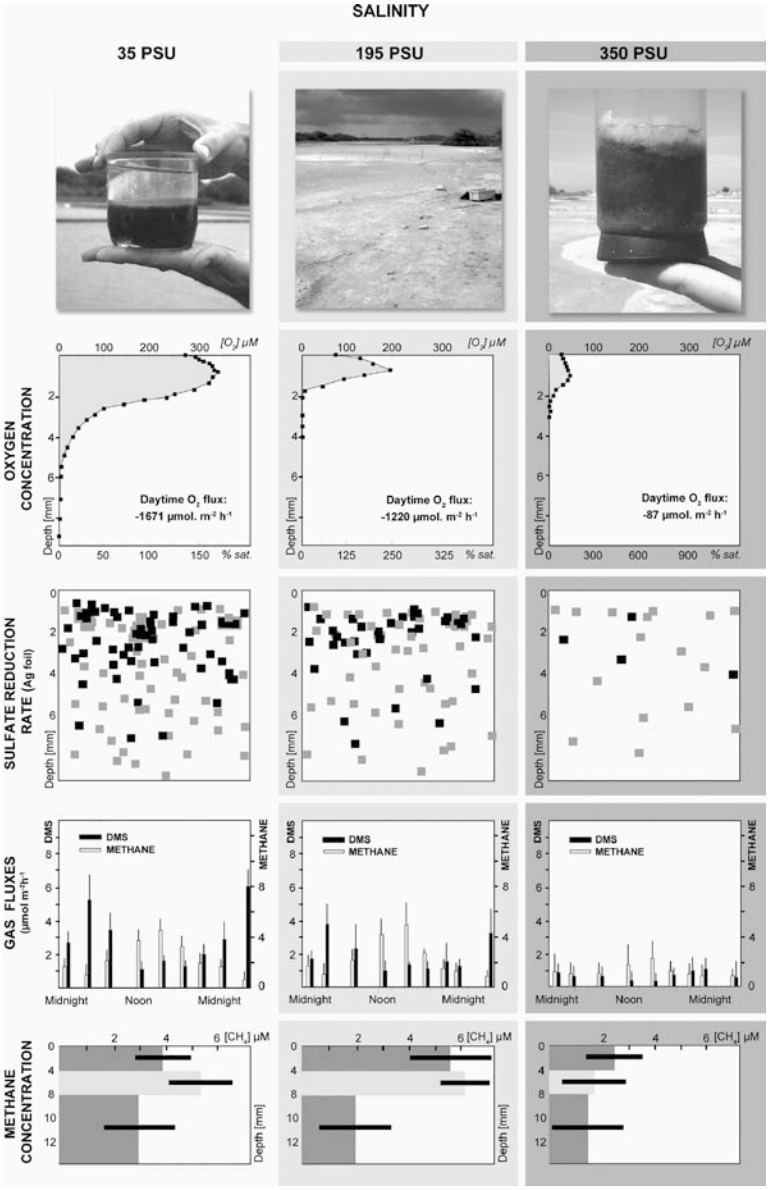

Figure 7. Biogeochemical properties of hypersaline mats of Candelaria, Cabo Rojo, (Puerto Rico). Properties measured at selected, representative salinities are given: overlying water salinity 35 PSU represents (*left hand column*) low, 195 PSU (*middle column*) medium and 350 PSU (*right hand column*) high salinity conditions. *Top row*: Overview of the site and close up of mats in flux chambers (picture taken after 48–72 h incubation). Mats at low and high salinity (*left* (35 PSU) and *right* (350 PSU) images, respectively), and view of the site at low tide of 195 PSU mats. *Second row from top*: Depth distribution of O_2. *Third row from top*: 2D distribution of sulfate reduction activity. *Second row from bottom*: Diel measurements of dimethyl sulfide (DMS) and methane (CH_4) fluxes. *Bottom row*: Depth profiles of CH_4. Average of three cores and standard deviation (*small bar*) shown.

a twofold decrease when the salinity increases from 195 to 350 PSU. Compared to observations at 35 PSU, the daytime flux of O_2 decreased by 25% at 195 PSU, and by 95% at 350 PSU. These observations follow the same patterns as the artificial manipulations of 120 PSU mats from Guerrero Negro by Bebout and coworkers (Bebout et al., 2002, 2004). The sulfate reduction pattern mimics that of the oxygen production (Fig. 7); the rate, estimated from the $Ag^{35}S$-foil, declined by about 40% when the salinity increased from 35 to 195 PSU and by 90–95% when the salinity reached 350 PSU. In contrast, the CH_4 depth profile revealed the highest concentrations at the intermediate salinity (195 PSU) (Fig. 7). It should be noted that these CH_4 depth profiles reflect a balance of production and consumption, and that methane-oxidizing bacteria are not common in hypersaline environments (Conrad et al., 1995; Cohen et al., 1994; Heyer et al., 2005). We were unable to measure CH_4 uptake in homogenized Cabo Rojo mats that were sampled at salinities of 210 PSU and higher. Interestingly, we consistently observed the highest methane concentrations in the oxic zone of the mat (Fig. 7). Other workers reported a similar distribution (Visscher et al., 1996; Hoehler et al., 2001; Buckley et al., 2008), which may be supported by the extremely high rates of photosynthesis characteristic for mats producing LMWOC, the best substrates for methanogenesis.

Measurements of methane and methyl sulfide (particularly that of dimethyl sulfide (DMS)) fluxes at various salinities generally followed the same patterns as other biogeochemical indices described above. When comparing the observations at 35, 195, and 350 PSU, the methane fluxes increased slightly at the intermediate salinity, whereas the DMS flux peaked at the lowest salinity after which it declined. When following the fluxes throughout a diel cycle, it appeared that the methane consistently peaked during the late afternoon and the DMS fluxes during the late night/early morning. It appeared that methane flux, and by extension its production, was directly coupled to photosynthetic production of LMWOC. DMS production, on the other hand, largely results from the abiotic reaction of LMWOC and biogenic sulfide (Kiene and Visscher, 1987; Visscher et al., 2003), which may take more time than a microbial reaction such as methanogenesis because of a required buildup of reactants to favorable concentrations. The lack of sulfide reoxidation during the anoxic night results in high concentrations of free sulfide (Visscher et al., 2002), which may favor DMS production in the Cabo Rojo mats. This scenario is similar to that reported earlier for the hypersaline mats of Guerrero Negro, Mexico (Visscher et al., 2003). When comparing the flux of methane and DMS at 35, 195, and 350 PSU (Fig. 7), it appears that the methane flux is affected less by the salinity than by the flux of DMS. Additional flux measurements were made to confirm the pattern. The highest diel methane fluxes were observed at 210–250 PSU and the highest DMS fluxes at 90–135 PSU (Fig. 8). The maximum O_2 production rate (measured between 11:00 a.m. and 2:30 p.m.) peaked at 90 PSU, and rapidly decreased at salinities of 200–350 PSU. When the ratio of methane to DMS fluxes is plotted (Fig. 9), a low ratio is observed from 30–195 PSU, followed by a sharp increase at 195 PSU, after which a steady decrease due to decreased overall microbial activity is seen at the highest salinities.

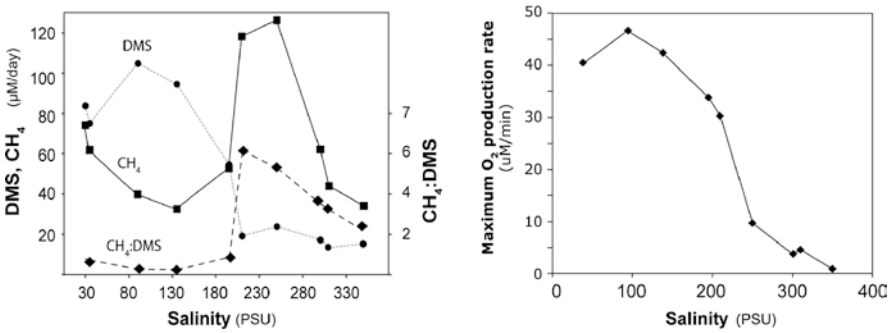

Figure 8. Fluxes of biogenic gases from Candelaria (Puerto Rico) mats. *Left panel*: Total diel fluxes CH_4 (*squares*, *solid line*), DMS (*circles*, *dotted line*) and the ratio of CH_4 to DMS (*diamonds*, *dashed line*) for the observed salinity range are given. Measurements represent a combination of seven salinity cycles. *Right panel*: Maximum O_2 production rates covering the same salinity range. These rates represent net photosynthesis and were determined using microelectrodes and the light-dark shift method.

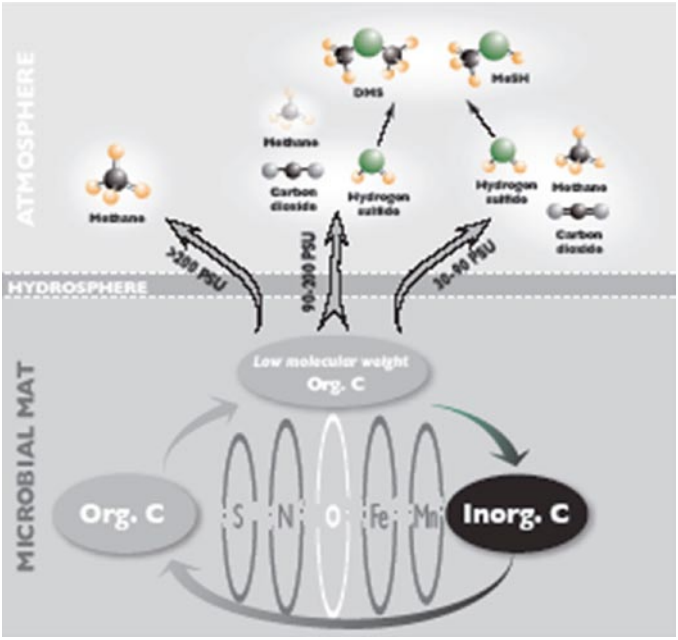

Figure 9. Conceptual model of changes in major biogenic gas production in this study under three salinity regimes: At low salinity (ca. 30–35 PSU; *right*), both CH_4 and H_2S are produced. Free sulfide reacts with low-molecular weight carbon to form dimethyl sulfide (DMS) and methanethiol (MeSH). At intermediate salinity (90–135 PSU; *center*), methane production occurs, but is less important relative to DMS production (see CH_4:DMS ratio (Fig. 8, *top panel*)). Finally, at high salinity (210–250 PSU; *left*), sulfate reduction ceases and methane production dominates.

Microbial community activity and composition are highly influenced by salinity (Sorokin et al., 2004). Metabolic rates and species composition, in particular, are affected by changes in salinity and therefore account for many changes in microbial community ecology across mat sites of variable salinity (Pinckney et al., 1995; Bebout et al., 2002; Visscher et al., 2003; Sørensen et al., 2004). In this study, both methane and DMS were produced as specific volatile compounds, biosignatures for methanogenic and sulfate-reducing activity, respectively. There are, however, complicating factors: methanotrophic microbes and SRB may be either metabolically inactive or unable to survive at higher salinities. Investigations using molecular techniques to address the presence or absence of methanotrophs and SRB are currently underway. Furthermore, at elevated salinities, production and excretion of osmolytes, substrates for methanogenesis but not sulfate reduction (Kiene and Visscher, 1987; Visscher and van Gemerden, 1991; Visscher et al., 1996, 2003), may accelerate the competitive advantage of methanogens over SRB. Similarly, DMS produced by SRB may act as a substrate for methanogenesis (Kiene and Visscher, 1987; Visscher et al., 1991b, 1996) resulting in a decrease in DMS concentrations and therefore apparent DMS production. Nevertheless, a pattern emerges indicating that at low salinity (i.e., 30–35 PSU in this study), high rates of methane and H_2S (and by extension CO_2) are observed (Fig. 9; H_2S the latter being the precursor for DMS and methanethiol). At intermediate salinities (i.e., 90–135 PSU in this study) DMS production peaks and methane production reaches a minimum. However, when the salinity increases, the DMS flux rapidly decreases to low values above 210 PSU. In contrast, the methane fluxes increase from 195 PSU and peak at 210–250 PSU in this study, when the competition with SRB ceases, as the latter organisms are presumably no longer very active (Fig. 9). At the highest salinities in this study, the activities of all organisms (including cyanobacteria; Fig. 8 bottom panel) are very low. Clearly, this pattern warrants further investigations, but it is plausible that the ratio of methane to DMS (and/or other methyl sulfides) may be a helpful tool to obtain a snapshot of the physiology of the entire community, as it is tied to a physiological response to salinity. Interestingly, Sørensen and coworkers reported that the optimum salinity for sulfate reduction in hypersaline mat slurries was between 100 and 120 PSU (near the in situ value of the mat) and that sulfate reduction was strongly inhibited when the salinity was increased to values of 215 PSU and higher (Sørensen et al., 2004).

5. Conclusion

Hypersaline microbial mats are outstanding model systems for life on early Earth and potentially elsewhere. “Extreme conditions” which favor microbial mat communities may mimic early Earth conditions and possibly even the conditions on extra-planetary bodies; for example, recent studies of Mars suggest that water may have occurred in shallow, hypersaline ponds (Duxbury et al., 2004; Madden

et al., 2004; Mancinelli et al., 2004; Squyres, 2004). Furthermore, calcium carbonate recently confirmed by the Phoenix lander near the Martian North Pole (Hand, 2008) could potentially contain evidence for microbial Martian life. Simple sulfur gases, such as methanethiol, could be utilized as biosignatures in the search for life beyond the Solar system (Pilcher, 2003) through remote sensing technology. Although it may remain controversial whether mineral and volatile signatures are indeed of biogenic origin (Allwood et al., 2006; Grotzinger and Knoll, 1999; Peters et al., 2005), the investigation of contemporary hypersaline microbial mats and the biosignatures they produce provides evidence in the ongoing quest to understand both our future and our past.

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7. References

Allwood, A.C., Walter, M.R., Kamber, B.S., Marshall, C.P. and Burch, I.W. (2006) Stromatolite reef from the Early Archaean era of Australia. Nature **441**: 714–718.

Andres, M.S., Sumner, D.Y., Reid, R.P. and Swart, P.K. (2006) Isotopic fingerprints of microbial respiration in aragonite from Bahamas stromatolites. Geology **34**: 973–976.

Arp, G., Thiel, V., Reimer, A., Michaelis, W. and Reitner, J. (1999) Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. Sed. Geol. **126**: 159–176.

Arp, G., Reimer, A. and Reitner, J. (2001) Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. Science **292**: 1701–1704.

Arp, G., Reimer, A. and Reitner, J. (2003) Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. J. Sediment. Res. **73**: 105–127.

Awramik, S.M. (1992) The history and significance of stromatolites, In: M. Schidlowski, M.M. Kimberley, D.M. McKirdy and P.A. Trudinger (eds.) *Early Organic Evolution. Implications for Energy and Mineral Resources*. Springer, Berlin, pp. 435–449.

Bak, F. and Widdel, F. (1986) Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., sp. nov. Arch. Microbiol. **146**: 170–176.

Baumgartner, L.K., Reid, R.P., Dupraz, C., Decho, A.W., Buckley, D.H., Spear, J.R., Przekop, K.M. and Visscher, P.T. (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sed. Geol. **185**: 131–145.

Baumgartner, L.K., Dupraz, C., Buckley, D.H., Spear, J.R., Pace, N.R. and Visscher, P.T. (2009) Microbial species richness and metabolic activities in hypersaline microbial mats: insight into biosignature formation through lithification. Astrobiology **9**: 861–874.

Bebout, B.M., Carpenter, S., Des Marais, D.J., Discipulo, M., Embaye, T., Garcia-Pichel, F., Hoehler, T.M., Hogen, M., Jahnke, L.L., Keller, R.M., Miller, S.R., Prufert-Bebout, L.E., Raleigh, C.,

Rothrock, M. and Turk, K. (2002) Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating Earths present and past field environments. Astrobiology **2**: 383–402.

Bebout, B.M., Hoehler, T.M., Thamdrup, B., Albert, D., Carpenter, S.P., Hogan, M., Turk, K. and Des Marais, D.J. (2004) Methane production by microbial mats under low sulphate concentrations. Geobiology **2**: 87–96.

Benson, L. (1994) Carbonate deposition, Pyramid Lake Subbasin, Nevada: 1. Sequence of formation and elevational distribution of carbonate deposits (tufas). Palaeogeogr. Palaeocl. **109**: 55–87.

Bober, C., Mojica, K. and Cooney, M. (2005) Quantification of single-species marine biofilm with Alcian Blue. J. Young Investigator **12**: 1–7.

Bontognali, T.R.R., Vaconcelos, C., Warthmann, R.J., Dupraz, C., Bernasconi, S.M. and McKenzie, J.A. (2008) Microbes produce nanobacteria-like structures, avoiding cell entombment. Geology **36**: 663–666.

Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M. and Visscher, P.T. (2007) Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. Geobiology **5**: 401–411.

Braissant, O., Decho, A.W., Przekop, K.M., Gallagher, K.L., Glunk, C., Dupraz, C. and Visscher, P.T. (2009) Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat. FEMS Microbiol. Ecol. **67**: 293–307.

Buckley, D.H., Baumgartner, L.K. and Visscher, P.T. (2008) Vertical distribution of methane metabolism in microbial mats of the Great Sippewissett Salt Marsh. Environ. Microbiol. **10**: 967–977.

Burne, R.V. and Moore, L.S. (1987) Microbialites: organosedimentary deposits of benthic microbial communities. Palaios **2**: 241–254.

Cady, S.L., Farmer, J.D., Grotzinger, J.P., Schopf, J.W. and Steele, A. (2003) Biosignatures and the search for life on Mars. Astrobiology **3**: 351–368.

Camoin, G., Cabioch, G., Eisenhauer, A., Braga, J.-C., Hamelin, B. and Lericolais, G. (2006) Environmental significance of microbialites in reef environments during the last deglaciation. Sed. Geol. **185**: 277–295.

Canfield, D.E. and Des Marais, D.J. (1991) Aerobic sulfate reduction in microbial mats. Science **251**: 1471–1473.

Canfield, D.E. and Des Marais, D.J. (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim. Cosmochim. Acta **57**: 3971–3984.

Canfield, D.E. and Des Maris, D.J. (1994) Cycling of carbon, sulfur oxygen, and nutrients in a microbial mat, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structures, Development and Environmental Significance.* Springer, Heidelberg, pp. 255–264.

Caroff, L.I. and Des Marais, D.J. (eds.) (2005) *Pale Blue Dot 2 Workshop: Habitable and Inhabited Worlds Beyond Our Solar System*. NASA Conference Publication 2000-209595, NASA Ames Research Center, Moffett Field, CA, USA.

Casillas-Martinez, L., Gonzalez, M.L., Fuentes, Z., Nieves, D., Hernandez, C., Ramirez, W., Sytsma, R.E., Perez-Jimenez, J. and Visscher, P.T. (2005) Community structure, geochemical characteristics and mineralogy of a hypersaline microbial mat, Cabo Rojo, PR. Geomicrobiol. J. **22**: 269–281.

Castenholz, R.W. (1994) Microbial mat research: the recent past and new perspectives, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 3–18.

Catling, D.C., Zahnle, K.J. and McKay, C.P. (2001) Biogenic methane, hydrogen escape, and the irreversible oxidation of early Earth. Science **293**: 839–843.

Chapelle, F.H., O'Neil, K., Bradley, P.M., Methe, B.A., Ciufo, S.A., Knobel, L.L. and Lovley, D.R. (2002) A hydrogen-based subsurface microbial community dominated by methanogens. Nature **415**: 312–315.

Cohen, Y., Helman, Y. and Sigalevich, P. (1994) Light-driven sulfate reduction and methane emission in hypersaline cyanobacterial mats, In: L.J. Stal and P. Caumette (eds.) *Microbial Mats: Structure, Development and Environmental Significance*. Springer, Heidelberg, pp. 421–427.

Conrad, R., Frenzel, P. and Cohen, Y. (1995) Methane emission from hypersaline microbial mats: lack of aerobic methane oxidation activity. FEMS Microbiol. Ecol. **16**: 297–306.

Decho, A.W., Visscher, P.T. and Reid, R.P. (2005) Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Palaeogeol. Palaeocl. **219**: 71–86.

Défarge, C., Issa, O.M. and Trichet, J. (1999) Field emission cryo-scanning electron microscopy of organic matter and organomineral associations. Application to microbiotic soil crusts. C. R. Acad. Sci. II A. / Earth Planet. Sci. **328**: 591–597.

Des Marais, D.J. (1990) Microbial mats and the early evolution of life. Trends Ecol. Evol. **5**: 140–144.

Des Marais, D.J. (1995) The biogeochemistry of hypersaline microbial mats. Adv. Microb. Ecol. **14**: 251–274.

Des Marais, D.J., Cohen, Y., Nguyen, H., Cheatham, M., Cheatham, T. and Munoz, E. (1989) Carbon isotopic trends in the hypersaline ponds and microbial mats at Guerrero Negro, Baja California Sur, Mexico: implications for Precambrian stromatolites, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiology Ecology of Benthic Microbial Communities*. ASM Press, Washington, DC, pp. 191–203.

Des Marais, D.J., Harwit, M.O., Jucks, K.W., Kasting, J.F., Lin, D.N.C., Lunine, J., Schneider, J., Seager, S., Traub, W.A. and Woolf, N.J. (2003) Remote sensing of planetary properties and biosignatures on extrasolar terrestrial (Vol. 2, pg 153, 2002). Astrobiology **3**: 216–216.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**: 350–356.

Dupraz, C. and Visscher, P.T. (2005) Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol. **13**: 429–438.

Dupraz, C., Visscher, P.T., Baumgartner, L.K. and Reid, R.P. (2004) Microbe–mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas). Sedimentology **51**: 745–765.

Dupraz, C., Reid, R.P., Braissant, O., Norman, S. and Visscher, P.T. (2009) Processes of carbonate precipitation in modern microbial mats. Earth-Sci. Rev. **96**:141–162.

Duxbury, N.S., Abyzov, S.S., Romanovsky, V.E. and Yoshikawa, K. (2004) A combination of radar and thermal approaches to search for methane clathrate in the Martian subsurface. Planet. Space Sci. **52**: 109–115.

Epping, E.H.G., Khalili, A. and Thar, R. (1999) Photosynthesis and the dynamics of oxygen consumption in a microbial mat as calculated from transient oxygen microprofiles. Limnol. Oceanogr. **44**: 1936–1948.

Giani, D., Giani, L., Cohen, Y. and Krumbein, W.E. (1984) Methanogenesis in the hypersaline Solar Lake (Sinai). FEMS Microbiol. Ecol. **25**: 219–224.

Grey, K., Moore, L.S., Burne, R.V., Pierson, B.K. and Bauld, J. (1990) Lake Thetis, Western Australia: and example of saline lake sedimentation dominated by benthic microbial processes. Aust. J. Mar. Fresh. Res. **41**: 275–300.

Grotzinger, J.P. and Knoll, A.H. (1999) Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? Annu. Rev. Earth Planet. Sci. **27**: 313–358.

Hand, E. (2008) Phoenix: a race against time. Nature **456**: 691–695.

Hashimoto, W., Miki, H., Tsuchiya, N., Nankai, H. and Murata, K. (1998) Xanthan lyase of *Bacillus* sp. strain GL1 liberates pyruvylated mannose from xanthan side chains. Appl. Environ. Microbiol. **64**: 3765–3768.

Heyer, J., Berger, U., Hardt, M. and Dunfield, P.F. (2005) *Methylohalobius crimeensis* gen. nov., sp. nov., a moderately halophilic, methanotrophic bacterium. Int. J. Syst. Evol. Microbiol. **55**: 1817–1826.

Hoehler, T.M., Bebout, B.M. and Des Marais, D.J. (2001) The role of microbial mats in the production of reduced gases on the early Earth. Nature **412**: 324–327.

Humayoun, S.B., Bano, N. and Hollibaugh, J.T. (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. Appl. Environ. Microbiol. **69**: 1030–1042.

Jonkers, H.M., Ludwig, R., De Wit, R., Pringault, O., Muyzer, G., Niemann, H., Finke, N. and De Beer, D. (2003) Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol. Ecol. **44**: 175–189.

Jørgensen, B.B. (2001) Space for hydrogen. Nature **412**: 286–289.

Jørgensen, B.B. and Cohen, Y. (1977) Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mats. Limnol. Oceanogr. **22**: 657–666.

Jorgensen, B.B., Revsbech, N.P. and Cohen, Y. (1983) Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. **28**: 1075–1093.

Kasting, J.F., Pavlov, A.A. and Siefert, J.L. (2001) A coupled ecosystem-climate model for predicting the methane concentration in the archaean atmosphere. Orig. Life Evol. Biosph. **31**: 271–285.

Kiene, R.P. and Visscher, P.T. (1987) Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate. Appl. Environ. Microbiol. **53**: 2426–2434.

King, G.M. (1988) Methanogenesis from methylated amines in a hypersaline algal mat. Appl. Environ. Microbiol. **54**: 130–136.

Kruschel, C. and Castenholz, R.W. (1998) The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypersaline waters. FEMS Microbiol. Ecol. **27**: 53–72.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

Luther III, G.W., Church, T.M., Scudlark, J.R. and Cosman, M. (1986) Inorganic and organic sulfur cycling in salt-marsh pore waters. Science **232**: 746–749.

Madden, M.E.E., Bodnar, R.J. and Rimstidt, J.D. (2004) Jarosite as an indicator of water-limited chemical weathering on Mars. Nature **431**: 821–823.

Mancinelli, R.L., Fahlen, T.F., Landheim, R. and Klovstad, M.R. (2004) Brines and evaporites: analogs for Martian life. Adv. Space Res. **33**: 1244–1246.

Mann, C.J. and Nelson, W.M. (1989) Microbialitic structures in Storr's Lake, San Salvador Island, Bahamas Islands. Palaios **4**: 287–293.

Megonigal, J.P., Visscher, P.T. and Hines, M.E. (2003) Anaerobic metabolism and the production of trace gases, In: *Treatise on Geochemistry*, Vol. 8. Elsevier, Amsterdam, pp. 1–113.

Nankai, H., Hashimoto, W., Miki, I., Kawai, S. and Murata, K. (1999) Microbial system for polysaccharide depolymerization: enzymatic route for xanthan depolymerization by *Bacillus* sp. strain GL1. Appl. Environ. Microbiol. **65**: 2520–2526.

Navarrete, A., Peacock, A., Macnaughton, S.J., Urmeneta, J., Mas-Castellà, J., White, D.C. and Guerrero, R. (2000) Physiological status and community composition of microbial mats of the Ebro Delta, Spain, by signature lipid biomarkers. Microb. Ecol. **39**: 92–99.

Nealson, K.H. and Stahl, D.A. (1997) Microorganisms and biogeochemical cycles: what can we learn from layered microbial communities? In: J.F. Banfield and K.H. Nealson (eds.) *Geomicrobiology: Interactions Between Microbes and Minerals*, Vol. 35. Reviews in Mineralogy. Mineralogical Society of America, Washington, DC, pp. 5–34.

Oremland, R.S. and King, G.M. (1989) Methanogenesis in hypersaline environments, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*. ASM, Washington, DC, pp. 180–190.

Passow, U. and Alldredge, A.L. (1995) A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnol. Oceanogr. **40**: 1326–1335.

Perry, R.S., Mcloughlin, N., Lynne, B.Y., Sephton, M.A., Oliver, J.D., Perry, C.C., Campbell, K., Engel, M.H., Farmer, J.D., Brasier, M.D. and Staley, J.T. (2007) Defining biominerals and organominerals: direct and indirect indicators of life. Sed. Geol. **201**: 157–179.

Peters, K.E., Walters, C.C. and Moldowan, J.M. (2005) *The Biomarker Guide*. Cambridge University Press, Cambridge.

Pierson, B., Oesterle, A. and Murphy, G.L. (1987) Pigments, light penetration, and photosynthetic activity in the multi-layered microbial mats of Great Sippewissett Salt Marsh, Massachusetts. FEMS Microbiol. Ecol. **45**: 365–376.

Pilcher, C.B. (2003) Biosignatures of early earths. Astrobiology **3**: 471–486.

Pinckney, J., Paerl, H.W. and Bebout, B.M. (1995) Salinity control of benthic microbial mat community production in a Bahamian hypersaline lagoon. J. Exp. Mar. Biol. Ecol. **187**: 223–237.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.K., Bebout, B.M., Dupraz, C., Mactintyre, I.G., Paerl, H.W., Pinckney, J.L., Prufert-Bebout, L., Steppe, T.F. and Des Marais, D.J. (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature **406**: 989–992.

Reitner, J., Paul, J., Arp, G. and Hause-Reitner, D. (1996) Lake thetis domal microbialites–a complex framework of calcified biofilms and organomicrites (Cervantes, Western Australia), In: J. Reitner, F. Neuweiler and F. Gunkel (eds.) *Global and Regional Controls on Biogenetic Sedimentation. I. Reef Evolution*. Research Reports, Vol. 2. Göttinger Arb. Geol. Paläont., Sonderband, Göttingen, pp. 85–89.

Relexans, J.C. (1996) Measurements of the respiratory electron transport system (ETS) activity in marine sediments: state-of- the-art and interpretation. I. Methodology and review of the literature data. Mar. Ecol. Prog. Ser. **136**: 277–287.

Slobodkin, A.I. and Zavarzin, G.A. (1992) Methane production in halophilic cyanobacterial mats in lagoons of Lake Sivash. Mikrobiologiya **61**: 294–298.

Soetaert, K., Hofmann, A.F., Middelburg, J.J., Meysman, F.J.R. and Greewood, J. (2007) The effect of biogeochemical processes on pH. Mar. Chem. **105**: 30–51.

Sørensen, K.B., Canfield, D.E. and Oren, A. (2004) Salt responsesof benthic microbial communities in a solar saltern (Eilat, Israel). Appl. Environ. Microbiol. **70**: 1608–1616.

Sorokin, D.Y., Gorlenko, V.M., Namsaraev, B.B., Namsaraev, Z.B., Lysenko, A.M., Eshinimaev, B.T., Khmelenina, V.N., Trotsenko, Y.A. and Kuenen, J.G. (2004) Prokaryotic communities of the north-eastern Mongolian soda lakes. Hydrobiologia **522**: 235–248.

Spear, J.R., Walker, J.J., McCollom, T.M. and Pace, N.R. (2005) Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. Proc. Natl. Acad. Sci. USA **102**: 2555–2560.

Squyres, S.W. (2004) Science results from the MER Athena science investigation at Gusev Crater and Meridiani Planum. Astrobiology Supplement **1**: 5.

Stal, L.J., van Gemerden, H. and Krumbein, W.E. (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol. Ecol. **31**: 111–125.

Turner, B.F. and Fein, J.B. (2006) Protofit: a program for determining surface protonation constants from titration data. Comput. Geosci. **32**: 1344–1356.

van Gemerden, H. (1993) Microbial mats: a joint venture. Mar. Geol. **113**: 3–25.

Vasconcelos, C., Warthmann, R., McKenzie, J.A., Visscher, P.T., Bittermann, A.G. and van Lith, Y. (2006) Lithifying microbial mats in Lagoa Vermelha, Brazil: modern Precambrian relics? Sed. Geol. **185**: 175–183.

Visscher, P.T. and Stolz, J.F. (2005) Microbial mats as bioreactors: populations, processes and products. Palaeogeogr. Palaeocl. **219**: 87–100.

Visscher, P.T. and van Gemerden, H. (1991) Photoautotrophic growth of *Thiocapsa roseopersicina* on dimethyl sulfide. FEMS Microbiol. Lett. **81**: 247–250.

Visscher, P.T., Beukema, J. and van Gemerden, H. (1991a) In situ characterization of sediments: measurements of oxygen and sulfide profiles with a novel combined needle electrode. Limnol. Oceanogr. **36**: 1476–1480.

Visscher, P.T., Quist, P. and van Gemerden, H. (1991b) Methylated sulfur compounds in microbial mats: *in situ* concentrations and metabolism by a colorless sulfur bacterium. Appl. Environ. Microbiol. **57**: 1758–1763.

Visscher, P.T., van den Ende, F.P., Schaub, B.E.M. and van Gemerden, H. (1992) Competition between anoxygenic phototrophic bacteria and colorless sulfur bacteria in a microbial mat. FEMS Microbiol. Ecol. **101**: 51–58.

Visscher, P.T., Guidetti, J.R., Culbertson, C.W. and Oremland, R.S. (1996) Dimethylsulfoniopropionate as a potential methanogenic substrate in mono lake sediments, In: R.P. Kiene, P.T. Visscher, M.D. Keller and G.O. Kirst (eds.) *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*. Plenum, New York, pp. 361–368.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., Macintyre, I.G. and Thompson, J.A. (1998) Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfure cycling. Am. Mineral. **83**: 1482–1493.

Visscher, P.T., Gritzer, R. and Leadbetter, E.R. (1999) Low-molecular-weight fulfonates, a major substrate for sulfate reducers in marine microbial mats. Appl Environ Microbiol **65**: 3273–3278.

Visscher, P.T., Reid, R.P. and Bebout, B.M. (2000) Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology **28**: 919–922.

Visscher, P.T., Surgeon, T.M., Hoeft, S.E., Bebout, B.M., Thompson, J., Jr. and Reid, R.P. (2002) Microelectrode studies in modern marine stromatolites: unraveling the Earth's past? In: M.T. Taillefert and T. Rozan (eds.) *Electrochemical Methods for the Environmental Analysis of Trace Metal Biogeochemistry*, ACS Symposium Series 220. Cambridge University Press, New York, pp. 265–282**.**

Visscher, P.T., Baumgartner, L.K., Buckley, D.H., Rogers, D.R., Hogan, M.E., Raleigh, C.D., Turk, K.A. and Des Marais, D.J. (2003) Dimethyl sulphide and methanethiol formation in microbial mats: potential pathways for biogenic signatures. Environ. Microbiol. **5**: 296–308.

Ward, D.M., Shiea, J., Zeng, Y.B., Dobson, G., Brassel, S. and Eglinton, G. (1989) Lipid biochemical markers and the composition of microbial mats, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiology Ecology of Benthic Microbial Communities*. ASM Press, Washington DC, pp. 439–454.

Yannarell, A.C. and Paerl, H.W. (2007) Effect of salinity and light on organic carbon and nitrogen uptake in a hypersaline microbial mat. FEMS Microbiol. Ecol. **62**: 345–353.

Zhilina, T.N. (1986) Methanogenic bacteria from hypersaline environments. Syst. Appl. Microbiol. **7**: 216–222.

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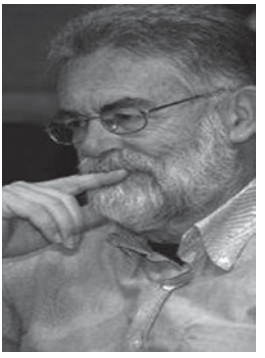

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PHOTOTROPHIC BIOFILMS FROM RÍO TINTO, AN EXTREME ACIDIC ENVIRONMENT, THE PROKARYOTIC COMPONENT

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1. Introduction

Biofilms are the organic layers that form on submerged surfaces in rivers, streams, or other aquatic environments. They are usually composed of microorganisms (prokaryotes and eukaryotes), extracellular products, and accumulated debris enmeshed in a matrix of extracellular polymeric substances (EPS) (Lock, 1993; Marshall, 1992). These biofilms are found in almost every body of flowing water and act as a trophic link between dissolved nutrients in the water column and the higher trophic levels of the ecosystem (Hynes, 1970). They play a key role in the uptake and retention of inorganic and organic nutrients (Lock, 1993; Romaní et al., 2004).

Many microbial processes that occur in the environment are not possible with single-species populations but require consortial activities (Geesey and Costerton, 1986). Typically, such activities are interactions within a given community among two or more populations of organisms, which enable them to maximize their metabolic capabilities and to maintain community integrity and stability. Many aquatic microorganisms, particularly bacteria, are metabolically most active when associated with surfaces (Lock, 1993). As biofilms develop, microorganisms secrete an extracellular matrix composed mainly of polysaccharides, which promote retention of organic matter and exoenzymes, and act as an ion exchange media. As biofilm development continues, a complex stratified community forms, with aerobic autotrophic and heterotrophic microorganisms near the surface and microorganisms sensitive to oxygen and/or radiation immersed in the biofilm matrix, close to the substrate. After attachment, the phototrophic population creates a nutrient-rich microzone by producing and excreting organic compounds (Haak and McFeters, 1982). The ability to remain within this microzone

by adhesion processes enables microorganisms to proliferate even in environments otherwise unsuitable for their survival. This is supported by the occurrence of sessile communities in many extreme environments, ranging from the hot springs of thermal areas (Ferris et al., 2005) to the cold oligotrophic waters of mountain springs (Haack and McFeters, 1982).

Biofilm development is believed to confer to the constituent microorganisms a number of advantages over free-living organisms (Davey and O'Toole, 2000). The most important are the enhancement of nutrient availability, the increase of metabolic cooperation, and the acquisition of new genetic traits through horizontal gene transfer (Costerton et al., 1995; Dahlberg et al., 1997). However, the organizational features of a biofilm are such that the constituent microorganisms maintain conditions within the biofilm that can be radically different from those of the water column, making it feasible to physically prevent the access of certain toxic substances or adverse environmental conditions (pH, O_2, radiation).

This fact is particularly important in the case of extreme environments, in which the disposition of microorganisms forming biofilms could be critical for their protection from the harsh environmental conditions. Of the existing extreme environments available for microbial diversity and ecology studies, acidic ecosystems (pH < 3) are becoming increasingly important since this extreme condition is often caused by microbial activity (Bond et al., 2000; Hallberg and Johnson, 2001; Amils et al., 2003). The natural oxidation and dissolution of the sulfidic mineral deposits exposed to oxygen and water results in acid production, and the process can be greatly enhanced by microbial metabolism (Nordstrom and Southam, 1997; González-Toril et al., 2001). At the same time, low pH facilitates metal solubility, particularly cationic metals and therefore acidic water tends to have high concentrations of heavy metals (Johnson, 1998; Amils et al., 2003).

Highly acidic environments are relatively scarce worldwide and are generally associated with volcanic activity and mining operations (Amils et al., 2003; Baffico et al., 2004; Druschel et al., 2004). Thus, since extreme acidic environments are often the consequence of anthropogenic influences (e.g., mining activity, acid rain), most ecological studies of acidic waters have been focused on environments affected by human activity. In this regard, Río Tinto (Iberian Pyritic Belt) is one of the most unique examples of extreme acidic environments, not only because of its natural origin (Fernández-Remolar et al., 2003, 2005) but also for its peculiar microbial ecology (López-Archilla et al., 2001; Amaral-Zettler et al., 2002). The river originates in the massive bodies of iron sulfides that make up the Iberian Pyritic Belt, and maintains a rather constant low pH (pH between 1.0 and 2.5), buffered by ferric iron and with high concentrations of heavy metals that are toxic to numerous aquatic organisms. These extreme conditions are the product of the metabolic activity of chemolithotrophic microorganisms, including iron- and sulfur-oxidizing bacteria that can be found in high numbers in its waters (González-Toril et al., 2003).

2. Eukaryotic Diversity in Río Tinto

As mentioned, besides the extreme conditions found in the system, what makes Río Tinto a unique extreme environment is the unexpected degree of microbial diversity, mainly eukaryotic, found in its waters (López-Archilla et al., 2001; Amaral-Zettler et al., 2002; Aguilera et al., 2006a,b. Eukaryotic microorganisms are the main contributors to the biomass of the river (López-Archilla et al., 2001). Most of the eukaryotic species thriving in Río Tinto are photosynthetic. Among these, chlorophytes related to different genera such as *Chlamydomonas*, *Dunaliella*, *Chlorella*, as well as *Euglena* are the dominant eukaryotic microorganisms. Species of these genera are known for their high metal and acid tolerance. Filamentous algae, represented by the genera *Zygnemopsis* and *Klebsormidium*, form large blooms especially during spring and summer. Other chlorophytes such as species belonging to the genera *Mesotaenium* and *Stichococcus* have also been detected although in low numbers.

The most acidic part of the river is inhabited by eukaryotic communities dominated by two species related to the *Dunaliella* (*Chlorophyta*) and *Cyanidium* (*Rhodophyta*) genera. The genus *Dunaliella* includes some of the most extreme acidophiles reported until now. Pennate diatoms are also present in the river forming large brown biofilms. These biofilms are usually dominated by only one species related to the genera *Pinnularia*, although some other minority genera have been identified, such as *Nitzschia* or *Cyclotella*.

In addition to photosynthetic species, heterotrophic protists are also widely distributed throughout the river. The mixotrophic flagellates are dominated by members of the genera *Bodo* and *Ochromonas*. Phagotrophic species such as ciliates, cercomonads, amoebae, and heliozoans have been also found in Río Tinto. At least two species of ciliates are members of the community. The dominant ciliate taxa belong to the order *Hypotrichida*. Amoebas can be found frequently, even in the most acidic parts of the river, feeding on diatoms. *Valhkampfia* species have been identified in addition to other species, including lobosea-like and acanthamoeba-like amoebas.

One species of heliozoan belonging to the genera *Actinophyris* is also present in the river. Heliozoa seem to be the characteristic top predators of the benthic food chain in the ecosystem. The only animal found in the river is a species of bdelloid rotifer related to the *Rotifera* genera (Amaral-Zettler et al., 2003; Aguilera et al., 2007a). Within the decomposer organisms, fungi are the most abundant, exhibiting great diversity, including yeast and filamentous forms (López-Archilla et al., 2005).

The seasonal bimodality of Río Tinto greatly influences the eukaryotic community biomass (Aguilera et al., 2007a). In general, green algae are responsible for most of the total eukaryotic biomass increase during the summers. This fact is closely related to the significant increase in temperature values and daylight as well as to the decrease in water flow, all of which promote biofilm formation.

3. Acidic Biofilms in the Tinto Basin

Microscopy observations of the Río Tinto biofilms revealed a variety of eukaryotic and prokaryotic microorganisms. The whole community is usually embedded in a thick mucilaginous coating that protects the inner microbial community from the external extreme conditions.

In situ colonization studies have shown that biofilm formation starts by the fixation of an organic conditioning biofilm, composed mainly of fungal hyphae, bacteria, and mineral detritus particles, which remain permanently attached to the substrate (Aguilera et al., 2007b). The next step is the colonization by pioneer motile eukaryotic species, such as amoeba or heterotrophic flagellates, followed by increasing numbers of sessile eukaryotes such *Chlorella* or diatoms. Finally, filamentous algae are incorporated into the biofilm.

The characterization of metal-exposed diatoms that form biofilms has shown that the extracellular mucous matrix could be partially responsible for an increase in biofilm tolerance to the toxic agents (Little et al., 1997; Barranguet et al., 2000). This matrix is mainly composed of extracellular polymeric substances (EPS) which provide a suitable micro-environment for microbial development (Wuertz et al., 2004).

Analysis of the internal structure of bacterial biofilms by confocal microscopy has shown that these communities form highly structured microbial assemblies. In the case of eukaryotic biofilms the internal structure has been less studied. The ultrastructural study with scanning electron microscopy in backscattered mode (Aguilera et al., 2007b) permitted transversal sections of the biofilms attached to the substrate to be observed. Microscale structural differences among naturally grown biofilms have been observed in different localities of Río Tinto. Although some of the biofilms form a well-defined layered structure, others show several layers of cells loosely packed between minerals or decaying organic matter. Although biofilms are dominated by a specific photosynthetic eukaryotic microorganism, bacteria are conspicuously detected throughout the Río Tinto biofilms. Some biofilms, such as those formed by *Dunaliella*, only appear in the most acidic localities and are clearly related with fungal communities.

In general, substrates are heterogeneously colonized and the factors involved are still poorly understood, but might be related to the specific physiology of the constituent microorganisms and the physico-chemical characteristics of the substrate surface (Hutchinson et al., 2006). Also water flow and age are considered important factors for biofilm structure. In the Tinto case, most of the biofilms are dramatically removed every year during the rainy season, due to the Mediterranean climatic regime of the river, which suggests that age is not an important parameter in the biofilm structure in this ecosystem.

Intrinsic physiological features of the microorganisms forming the biofilm can be also responsible for specific structures and their distribution on the riverbed. In the case of bacteria, the complex regulation of surface attachment, biofilm maturation and ultimately biofilm detachment is known to be affected by the

physiology of the cellular components (Davies et al., 1998). However, for eukaryotic biofilms data about intercellular relation and regulation in the formation of the biofilms are missing. Further in situ microsensor techniques studies and controlled mesocosm experiments will be necessary to fully understand the factors controlling eukaryotic biofilm formation and the associations among different microorganisms.

4. Prokaryotic Diversity in Phototrophic Acidic Biofilms of Río Tinto

Although photosynthetic biofilms have been described in Río Tinto (Amaral-Zettler et al., 2002; Aguilera et al., 2006a, b, 2007a, b) as well as in other acidic environments (Pierson et al., 1999; Pierson and Parenteau, 2000; Brake et al., 2001a, b; Baffico et al., 2004; García-Meza et al., 2005), the diversity of prokaryotic communities within these structures has been less studied. Usually, studies of the prokaryotic community in Río Tinto have been focussed on the water column (González-Toril et al., 2003), the oxic–anoxic interfaces (Malki et al., 2006) or the floating macroscopic filaments (García-Moyano et al., 2007).

Previous studies have found that the most representative bacteria species in the Río Tinto water column correspond to *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, both related to the oxidation of iron (González-Toril et al., 2003; Malki et al., 2006). These microorganisms are also well represented in the photosynthetic biofilms studied, except in the ones from the most extreme locations, where the predominant bacteria correspond to *Leptospirillum* spp. The capacity of *Leptospirillum ferrooxidans* to grow at a lower pH than *A. ferrooxidans* (Rawlings et al., 1999) might be the reason why this bacterium is not present in the biofilms at the most extreme parts of the river (pH 1.8).

Of the three known leptospirilli species, only *L. ferrooxidans* has been identified as an important member of the prokaryotic community in Río Tinto (González-Toril et al., 2003; García-Moyano et al., 2007, 2008). *Leptospirillum ferriphilum* (Coram and Rawlings, 2002) and *Leptospirillum ferrodiazotrophum* (Tyson et al., 2005) were only detected in low numbers in the water column and sediments of some locations (García-Moyano et al., 2007). Interestingly, all *Leptospirillum* species are well represented in the photosynthetic biofilms from the most extreme sampling location, indicating more favorable conditions for these species in environments with thinner photosynthetic biofilms and more extreme conditions. Fluorescence in situ hybridization experiments analysis (FISH and CARD-FISH) have also indicated that some clusters of bacteria belonging to *L. ferriphilum* and *L. ferrodiazotrophum* were present in these biofilms. This might indicate the presence of less restricted microenvironments in the biofilms where these species may prefer to develop.

The comparison of *Pinnularia* biofilms present in most of the sampling locations analyzed showed the highest diversity in terms of their prokaryotic content. These biofilms are formed by different layers of diatoms intermixed with sediments,

forming thicker biofilms and generating specific conditions that might facilitate the growth of microorganisms that otherwise would not be able to develop. The lowest level of prokaryotic diversity was found in the biofilms dominated by the red alga *Cyanidium*. Since the water physico-chemical characteristics from the locations where the *Cyanidium* biofilms were sampled are different, the low prokaryotic diversity should be related to the presence of *Cyanidium* and not to the water parameters. Another interesting result was observed in the biofilms dominated by the photosynthetic protist *Euglena mutabilis*. In this case, the prokaryotic diversity observed in euglenoid biofilms isolated from different sampling locations was always high. When high numbers of diatoms were present in the euglenoid biofilms, the prokaryotic diversity was also elevated.

The photosynthetic biofilm communities in the acidic waters of Río Tinto seem to have two contrasting roles in controlling the redox transformations of iron. On one hand, by oxygenating the water they promote iron oxidation, but they also provide organic carbon from their associated extrapolymeric substances that can be used by iron-reducing heterotrophic acidophiles. This second aspect is clearly related to the presence of *Acidiphilium* spp. in all the phototrophic biofilms analyzed, with the exception of the biofilms located at the lowest pH sampling stations (Fig. 1). *Acidiphilium* spp. can use ferric iron as an electron acceptor for anaerobic respiration of reduced organic compounds. The amount of organic biomass coming from the thinner photosynthetic biofilms at the most extreme locations in the river is lower than in all the other localities studied. The fact that *Acidiphilium* is not present in these biofilms might be due to the low pH and/or the absence of microaerobic conditions.

As mentioned, the structure of some of the biofilms analyzed seems to generate microniches that could facilitate the development of less extreme adapted organisms. Thus, sequences related to uncultured organisms present in acidic or contaminated soils such as *Betaproteobacteria* and *Acidobacteria* have also been detected in the Tinto photosynthetic biofilms. Previous studies of the prokaryotic communities associated with the rhizosphere of plants which grow at the banks of Río Tinto have shown that the main groups present corresponded to *Actinobacteria* and the *Acidobacteria* (Mirete et al., 2007).

It seems that thinner photosynthetic biofilms have less influence in the formation of microniches and consequently their prokaryotic community is more closely related to the one present in the water column. We can also find intermediate phototrophic biofilms in terms of prokaryotic biodiversity. For example, the biofilms dominated by diatoms seem to be more diverse in their prokaryotic communities, showing few clusters of *L. ferrooxidans* and *Acidiphilium* spp. and the presence of *Actinobacteria* and *Archaea* in the area near the substratum. *Chlorella* biofilms were more similar to the water column prokaryotic community showing clusters of *Leptospirillum* spp., *A. ferrooxidans*, and *Acidiphilium* spp. These results explain why we found higher prokaryotic diversity associated with photosynthetic biofilms than within the water column (Fig. 2).

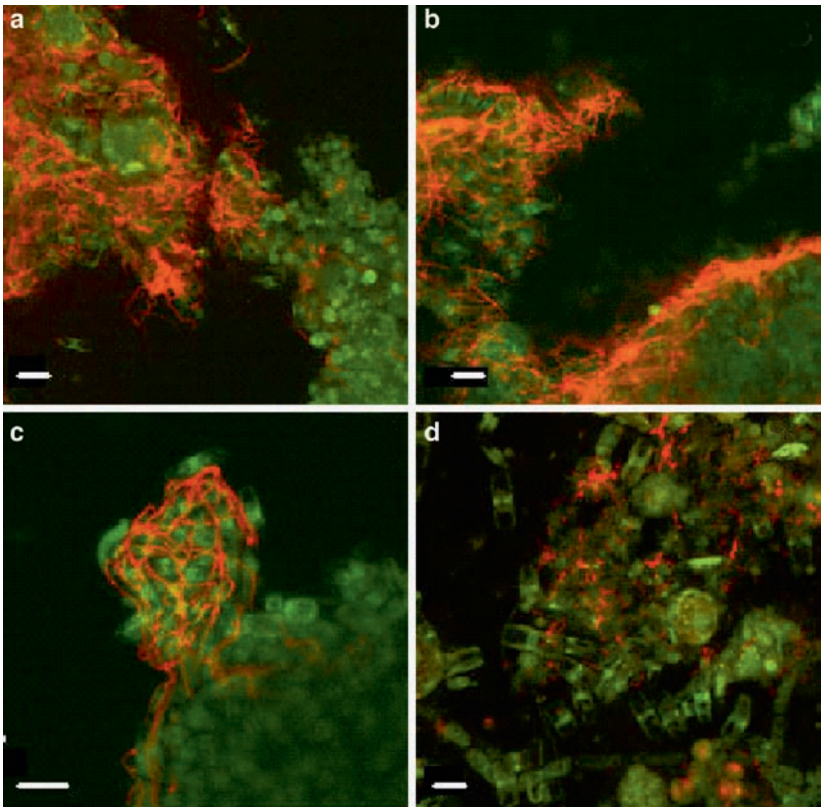

Figure 1. Laser scanning confocal images from photosynthetic biofilms hybridized with Cy3 labelLed probes. The bacteria are shown in red and the green autofluorescence came from chlorophyll (Bar = 5 μm). (**a**) Universal probe for *Bacteria* domain. (**b**) Specific probe for *Gammaproteobacteria*. (**c**) Specific probe for *Acidithiobacillus* spp. (**d**) Specific probe for *Acidiphilium* spp.

5. Final Comments

The data described above provide preliminary information regarding the prokaryotic diversity associated to acidic phototrophic biofilms. Our knowledge of the prokaryotic diversity of acid mine drainage habitats and natural acidic waters continues to increase with new cloning studies confirming the presence of uncultured new organisms. Based on the environmental sequences obtained by cloning, new hybridization probes can be designed to verify the abundance and spatial location of microbial community members in specific microenvironments in these and other extreme environments. The need to continue isolating microorganisms implicated in biohydrometallurgical process makes culture-independent

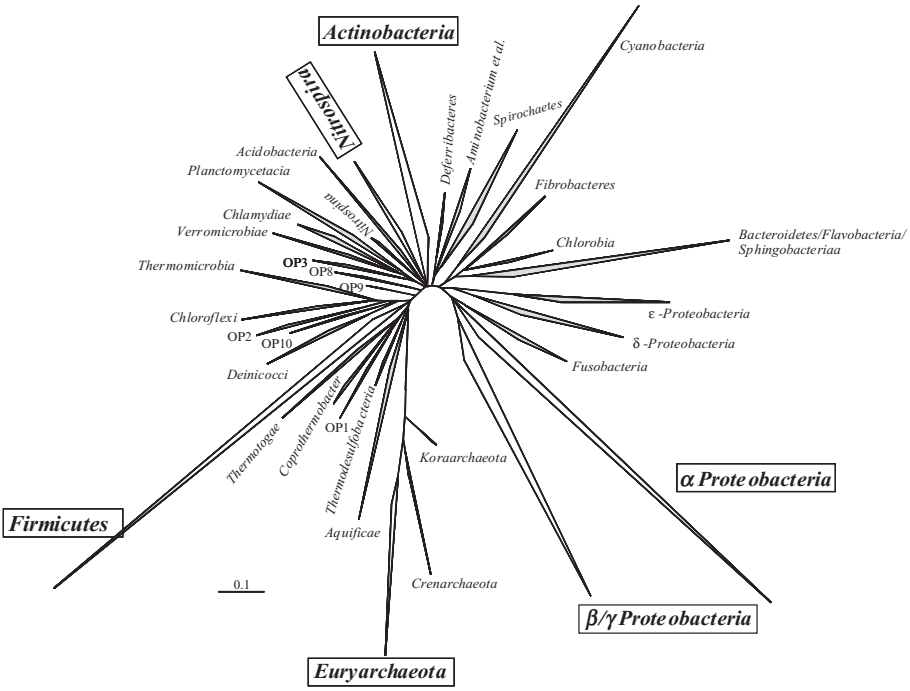

Figure 2. Phylogeny of 16S rRNA gene sequences from prokaryotic communities associated with photosynthetic biofilms of Río Tinto. Tree generated using parsimony and maximum likelihood in ARB package method with different sets of filters (Bar represents 10% difference in nucleotide sequences).

techniques valuable tools with which to evaluate the presence and distribution of specific prokaryotic microorganisms in photosynthetic acidic biofilms. This makes it possible to perform in situ structure/function analysis along with hybridization combined with microsensors and ultrastructure information of the biofilms in their natural habitat. These studies are the basis for a more comprehensive knowledge of the microbial communities associated to extreme acidic environments and other underexplored extreme habitats.

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7. References

Aguilera, A., Manrubia, S.C., Gómez, F., Rodríguez, N. and Amils, R. (2006a) Eukaryotic community distribution and its relatioship with to water physicochemical parameters in an extreme acidic environment, Río Tinto (Southwestern Spain). Appl. Environ. Microbiol. **72**: 5325–5330.

Aguilera, A., Gómez, F., Lospitao, E. and Amils, R. (2006b) A molecular approach to the characterization of the eukaryotic communities of an extreme acidic environment: methods for DNA extraction and denaturing gradient gel electrophoresis analysis. Syst. Appl. Microbiol. **29**: 593–605.

Aguilera, A., Zettler, E., Gómez, F., Amaral-Zettler, L., Rodríguez, N. and Amils, R. (2007a) Distribution and seasonal variability in the benthic eukaryotic community of Rio Tinto (SW Spain), an acidic, high metal extreme environment**.** Syst. Appl. Microbiol. **30**: 531–546.

Aguilera, A., Souza-Egipsy, V., Gómez, F. and Amils, R. (2007b) Development and structure of eukaryotic biofilms in an extreme acidic environment, Río Tinto (SW, Spain). Microb. Ecol. **53**: 294–305.

Amaral-Zettler, L.A., Gómez, F., Zettler, E., Keenan, B.G., Amils, R. and Sogin, M.L. (2002) Eukaryotic diversity in Spain's River of Fire. Nature **417**: 137.

Amaral-Zettler, L.A., Messerli, M., Laatsch, A., Smith, P. and Sogin, M.L. (2003) From genes to genomes: beyond biodiversity in Spain's Río Tinto. Biol. Bull. **204**: 205–209.

Amils, R., González-Toril, E., Fernández-Remolar, D., Gómez, F., Rodríguez, N. and Durán, C. (2003) Interaction of the sulfur and iron cycles in the Tinto River ecosystem. Rev. Environ. Sci. Biotechnol. **1**: 299–309.

Baffico, G.D., Díaz, M.M., Wenzel, M.T., Koschorreck, M., Schimmele, M., Neu, T.R. and Pedrozo, F. (2004) Community structure and photosynthetic activity of epilithon from a highly acidic (pH<2) mountain stream in Patagonia, Argentina. Extremophiles **8**: 465–475.

Barranguet, C., Charantoni, E.M., Plans, E. and Admiraal, W. (2000) Short-term response of monospecific and natural algal biofilms to copper exposure. Eur. J. Physiol. **35**: 397–406.

Bond, P.L., Smriga, S.P. and Banfield, J.F. (2000) Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl. Environ. Microbiol. **66**: 3842–3849.

Brake, S.S., Dannelly, H.K. and Connors, K.A. (2001a) Controls on the nature and distribution of an alga in coal mine-waste environments and its potential impact on water quality. Environ. Geol. **40**: 458–469.

Brake, S.S., Dannelly, H.K., Connors, K.A. and Hasiotis, S.T. (2001b) Influence of water chemistry on the distribution of an acidophilic protozoan in an acid mine drainage system at the abandoned Green Valley coal mine, Indiana, USA. Appl. Geochem. **16**: 1641–1652.

Coram, N.J. and Rawlings, D.E. (2002) Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African commercial biooxidation tanks that operate at 40ºC. Appl. Environ. Microbiol. **68**: 838–845.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R. and Lappin-Scott, H.M. (1995) Microbial biofilms. Ann. Rev. Microbiol. **49**: 711–745.

Dahlberg, C., Linberg, C., Torsvik, V.L. and Hermansson, M. (1997) Conjugative plasmids isolated from bacteria in marine environments show various degrees of homology to each other and are not closely related to well-characterized plasmids. Appl. Environ. Microbiol. **63:** 4692–4697.

Davey, M.E. and O'Toole, G.A. (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. **64**: 847–867.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. and Greeberg, E.P. (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **280**: 295–298.

Druschel, G.K., Baker, B.J., Gihring, T.H. and Banfield, J.F. (2004) Biogeochemistry of acid mine drainage at Iron Mountain, California. Geochem. Trans. **5**: 13–32.

Fernández-Remolar, D.C., Rodríguez, N., Gómez, F. and Amils, R. (2003) Geological record of an acidic environment driven by the iron hydrochemistry: the Tinto River system. J. Geophys. Res. **108**: 5080–5095.

Fernández-Remolar, D.C., Morris, R.V., Gruener, J.E., Amils, R. and Knoll, A.H. (2005) The Río Tinto Basin, Spain: mineralogy, sedimentary geobiology and implications for interpretation of outcrop rocks at Maridianu Planum, Mars. Earth Planet. Sci. Lett. **240**: 149–167.

Ferris, M.J., Sheehan, K.B., Kühl, M., Cooksey, K., Wigglesworth-Cooksey, B., Harvey, R. and Henson, J.M. (2005) Algal species and light microenvironment in a low-pH, geothermal microbial mat community. Appl. Environ. Microbiol. **71**: 7164–7171.

García-Meza, J.V., Barrangue, C. and Admiraal, W. (2005) Biofilm formation by algae as a mechanism for surviving on mine tailings. Environ. Toxicol. Chem. **24**: 573–582.

García-Moyano, A., González-Toril, E., Aguilera, A. and Amils, R. (2007) Prokaryotic community composition and ecology of macroscopic filaments from an extreme acidic environment, Río Tinto (SW Spain). Syst. Appl. Microbiol. **30**: 601–614**.**

García-Moyano, A., Gónzalez-Toril, E., Moreno-Paz, M., Parro, V. and Amils, R. (2008) Evaluation of *Leptospirillum* spp. in the Río Tinto basin, a model of interest to biohydrometallurgy. Hydrometallurgy **94**: 155–161.

Geesey, G.G. and Costerton, J.W. (1986) The microphysiology of consortia within adherent bacterial populations, In: F. Megusar and M. Gantar (eds.) *Perspectives in Microbial Ecology*. Mladinska Kniga, Ljubljana, Slovenia, pp. 238–242.

González-Toril, E., Gómez, F., Rodríguez, N., Fenández-Remolar, D., Zuluaga, J., Marín, I. and Amils, R. (2001) Geomicrobiology of the Tinto River, a model of interest in biohydrometallurgy, In: V.S.T. Cimenelli and O. García (eds.) *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development*. Part B. Elsevier, Amsterdam, pp. 639–650.

González-Toril, E., Llobet-Brossa, E., Casamayor, E.O., Amann, R. and Amils, R. (2003) Microbial ecology of an extreme acidic environment. The Tinto River. Appl. Environ. Microbiol. **69**: 4853–4865.

Haak, T.K. and McFeters, G.A. (1982) Microbial dynamics of an epilithic mat community in a high alpine stream. Appl. Environ. Microbiol. **43**: 702–707.

Hallberg, K.B. and Johnson, D.B. (2001) Biodiversity of acidophilic prokaryotes. Adv. Appl. Microbiol. **49**: 37–84.

Hutchinson, N., Nagarkar, S., Aitchison, J.C. and Williams, G.A. (2006) Microspatial variation in marine biofilm abundance on intertidal rock surfaces. Aquat. Microb. Ecol. **42**: 680–696.

Hynes, H.B.N. (1970) *The Ecology of Running Waters.* Liverpool University Press, Liverpool.

Johnson, D.B. (1998) Biodiversity and ecology of acidophilic microorganisms. FEMS Microbiol. Ecol. **27**: 307–317.

Little, B.J., Wagner, P.A. and Lewandodowski, Z. (1997) Spatial relationships between bacteria and mineral surfaces, In: J. Banfield and K.H. Nealson (eds.) *Geomicrobiology: Interactions Between Microbes and Minerals*. Mineral Society of America, Washington, DC, pp. 123–189.

Lock, M.A. (1993) Attached microbial communities in rivers, In: T.E. Ford (ed.) *Aquatic Microbiology: An Ecological Approach*. Blackwell, Oxford, pp. 113–138.

López-Archilla, A.I., Marín, I. and Amils, R. (2001) Microbial community composition and ecology of an acidic aquatic environment: the Tinto River, Spain. Microb. Ecol. **41**: 20–35.

López-Archilla, A.I., González, A.E., Terrón, M.C. and Amils, R. (2005) Diversity and ecological relationships of the fungal populations of an acidic river of Southwestern Spain: the Tinto River. Can. J. Microbiol. **50**: 923–934.

Malki, M., Gonzalez-Toril, E., Sanz, J.L., Gómez, F., Rodríguez, N. and Amils, R. (2006) Importance of the iron cycle in hydrometallurgy. Hydrometallurgy **83**: 223–228.

Marshall, K.C. (1992) Biofilms: an overview of bacterial adhesion, activity, and control at surfaces. ASM News **58**: 202–207.

Mirete, S., de Figueras, C.G. and Gónzalez-Pastor, J.E. (2007) Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. Appl. Environ. Microbiol. **73**: 6001–6011.

Nordstrom, D.K. and Southam, G. (1997) Geomicrobiology of sulfide mineral oxidation, In: J.F. Banfield and K.H. Nealson (eds.) *Geomicrobiology: Interactions Between Microbes and Minerals*, Vol. 35. Mineralogical Society of America, Washington, DC, pp. 361–390.

Pierson, B.K. and Parenteau, M.N. (2000) Phototrophs in high iron microbial mats: microstructure of mats in iron-depositing hot springs. FEMS Microbiol. Ecol. **32**: 181–196.

Pierson, B.K., Parenteau, M.N. and Griffin, B.M. (1999) Phototrophs in high-iron-concentration microbial mats: physiological ecology of phototrophs in an iron-depositing hot spring. Appl. Environ. Microbiol. **65**: 5474–5483.

Rawlings, D.E., Tributsch, H. and Hansford, G.S. (1999) Reasons why "*Leptospirillum*"-like species rather than *Thiobacillus ferrroxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology **145**: 5–13.

Romaní, A.M., Guasch, H., Muñoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F. and Sabater, S. (2004) Biofilm structure and function and possible implications for riverine DOC dynamics. Microb. Ecol. **47**: 316–328.

Tyson, G.W., Lo, I., Baker, B.B., Allen, E.E., Hugenholtz, P. and Banfield, J.F. (2005) Genome-directed isolation of the key nitrogen fixer *Leptospirillum ferrodiazotrophum* sp. nov. from an acidophilic microbial community. Appl. Environ. Microbiol. **71**: 6319–6324.

Wuertz, S., Okabe, S. and Hausner, M. (2004) Microbial communities and their interactions in biofilm systems: an overview. Water Sci. Technol. **49**: 327–336.

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FLUVIAL BEDFORM GENERATION BY BIOFILM ACTIVITY IN THE BERROCAL SEGMENT OF RÍO TINTO: ACIDIC BIOFILMS AND SEDIMENTATION

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1. Introduction

One of the most astonishing environments that can be found on Earth's habitat diversity is the Río Tinto acidic system, a 100-km long acidic river on the Southwestern Spain (Fig. 1). The subsurface microbial community acting on an ancient volcano-sedimentary complex enriched in pyrite during a million of years (Fernández-Remolar et al., 2005) have induced a deep impact on the surface of the fluvial basin. The long-term microbial weathering of sulfides has been promoting the acidification of surface waters by the production of protons through the following equation:

$$4\ FeS_2 + 15\ O_2 + 2\ H_2O \Rightarrow 8\ SO_4^{2-} + 4\ Fe^{3+} + 4\ H^+$$

As a consequence, the resulting solutions that feed the Río Tinto headwaters (Fernández-Remolar et al., 2008b) are enriched in sulfate and iron complexes. Molecular complexation of ionic species is strongly dependent on the water fluxes that are seasonally equilibrated by the iron buffer (Gómez et al., 2004; Fernández-Remolar et al., 2008a), which controls the pH of acidic solutions:

$$Fe^{3+} + H_2O \Rightarrow Fe(OH)^{2+} + H^+$$

It is remarkable that the oxidizing potential of acidic waters is controlled by the ferric cation (Sato, 1960; Bigham and Nordstrom, 2000) more than the oxygen itself. Under anoxygenic conditions, this cation is the only oxidizing agent that acts at deeper areas of the sulfide aquifers that release sulfate, ferrous iron, and protons under microbial attack. Such a reaction allows recycling iron to a lower oxidation

Figure 1. Google Earth image of the Río Tinto Headwaters (**a**) and a detail of the Berrocal segment (**b**), showing different biosedimentary subenvironments as highly turbulent (white square) and low energy (white triangle) areas (©2008 Google – Map data © Tele Atlas).

state and also controlling the pH in the subsurface anaerobic environments that are placed in the deeper areas of the metallic sulfide aquifers.

The water outflow from the underground reservoirs allows transporting different transition and metallic elements in the acidic solution. Continuous meas-

urements of the water river recorded a pH ranging from 0.9 to 3, which favors high concentrations of ferric iron (8.8 g l^{-1}), sulfur (19 g l^{-1}), magnesium (1.7 g l^{-1}), aluminum (3 g l^{-1}), copper (55 mg l^{-1}), lead (0.16 mg l^{-1}), silicon (68.4 mg l^{-1}), zinc (64.7 mg l^{-1}), or manganese (0.18 mg l^{-1}), among others. High conductivity values that have been obtained between 5 and 45 mS cm^{-1} are in concordance with ionic concentration of water.

Some external parameters depending on climate as water availability and temperature are the major driving forces to favor biofilm seasonal growth. The Río Tinto area shows an annual average temperature of 17ºC that is between 10º and 25.5º for the coldest and the hottest month, respectively (for better climate information, see Fernández-Remolar et al., 2003). The headwater area has great availability of water during the fall and spring: rainfall rates are currently higher than 950 mm each year, which recharge the aquifers and distribute oxygen into the anoxic areas of the river. Aquifer replenishment during the wet periods balances the high evapotranspiration rates for summer under steady conditions, which guaranteed a continuous flow each year even in the driest conditions.

The oxidation process in areas that are exposed to the atmosphere (not only surface waters but also the upper part of the aquifer) is sustained by an active biological pump. Oxygen uptake by microbes decreases the oxygen transfer in the water column, whose concentration is negligible at the bottom of river waters and the subsurface fluids. Oxygen availability is lowered to a greater extent by evaporation during the dry season (Fernández-Remolar et al., 2003). Under these conditions, river fluids decrease the flow, increment the density at bottom, and lower the diffusion rates for chemicals. The resulting chemistry averages complete oxygen depletion in the water column below. Interestingly, polymer formation is pushed down by density rearrangement in the water column inducing compositional fractionation and acidity: short SO_4-Fe chained complexes and simple Fe-OH groups at the surface (chemical blocks for copiapite and jarosite), but long-chained SO_4-Fe bearing compounds that are the base of the schwertmannite. As a consequence, the Río Tinto water experiences strong stratification that drives a gradient in the chemical content from surface (having atmospheric level for oxygen) to the bottom where it is completely absent. Chemical fractionation is coupled to both aerobic and anaerobic microbial communities in biofilms that sequester, exchange, and recycle ferric to ferrous iron along the water column (González-Toril et al., 2003a).

The introduction of highly concentrated brines into a fluvial basin under a two-seasonally Mediterranean climate produces the precipitation of diverse mineral species (Fig. 2), mainly composed of ferric iron and sulfate (Amoros et al., 1981; García, 1996). Sulfates are highly abundant in the acidic brine mineral record of the river and, depending on local and temporal variations in hydrochemistry and climate, may appear as pure sulfate species or transitional phases between iron oxyhydroxides and sulfates (Fig. 2b). Recent analyses of modern sediments from the river showed that the main groups of minerals were iron hydroxysulfates such as copiapite, coquimbite, gypsum, halotrichite, hexahydrite, melanterite, rhomboclase, rozenite, szomolnokite, and voltaite (Buckby et al.,

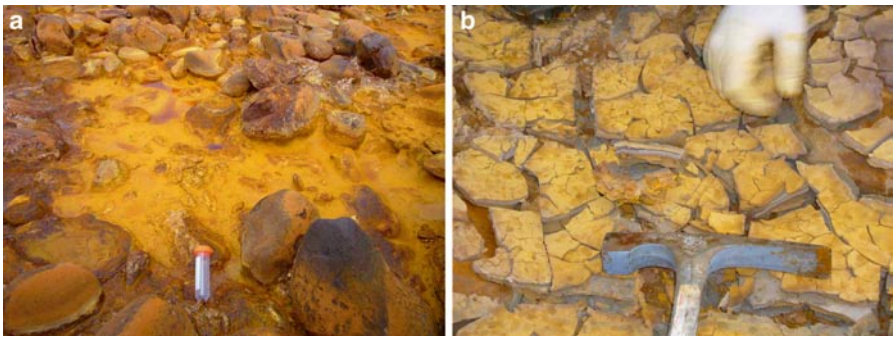

Figure 2. Acidic iron-rich deposits sedimented in the Río Tinto banks during the last rainfall episodes in the wet spring season. (**a**) orange precipitates recognized as jarosite under analysis with a VNIR field spectrometer. (**b**) Fine laminae alternating from top to bottom: yellowish (jarosite-oxyhydroxide), greenish (detrital), and reddish (shwertmanitte?) laminae.

2003). To a minor extent, some iron oxyhydroxides and oxides like ferrihydrite, goethite, and hematite have also been identified (Hudson-Edwards et al., 1999). Differences in water pH, redox, and ionic composition have been claimed as the main factors to generate differences in sulfate and oxyhydroxide parageneses in the Río Tinto banks (Buckby et al., 2003).

2. Rio Tinto Photosynthetic Biofilms

Over and above the extreme conditions detected in the Tinto ecosystem, what makes Río Tinto a unique acidic environment is the unexpected degree of eukaryotic diversity found in the system (López-Archilla et al., 2001; Amaral-Zettler et al., 2002; Aguilera et al., 2006). Members of the phylum Chlorophyta (*Chlamydomonas*, *Chlorella*, and *Euglena*) are the most frequent species detected, followed by two filamentous algae belonging to the genera *Klebsormidium* and *Zygnemopsis*. The most acidic part of the river is inhabited by a eukaryotic community dominated by species of photosynthetic protists of the genera *Dunaliella* and *Cyanidium*. Among the eukaryotic decomposers, fungi are very abundant and exhibit great diversity, including yeast and filamentous forms (López-Archilla et al., 2005). Phagotrophic species such as ciliates, cercomonads, amoebae, and heliozoans have been also detected in the Tinto basin (Aguilera et al., 2006).

Most of the eukaryotic microorganisms found on the Tinto basin are distributed in extensive biofilms of different shapes that cover most of the riverbed. Differences in water flow may also play a significant role in determining the biofilm structure and the amount of material accumulated on the sediments. Some of them adopt filamentous morphologies in flowing water, whereas others form thick colorful patches firmly attached to the mineral substrates. The mucous matrix where the cells are imbedded is an important part of these acidic biofilms and is mainly made up of

extracellular polymeric substances (EPS) with large amounts of negatively charged functional groups that can act as metal-binding sites (Aguilera et al., 2007).

The microbial diversity of the biofilms varies greatly along the river. Structural analyses show a close link between the photosynthetic microorganisms and the other components of the trophic web. Previous studies focused on the diversity of chemolithotrophic prokaryotes in relation to the iron and sulfur cycles in the water column and bacterial streamers (González-Toril et al., 2003b; García-Moyano et al., 2007). The diversity of fungi associated with the harsh water conditions has already been described (López-Archilla et al., 2005), but little is known about the microbial communities associated with photosynthetic biofilms. Preliminary results showed that in some areas of the river, chemolithotrophic bacteria are included in the photosynthetic biofilms as important structural components. In addition, heterotrophic bacteria and fungi have been found in association, probably generating microenvironments with less extreme conditions than those existing in the water column. Recent experiments performed with microsensors have shown the ability of acidic eukaryotic biofilms to modify some of the conditions (pH, oxygen, and metal concentration) existing in the water column (Souza-Egipsy et al., 2008).

3. Geomorphology and Sedimentology in the Río Tinto Fluvial System

3.1. RIVER GEOMORPHOLOGY IN THE BERROCAL SEGMENT

The morphology of the modern Río Tinto channel has resulted from the interplay between local compartimentalization of tectonic blocks plus an active climatic weathering over Quaternary. Deep entrenching in some river segments contrasts with preservation of terraces in others, which evidences block isolation and differential sedimentary processes at regional scale. An extreme case is the Berrocal area (Fig. 1b), which has provided many examples of channel configuration by biofilm mineralization shown in this work. In the Berrocal area (Fig. 3), there is an intensive channel entrenching over Quaternary with poor preservation of river sediments younger than 4,000 years; given by ^{14}C aging in fluvial deposits corresponding to a river terrace entrenched by the modern river. These materials are compounds of conglomeratic deposits cemented by ferruginous material that exert a strong structural control in the modern sedimentation. During the spring and fall flooding, the river waters cover the ferruginous terrace materials that are reactivated as a channel bed. Given that stream velocity is not excessive, sedimentation occurs as successive aggradation of fine laminas alternating as fine-grained detrital, iron oxyhydroxides, biofilm organic, and sulfate-rich laminas.

The main channel in the area is bedded by the 2,500–2,750-year-old young terrace (Fernández-Remolar et al., 2005) but subjected to collapse and erosion. Collapses may occur by seasonal phase changes in some mineral deposits on the river bed. Processes such as hydration/dehydration or dissolution of sulfates

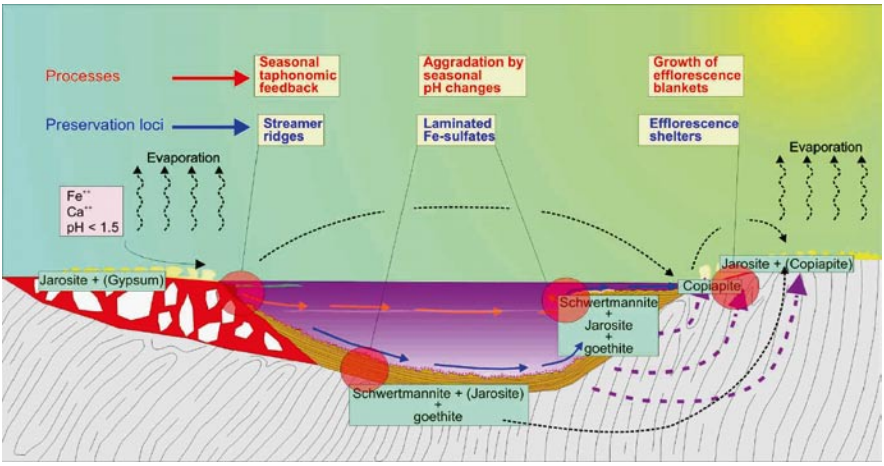

Figure 3. Sedimentary subenvironments during the dry season in the acidic channel of Río Tinto including mineral composition of materials. Mineral distribution in the different loci has been estimated through the mineral occurrence in the river (Fernández-Remolar et al., 2005). Formation of fine-grained detrital deposits and iron oxyhydroxides are not contemplated in this scheme given that they are more frequently originated during the wet seasons.

involve in some cases dramatic changes in volume. It derives to differential destabilization of the deposits enriched in sulfates producing riffles and pits along the river, which may be favored by Tectonic features. In these fluvial areas, the stream gradient is higher, the river flow increased, and the sedimentary river structures in equilibrium with these physical constraints were far different. Moreover, the river channel shows a permanent water lamina that experiences seasonal variation in the flow regimen throughout the year. Under these conditions, biofilm development and mineralization induce the production of peculiar structures that have a deep impact on the channel configuration.

3.2. RIVER SEDIMENTOLOGY

Mineralogical analyses done by XRD and Mössbauer spectrometer (Hudson-Edwards et al., 1999; Fernández-Remolar et al., 2005) have revealed the presence of sulfates, iron oxides, and phyllosilicates. The mineral associations are strongly dependent on the seasonal climatic changes that control physico-chemical parameters for sedimentation. Sulfates are the most abundant mineral phases that have been recognized on the riverbanks (Fernández-Remolar et al., 2005). Ferrous and ferric sulfates are represented by melantherite, copiapite, coquimbite, hydronium jarosite, and schwertmannite. Copiapite and coquimbite currently appear in the dry season as whitish to yellowish efflorescences growing on fluvial deposits or contacting acidic pools. However, hydronium jarosite frequently appears forming reddish

sheets that are covered episodically by water bodies, but also as cementing materials of sandy deposits mediated by evaporative pumping (Fernández-Remolar et al., 2005). The mineralogical analyses have also detected hydronium jarosite inside the channel sediments that are episodically covered by permanent water lamina, which results in extreme seasonal oversaturation during summer. Schwertmannite occurs as laminated darkish red deposits in seasonal pools exposed to incomplete or complete desiccation in summer.

Iron oxide and oxyhydroxides have been determined in different river loci co-occurring with the ferric sulfate associations. Concretely, goethite has been detected as a low crystalline phase included in the laminated sediments of Rio Tinto (Fig. 4a). The goethite composition prevails not only under the seasonal pH increase, but also when a freshwater tributary meets the acidic stream. In addition, the strong pH change that the Río Tinto waters experience downstream in the estuarine area may

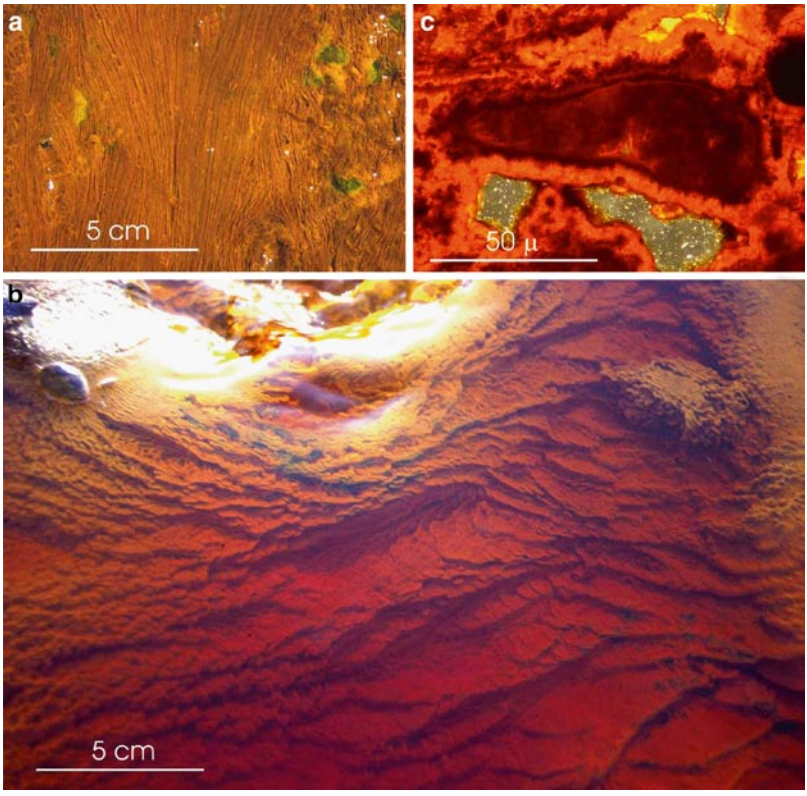

Figure 4. Different features of biology preservation in Río Tinto. (**a**) Incipient coating on biofilm filaments by solution oversaturation in ferric iron and sulfate. (**b**) Corrugated structures originated from several coating stages on a primary streamer structure. (**c**) Biological rest initially enveloped by a microbial film (cloudy cover on fossil) that was followed by coating in a second stage.

favor a massive precipitation of iron oxyhydroxides. Goethite also associates with hematite as reddish cements of sandy deposits that were formed during flash flooding events. In other cases, the goethite and hematite composition in sediments may be associated with transported grains of gossan by the river.

The co-occurrence of phyllosilicate phases with the sulfate and iron mineralogies is noteworthy. They appear as sheet alternating the sulfate and oxide laminas during the wet episodes (Fig. 2b) and composed of illite, chlorite, kaolinite, and montmorillonite. Such a diverse association suggests episodic erosion and fast transportation into the water given the instability of smectites when exposed to acidic conditions.

Direct observations in the fluvial system reveal that the mineral distribution in the river depends on chemical and physical processes. As seen before, the bottom and mineral crusts are the two main sedimentological loci where sulfates and oxides form parageneses. In the river channel, schwertmannite and jarosite associate with very poorly crystalline goethite forming orange to reddish colloidal-like aggregates. Moreover, goethite concentration of sediments is much higher when freshwater streams meet the acidic waters of Río Tinto. In these cases, schwertmannite precipitates at that distance from the neutral input where the acidic conditions are recovered. The mineralogical analyses suggest that sulfate concentration and pH of solutions are the main parameters controlling the composition of the associations of ferric minerals in the riverbeds (Bigham et al., 1996). The pH and ionic extreme conditions inside abandoned pools during summer favor the precipitation of schwertmannite and jarosite (Fig. 3) as dark reddish lamina covered by yellowish precipitates.

4. Preservation and Taphonomy of Biofilms

Preservation is a natural process that is seasonally induced under coating on biological structures (Fernández-Remolar and Knoll, 2008) by oversaturation and polymerization of ferric and sulfate complexes. Under acidic sedimentation, the preservation of biofilms is driven by the same seasonal changes in the physicochemical parameters of the Rio Tinto waters affecting the sedimentation. During the spring and fall wet seasons (Fernández-Remolar et al., 2003), coating is not favored given the pH increase, brine dilution, and high flow regimen (high erosive rates) acting together against the generation of environmental conditions favorable for preservation. Low erosion rates and oversaturation prevailing under dry conditions favor a fast mineralization for biological structures. In this case, the transition between wet to dry conditions is recorded in coatings as very fine lamination (<1,000 μm) of alternating iron oxyhydroxides and sulfates (Fig. 5).

Interestingly, some fossil remains recovered from the old terrace show a close association between coating and fungal mats trapping macrobiological remains such as insects and plants (Fig. 4c). In this sense, fungal activities, as many other biofilms (Gall, 2001), act as preservational agents by inducing the emergence of preservational microenvironments. Such microenvironments house

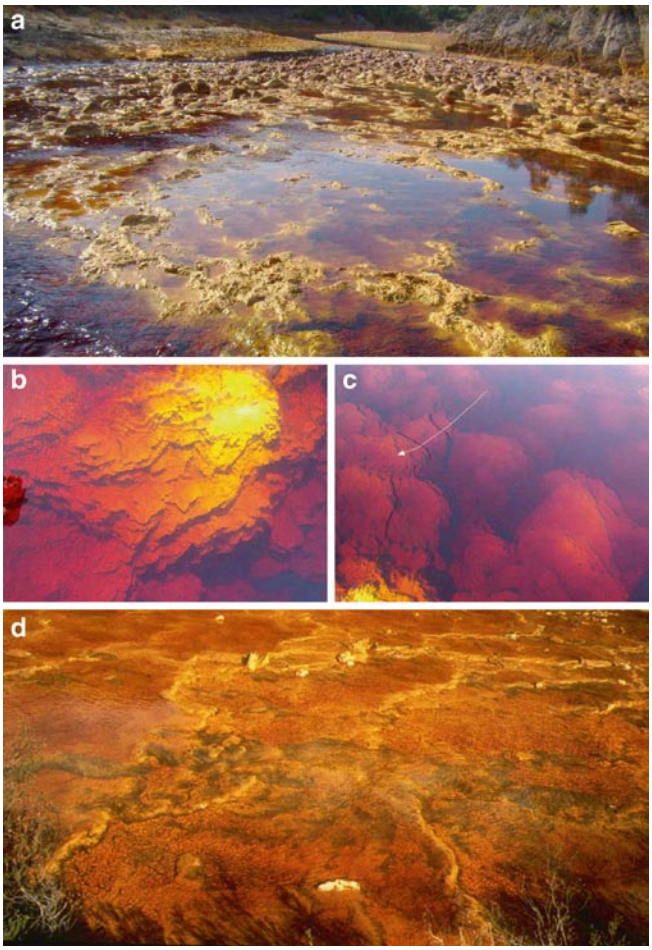

Figure 5. Complex structures formed by corrugated surfaces and terracetes in a ferric sedimentary flat laterally bounded by river channels. (**a**) General view showing main lineaments of ferric precipitates enclosing complexes of terracetes and corrugated structures. (**b**) Detail of a structure composed of successive terracetes (**c**) Prograding sheets of ferric deposits (*black lines*) growing downstream (*white arrow*). (**d**) Colonizing and growing phase of streamers during the last stages of a wet season.

physico-chemical conditions so far different from the macroenvironment that enhances mineralization by strong micron-scaled gradients.

5. Biosedimentation and Channel Morphology at the Berrocal Segment

A wide variety of sedimentary structures are recorded on the river bed and the banks of Río Tinto. Some structures are the response of the interaction between

the climatic conditions of the region and the particular chemistry of the river. This is the case of sulfate crusts and efflorescences that are primary structures formed by the precipitation and evaporation of the acidic brines of Rio Tinto. However, some others result from the interaction of biological activity and the acidic system by means of biofilm coating and sediment trapping. The biosedimentary structures show differences that are based on the water energy, solution hydrochemistry, and biofilm diversity.

The most bizarre structures of biological origin (Fig. 4b, 5a–d) occur in the main channel of the Berrocal segment where the flow regimen is high. They consist of corrugated surfaces that coalesce in curved ridges and close terraced surfaces forming successive steps known as terracetes. Depending on the water energy, these structures can change to prograding and stacking sheets of ferric laminas that grow downstream (Fig. 5c). All these structures are currently associated with the seasonal growth of algae streamers (Fig. 5d) and fungal and bacteria filaments, which develop on the highest part of ridges and wrinkles during the wet and temperate season (Fernández-Remolar et al., 2003). Highest rate for biofilm colonization and growth occur during periods of time between the wet and dry seasons. This is the time with more space for microbes to colonize and grow but with low saturation in waters, and also less ferric concentration that can interfere with the photosynthesis. However, biofilms composed of heterotrophic and chemolithotrophic microorganisms are active during the dry season. As the wet season goes over to dryer conditions, streamers and other filaments (bacterial and fungal) start to be covered by a thin coating of ferric materials, until being trapped inside a rigid oxide coating during the dry and warm time. During dry conditions (laminar flow and oversaturation), filaments and other biological remains are rapidly covered first by iron oxyhydroxides that are followed over season by ferric sulfates. Direct observations in the river reveals that, during the coating process, the extreme of streamers oriented downstream is attached to the river bed. A continuous coating of the fixed streamers would produce same bended structures as seen in the river bottom (Fig. 4b, 5b–c).

Such a complex process of biofilm growing followed by ferric coating generates the topological configuration of the river substrate that favors the next stage of colonization by streamers. Such a topological change implies the increase of the fluvial gradient, which is a favorable parameter for streamer growth. Similar mechanisms have been observed in some marine regions where the successive accumulations of biological remains activate a positive feedback that induce an increase in the water energy. In these cases, the final results are mounds generated and driven by organisms that are strongly dependent on the water oxygenation. Such a mechanism has been known as a taphonomic feedback and is currently found in reefal environments. Same mechanism of coating occurs on boulders, tree branches, and other objects that are transported by the river during the flooding episodes. Therefore, it can be inferred that alteration of the riverbed morphology by microbes is a process that overimposes on other factors of greater magnitude.

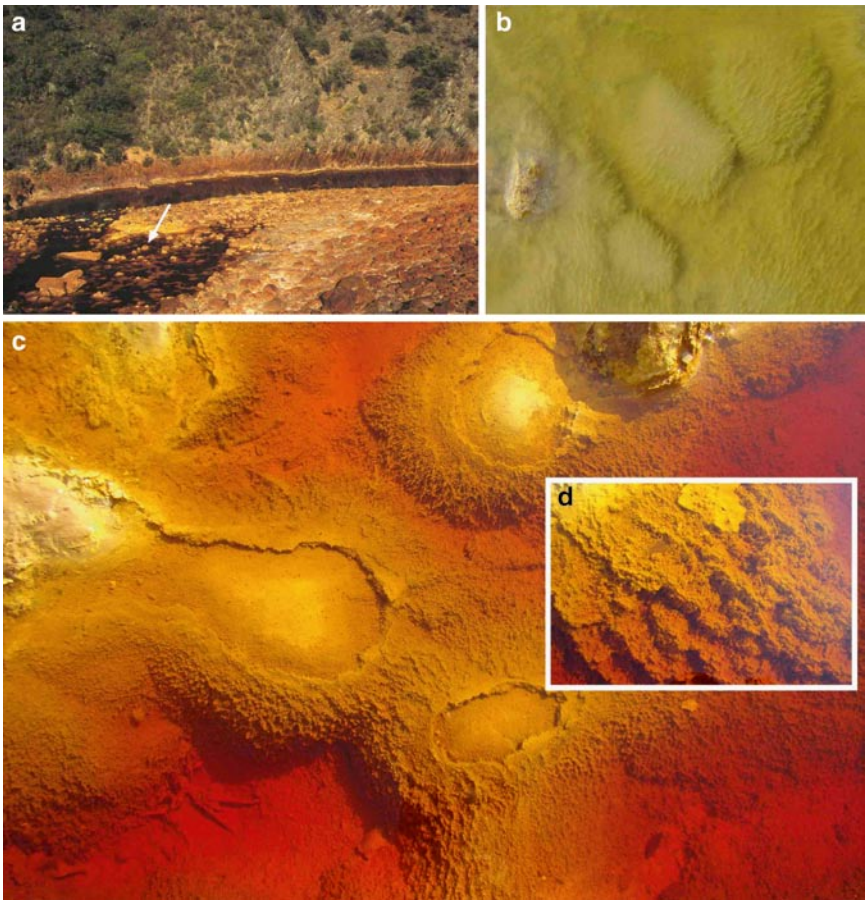

Figure 6. Location and main morphological features of the dome-like surfaces. (**a**) Sedimentary subenvironment (*white arrow*) where these structures occur. (**b**) Microbial film colonizing and growing in a low-energy locus with vertical and lateral projections. (**c**) Dome-like structures show coated biofilms with same projections seen in (**b**). (**d**) Details of vertical walls associated with a dome.

The second group of structures is dome-like surfaces that are eventually covered by radial projections of microbial origin (Fig. 6). Given that the fluid turbulence is not high, biofilms grow through lateral and vertical protrusions (Fig. 6b–d). They occur in fluvial areas of the secondary channel where the river flow is low and only episodically active during the high water episodes. In this case, the control of riverbed morphology depends on biofilm growth that accelerates the sedimentation rates by sediment trapping and mineral coating. The inner structure is composed of alteration of successive yellowish and reddish laminas that correspond to the hydrochemical oscillations mediated by climate.

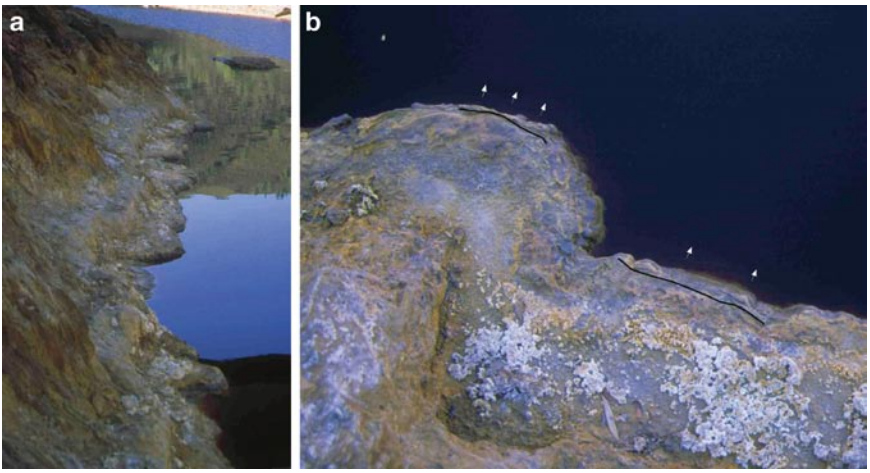

Figure 7. Images of prograding structures in a meandering transect of Río Tinto in Berrocal. (**a**) Coalescence of different prograding structures shouldering the rocky bank of river. (**b**) Details of one of these structures where white arrows point to the inner side of meander, black lines trace successive growing laminas in the structure.

A last group of structures occasionally covered by biofilms (Fig. 7a–b) are found shouldering outer banks of the meandering river transects (Reading, 1996). They are internally formed by yellowish to reddish alternating laminas with an onlap–downlap pattern (Fig. 7b), which suggests that river progradation is outward. This is consistent with the situation of structures in the external side of the river meander where the fluvial currents go to the internal side of the river channel. Meandering activity drives erosion on the outer riverbank (Reading, 1996), which would force destruction of sedimentary structures in this area. However, the biofilm activity can favor sedimentation by trapping and coating in an erosive regimen. This process will be optimal during the high water time in transition between wet and dry seasons when water saturation starts but the river water is still covering the fluvial banks. Under this dynamics, the successive mineral trapping and coating should induce sheet progradation toward the opposite riverbank.

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7. References

Aguilera, A., Manrubia, S.C., Gómez, F., Rodríguez, N. and Amils, R. (2006) Eukaryotic community distribution and its relationship to water physicochemical parameters in an extreme acidic environment, Río Tinto (Southwestern Spain). Appl. Environ. Microbiol. **72**: 5325–5330.

Aguilera, A., Souza-Egipsy, V., Gómez, F. and Amils, R. (2007) Development and structure of eukaryotic biofilms in an extreme acidic environment, Río Tinto (SW, Spain). Microb. Ecol. **53**: 294–305.

Amaral-Zettler, L., Gómez, F., Zettler, E., Keenan, B.G., Amils, R. and Sogin, M.L. (2002) Eukaryotic diversity in Spain`s "River of Fire". Nature **417**: 137.

Amoros, J.L., Lunar, R. and Tavira, P. (1981) Jarosite – a silver bearing mineral of the Gossan-of-Rio-Tinto (Huelva) and La Union (Cartagena, Spain). Min. Dep. **16**: 205–213.

Bigham, J.M. and Nordstrom, D.K. (2000) Iron and aluminium from hydroxysulfates from acid sulfate waters, In: C.N. Alpers, J.L. Jambor and D.K. Nordstrom (eds.) *Sulfate Minerals. Crystallography, Geochemistry, and Environmental Significance*, Vol. 40. Mineralogical Society of America, Washington, DC, pp. 351–403.

Bigham, J.N., Schwertmann, U., Traina S.J., Winland R.L. and Wolf, M. (1996) Schwertmannite and the chemical modelling of iron in acid sulfate waters. Geochim. Cosmochim. Acta **60**: 2111–2121.

Buckby, T., Black, S., Coleman, M.L. and Hodson, M.E. (2003) Fe-sulfate-rich evaporative mineral precipitates from the Río Tinto, southwest Spain. Min. Mag. **67**: 263–278.

Fernández-Remolar, D.C. and Knoll, A.H. (2008) Fossilization potential of iron-bearing minerals in acidic environments of Rio Tinto, Spain: implications for Mars exploration. Icarus **194**: 72–85.

Fernández-Remolar, D., Rodríguez, N., Gómez, F. and Amils, R. (2003) The geological record of an acidic environment driven by iron hydrochemistry: the Tinto River system. J. Geophys. Res. **108**(E7), 10.1029/2002JE001918.

Fernández-Remolar, D.C., Morris, R.V., Gruener, J.E., Amils, R. and Knoll, A.H. (2005) The Río Tinto Basin, Spain: mineralogy, sedimentary geobiology, and implications for interpretation of outcrop rocks at Meridiani Planum, Mars. Earth Planet. Sci. Lett. **240**: 149–167.

Fernández-Remolar, C., Gomez, F., Prieto-Ballesteros, O., Schelble, R.T., Rodriguez, N. and Amils, R. (2008a) Some ecological mechanisms to generate habitability in planetary subsurface areas by chemolithotrophic communities: the Río Tinto subsurface ecosystem as a model system. Astrobiology **8**: 1–18.

Fernández-Remolar, D.C., Prieto-Ballesteros, O., Rodríguez, N., Gómez, F., Amils, R., Gómez-Elvira, J. and Stoker, C.R. (2008b) Underground habitats in the Río Tinto basin: a model for subsurface life habitats on Mars. Astrobiology **8**: 1023–1047.

Gall, J.C. (2001) Taphonomy: role of microbial mats, In: D.G.E. Briggs and P.R. Crowther (eds.) *Paleobiology II*. Blackwell, Oxford, pp. 280–284.

García, G.G. (1996) The Río Tinto mines, Huelva, Spain. Min. Rec. **27**: 275–285.

García-Moyano, A., González-Toril, E., Aguilera, A. and Amils, R. (2007) Prokaryotic community composition and ecology of macroscopic floating filaments from an extreme acidic environment, Río Tinto (SW Spain). Syst. Appl. Microbiol. **30**: 601–614.

Gómez, F., Fernández-Remolar, D., González-Toril, E.F. and Amils, R. (2004) The Tinto River, an extreme gaian environment, In: L. Margulis, J. Miller, P. Boston, S. Schneider and E. Crist (eds.) *Scientists on Gaia 2000*. MIT Press, Cambridge, pp. 321–334.

González-Toril, E., Gómez, F., Rodríguez, N., Fernández-Remolar, D., Zuluaga, J., Marín, I. and Amils, R. (2003a) Geomicrobiology of the Tinto River, a model of interest for biohydrometallurgy. Hydrometallurgy **71**: 301–309.

González-Toril, E., Llobet-Brossa, E., Casamayor, E.O., Amann, R. and Amils, R. (2003b) Microbial ecology of an extreme acidic environment, the Tinto River. Appl. Environ. Microbiol. **69**: 4853–4865.

Hudson-Edwards, K.A., Schell, C. and Macklin, M.G. (1999) Mineralogy and geochemistry of alluvium contaminated by metal mining in the Rio Tinto area, southwest Spain. Appl. Geochem. **14**: 1015–1030.

López-Archilla, A.I., Marín, I. and Amils, R. (2001) Microbial community composition and ecology of an acidic aquatic environment: the Tinto river, Spain. Microb. Ecol. **41:** 20–35.

López-Archilla, A.I., González, A.E., Terrón, M.C. and Amils, R. (2005) Diversity and ecological relationships of the fungal populations of an acidic river of southwestern Spain: the Tinto River. Can. J. Microbiol. **50**: 923–934.

Reading, H.G. (1996) *Sedimentary Environments: Processes, Facies and Stratigraphy*. Blackwell Science, Malden, MA, p. 688.

Sato, M. (1960) Oxidation of sulfide ore bodies. I. Geochemical environments in terms of Eh and pH. Econ. Geol. **55**: 928–961.

Souza-Egipsy, V., González-Toril, E., Zettler, E., Amaral-Zettler, L., Aguilera, A. and Amils, R. (2008) Prokaryotic community structure in algal photosynthetic biofilms from extreme acidic streams in Río Tinto (Huelva, Spain). Int. Microbiol. **11**: 251–260.

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CYANOBACTERIAL MATS OF THE MELTWATER PONDS ON THE MCMURDO ICE SHELF (ANTARCTICA)

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1. Introduction

Cryo-ecosystems dominate cold environments such as the Antarctic and are characterised by persistent cold temperatures with freeze−thaw cycles, extreme light conditions (including ultraviolet radiation) and high variability in nutrient availability and salinity (Vincent, 2007). In Antarctica, freshwater cryo-ecosystems can be found in the form of ice-based habitats such as the meltwater ponds on the McMurdo Ice Shelf (Fig. 1) which contain liquid water during the summer months but completely freeze-over in the winter (Howard-Williams et al., 1990). These systems are dominated by microbial life including complex sustainable cyanobacterial-dominated microbial mat communities. Analogous cryo-ecosystems have also been discovered in the Arctic and have been considered as models for how microorganisms may have survived and evolved during major cooling and freeze-up events on early Earth (Vincent and Howard-Williams, 2000; Vincent et al., 2004a).

In this chapter, we first introduce the general topology of the McMurdo Ice Shelf and the ecosystem characteristics of the meltwater ponds on the McMurdo Ice Shelf. We then review microbial mat communities and cyanobacterial diversity found on the McMurdo Ice Shelf. The current understanding on processes related to N_2-fixation in microbial mats will also be illustrated. The chapter concludes with a presentation of major physiological and adaptation mechanisms of cyanobacteria to survive and flourish in the meltwater ponds of the McMurdo Ice Shelf.

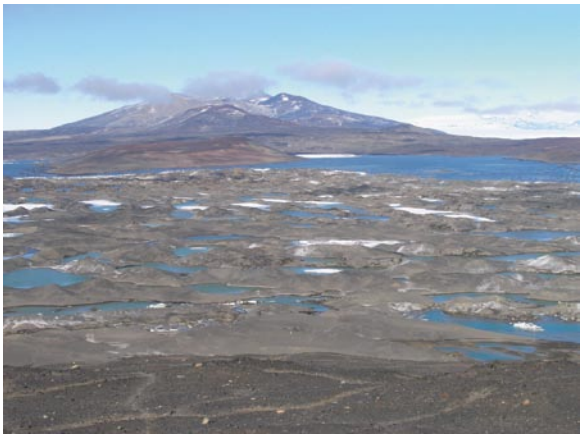

Figure 1. McMurdo Ice Shelf with meltwater ponds, Antarctica (Photograph: A.D. Jungblut).

2. Meltwater Ponds on the McMurdo Ice Shelf in Antarctica

The McMurdo Ice Shelf was first described by Scott (1905) and forms the northwestern extension of the Ross Ice Shelf in southern Victoria Land, Antarctica. It includes an area of about 1,500–2,000 km^2 with a thickness of 10–50 m (Swithenbank, 1970; Howard-Williams et al., 1990). The ice movement is slow, only 2 m year^{-1} in the area between Black Island and Brown Peninsula (Swithenbank, 1970). It is an ablation zone largely covered by sediment, which consists mainly of silt to cobble-sized black basalt clasts of marine origin and frozen seawater (de Mora et al., 1994). The topology of the McMurdo Ice Shelf is marked by two morphologically distinct different systems, undulating and pinnacle ice. Undulating ice is usually covered by 10–20 cm of moraine and marine sediment. The undulations are up to 20-m deep and are often filled with water bodies of varying size ranging from less than 1 to 4 m in depth. In comparison, pinnacle ice has a flatter surface with a more patchy sediment cover and with usually only smaller ponds, streams and trickles present (Howard-Williams et al., 1990).

During the summer, the area is covered with a network of meltwater ponds that vary in size, shape and physicochemical conditions and can cover an area of 30,000 m^2 (de Mora et al., 1991). Many of these ponds are stratified into an upper fresh and a lower saline layer (Hawes et al., 1997). The water of the ponds is recharged via melting of basal ice and snow that collects in the pond catchment over winter. The salts present in these ponds originate from a variety of processes including freeze concentration, ice ablation and summer ablation, as well as aerosol development and the intrusion of seawater through cracks. Additionally, chemical weathering of sedimentary material such as the dissolution of mirabilite ($Na_2SO_4 \cdot 10H_2O$), thernardite (Na_2SO_4) and small amounts of gypsum ($CaSO_4 \cdot 2H_2O$) contribute to the salt matrix composition (de Mora et al., 1994; Keys and Williams, 1981;

Wait et al., 2006). The conductivities in the ponds can range from 100 to 56,000 μS cm^{-1} (Howard-Williams et al., 1990). Environmental conditions in the meltwater ponds are characteristic for freshwater cryo-ecosystems with frequent freeze−thaw cycles, short growth period, periodic exposure to high UV radiation and variable irradiance, salinities and nutrient availability (Hawes et al., 2008).

The biomass of the meltwater ponds on the McMurdo Ice Shelf is dominated by the benthic microbial mats rather than planktonic populations. The network of meltwater ponds on the McMurdo ice shelf is considered Antarctica's largest wetland, and its great variety of habitats and diverse microbial communities makes it a biogenic pool for the surrounding more extreme regions in Victoria Land, such as the Dry Valleys, via aerial transport (Howard-Williams et al., 1990).

Meltwater ponds, with their microbial communities, are also hypothesized to be analogous to habitats present during periods of extensive glaciation on early Earth, which would have been a refuge for the survival, growth and diversification of prokaryotic and eukaryotic organisms (Vincent and Howard-Williams, 2000; Vincent et al., 2004b).

3. Microbial Diversity of the McMurdo Ice Shelf

3.1. MICROBIAL MAT DIVERSITY IN MELTWATER PONDS OF THE MCMURDO ICE SHELF WITH A FOCUS ON LIPID BIOMARKERS

Microbial mats are common in most aquatic habitats on Earth, ranging from fresh to marine waters and geothermal to permafrost ecosystems. They also dominated life on early Earth (Stal et al., 1985; Vincent et al., 2004a). A mat can be viewed simultaneously as a complex microbial community, a micro-environmental modifier and an independent morphological entity (Golubic and Seong-Joo, 1999). Microbial mats are often characterized by a vertical stratification of physicochemical conditions and functional groups of microorganisms (Stal, 2000).

To date, lipid biomarker analyses have concentrated on three meltwater ponds on the McMurdo Ice Shelf. The ponds are called Fresh, Orange and Salt Ponds (Fig. 2a–c, respectively), and were chosen because they represent the range of conductivities found on the McMurdo Ice Shelf with approximately 900, 3,469 and 54,100 μS cm^{-1}, respectively (Jungblut et al., 2008). Lipids are an ideal tool to identify functional groups in microbial mats because they are an integral part of membranes and storage compounds in all prokaryotes and eukaryotes. Certain lipids are also diagnostic for specific classes of organisms. Therefore, lipid biomarkers have been used extensively to characterize complex living microbial communities from a variety of environments, including marine, freshwater, hydrothermal, hypersaline and cold habitats, and also allow comparison with ancient fossilized communities (Thiel et al., 1997; Cowen et al., 2003; Jahnke et al., 2004; Fang et al., 2006).

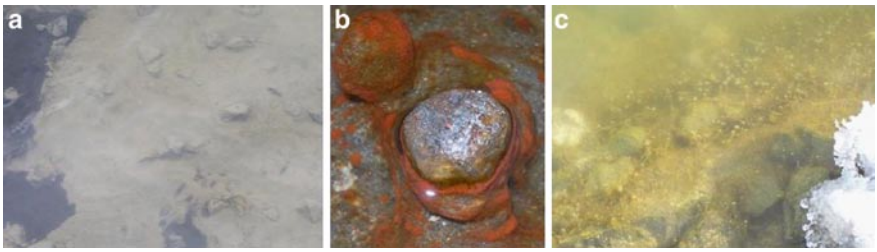

Figure 2. Microbial mats from the McMurdo Ice shelf, Antarctica: (**a**), Fresh Pond; (**b**), Benthic microbial mat with trapped gas bubbles and high concentrations of red-pigmented rotifers from Orange Pond; (**c**), Benthic microbial mat and precipitated salt from Salt Pond (Photographs: A.D. Jungblut).

The microbial mats in the meltwater ponds of the McMurdo Ice shelf are dominated by cyanobacteria, which represent the dominant primary producer in this environment (see Section 3.2). Oscillatoriales comprised more than 70% of the microbial mat communities (Howard-Williams et al., 1990). This group is particularly important for the formation of the mat matrix because of its motile filamentous morphology and extracellular polysaccharide production. The mats are oxic on the top with an oxygen peak in the deep chlorophyll maximum and become anoxic towards the bottom of the mats (Vincent et al., 1993a, b). Cyanobacteria are usually found in the top layers. Like microbial mats from other climatic zones, they may be covered by sediment and extracellular substances including UV protective and light-quenching pigments (Stal, 2000) (see Section 5.2).

In general, the layers below the cyanobacteria can be anoxic owing to heterotrophic decomposition of the organic matter produced by cyanobacteria. Mountfort et al. (1999, 2003) found that nitrate reduction and methanogenesis with sulfate reduction dominated this anaerobic process. This was confirmed through the identification of diagnostic fatty acid methylated esters (FAME) for sulphate-reducing bacteria (*iso*-$C_{17:1}\Delta^9$ and 10Me-$C_{16:0}$, (Londry et al., 2004; Jungblut et al., 2008). In addition, FAME (Cy-15:1) characteristic for the presence of *Clostridia* (Vestal and White, 1989) were also tentatively identified by Jungblut et al. (2008), which correlated with the previous isolation of a novel psychrophilic *Clostridium* sp. from Fresh Pond (Mountfort et al., 1997).

Molecular analysis based on 16S rRNA gene analysis also identified a variety of phylotypes grouping within Alphaproteobacteria, Gammaproteobacteria Deltaproteobacteria, Bacteroidetes, Spirochaetes, Actinobacteria and Archaea (*Crenarchaeota*) in mats and sediment samples (Sjöling and Cowan, 2003) suggesting the presence of a variety of different functional groups. Interestingly, lipid biomarker analyses were able to supplement this by identifying diagnostic signatures such as unsaturated wax esters for *Chloroflexus* sp. (Jungblut et al., 2008), an anoxygenic photoheterotroph, which has previously been identified as a dominant group in the microbial mats of hot springs with temperatures up to 66°C

(Skirnisdottir et al., 2000). In addition, lipid biomarker analyses suggested the presence of specific bacterial functional groups, such as sulfur and iron oxidizers, based on FAMEs, such as cy-$C_{17:0}$, $C_{18:1}\Delta^{11}$ and cy-$C_{19:0}$ (Grimalt et al., 1991; Fourçans et al., 2004). Further physiological and molecular studies are lacking to confirm the functional groups within these microbial mats.

A number of protozoa and metazoa can also be found in the microbial mats of the McMurdo Ice Shelf; however, most of the analyses to date are based on morphology. Previously described groups include Chlorophyta, Dinophyta, Bacillariophyta, Haptophyta, Chrysophyta and Cryptophyta (Howard-Williams et al., 1990). The presence of these groups was confirmed through the identification of specific sterols, in particular dinosterol and dinostanol, which can be found in Dinophyta and in trace levels in Bacillariophyta. In addition, sterols commonly occurring in Bacillariophyta, Haptophyta, Chrysophyta and Cryptophtya, including 24-methylcholesta-5,22*E*-dien-3β-ol, were also identified (Volkman, 2003). However, the diversity of this community would become apparent after additional molecular studies, as shown in Morgan-Kiss et al. (2008), where a novel psychrophile *Chlorella* sp. was identified from the McMurdo Ice Shelf. Such studies would also enable determination of phylogenetic relationships between organisms from other cold environments, such as the Arctic and similar climatic zones.

The metazoan diversity of microbial mats is dominated by several invertebrate taxa of Rotifera, Nematoda and Tardigrada (Suren, 1988). However, further comprehensive analyses, particularly those using molecular tools, are again lacking. Higher plants and organic matter input from higher plants are absent, since no diagnostic lipids, especially FAMEs were detected in the mats of the McMurdo Ice Shelf.

Although the quantification methods used by Jungblut et al. (2008) did not differentiate functional groups or abundances between microbial mats from meltwater ponds of different conductivities, a complex assemblage of bacteria, eukaryotes and functional groups has been identified in microbial mats on the McMurdo Ice Shelf. On the basis of lipid biomarker analyses, the pond communities appeared to be less diverse than microbial mats from temperate ecosystems, yet more diverse than those from hot spring environments.

3.2. CYANOBACTERIAL DIVERSITY ON THE MCMURDO ICE SHELF

Cyanobacteria were described early in the characterisation of Antarctic biodiversity. Scientists such as West and West (1911) and Fritsch (1912) characterised many Antarctic cyanobacteria. Their work pioneered the classification of Antarctic species. Common Antarctic cyanobacteria belong to the four orders Chroococcales, Nostocales, Oscillatoriales and Stigonematales (Swithenbank, 1970; Vincent, 1988; Broady, 1989; Howard-Williams et al., 1990).

A diverse group of cyanobacterial morphotypes has been described for the benthic mats of the meltwater ponds on the McMurdo Ice Shelf (Howard-Williams et al., 1990). These microbial mats are commonly composed of morphotypes

belonging to the orders Oscillatoriales and Nostocales. Most of these mats are dominated by Oscillatoriales. In particular, *Phormidium* cf. *deflexum*, *Lyngbya* cf. *limnetica*, *Oscillatoria priestleyi*, *Phormidium autumnale*, *Oscillatoria* cf. *fragile*, *Phormidium autumnale* and *Oscillatoria limosa* are regularly identified as part of these communities (Nadeau and Castenholz, 2000). *O. priestleyi* is especially characteristic of hypersaline meltwater ponds, including Salt and Brack Ponds on the ice shelf. *Nostoc* spp. (often *Nostoc commune*) and *Nodularia* sp. were observed from the order Nostocales (*Nodularia* cf. *harveyana* (Kuetz). On the other hand, unicellular cyanobacteria of the order Chroococcales are not common in the ice shelf communities; however, such morphological studies are often limited by the ambiguous taxonomy and the plastic morphology of cyanobacteria.

Molecular analyses on cyanobacterial communities of microbial mats from Fresh, Orange and Salts Ponds identified 12 different phylogenetic clusters based on 16S rRNA gene analysis (Jungblut et al., 2005). Four of these clusters were formed in the order Nostocales, including seven different phylotypes. Eight clusters belonged to Oscillatoriales, including 19 different phylotypes. Phylotypes from this analysis, which grouped within the Oscillatoriales included *Phormidium* sp., *Oscillatoria* sp. and *Lyngbya* sp., whereas phylotypes grouped within Nostocales included *Nostoc* spp., *Nodularia* sp. and *Anabaena* sp. Fresh and Orange Ponds showed a similar diversity in contrast to that of the hypersaline Salt Pond, where the diversity within cyanobacterial mats was reduced and it was composed of different groups than the less saline ponds. *O. priestleyi* seems to be the dominant morpho- and phylotype within this hypersaline meltwater pond.

Molecular analyses also allowed a better comparison with cyanobacterial mat communities from other Antarctic and cold environments. For example, sequence data of clones from other Antarctic environments and various Arctic strains were used to assess the geographical distribution of the uncultured cyanobacterial clones from the three ponds. The Antarctic references originated from Lake Fryxell and Lake Bonney in the Dry Valleys, Vestfold Hills (East Antarctica) and the meltwater system south of Bratina Island (McMurdo) (Priscu et al., 1998; Nadeau et al., 2001; Taton et al., 2003). Phylotypes described by Jungblut et al. (2005) suggested different levels of geographic distribution. They included phylotypes, which have only thus far been identified in Antarctic environments, only in polar environments, as well as those that had highest similarities to temperate cyanobacteria. This has further been supported by Taton et al. (2006a, b), where microbial mat communities from ponds and lakes in the Vestfold and Larsman Hills (Antarctica) were investigated.

4. N_2-Fixation in Microbial Mats of the McMurdo Ice Shelf

Biological N_2-fixation enables the transformation of atmospheric dinitrogen into biologically available dinitrogen sources via the reduction of dinitrogen to ammonium. The ability of microorganisms to fix dinitrogen is of particular interest in

these Antarctic meltwater ponds, as dinitrogen sources are limiting due to the prevalent low dissolved inorganic dinitrogen/phosphorus ratios. The fixed dinitrogen input into these ponds is limited to recycled NH_4−N from sediments and snow/ice melt. However, strong dinitrogen regeneration within the mats is indicated by dinitrogen concentrations in the interstitial fluids, which are much higher than that in the water column. Therefore, transport of dinitrogen into the water column through diffusion is assumed (Howard-Williams et al., 1990; Howard-Williams and Hawes, 2007).

The ability to fix dinitrogen is found in many bacteria such as proteobacteria, green-sulfur and non-sulfur bacteria, Spirochaetes, Firmicutes and cyanobacteria as well as Archaea (Zehr et al., 2003). In a molecular study of the microbial mat from Orange Pond on the McMurdo Ice Shelf, the ability to fix dinitrogen (presence of *nifH* gene) was identified in 18 different *nif*H phylotypes grouping into cyanobacteria, Firmicutes, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria, Spirochaetes and Verrucomicrobiae. However, out of all these potential dinitrogen fixers, the most active dinitrogen fixation in meltwater ponds to date has been attributed to *Nostoc* spp. based on physiological and molecular studies (Fernández-Valiente et al., 2001; Jungblut and Neilan, unpublished). In contrast, no N_2-fixing activity could be attributed to the oscillatorian community of the microbial mats in this environment, even though they dominate the cyanobacterial diversity (Fernández-Valiente et al., 2001).

This pattern of N_2-fixation contrasts with cyanobacterial mats from tropical and temperate marine and hypersaline environments. The typical cyanobacterial N_2-fixing phylotypes in these environments were non-heterocystous filamentous Oscillatoriales species, including *Lyngbya*, *Phormidium* and *Plectonema,* as well as the unicellular Chroococcales, such as *Halothece* and *Synechocystis* (Steppe et al., 1996; Affourtit et al., 2001; Omoregie et al., 2004).

Previous estimates suggested that biological N_2-fixation contributes 1 g m^{-2} $year^{-1}$ from the microbial mats out of a total dinitrogen requirement of approximately 3 g m^{-2} $year^{-1}$ (based on chlorophyll-specific photosynthetic rates in Howard-Williams et al., 1990) for these ice-based freshwater systems. These values were in the same range as those observed in temperate rice fields (Fernández-Valiente et al., 2001). On the basis of physiological studies, the heterotrophic bacterial contribution to the total budget is very limited. These findings agree with studies on microbial consortia from Lake Bonney, Canada Stream, and Dry Pond (Dry Valleys, Antarctica) (Olson et al., 1998).

Recent studies have shown that an increase in the dinitrogenase activity of cyanobacterial mats from the McMurdo Ice Shelf is concomitant with an increase in temperature (Velázquez et al., 2006). Therefore, it is possible that low temperatures in these Antarctic mats could have an influence on N_2-fixation activity, as shown for *Nostoc* sp. isolated from Antarctic moss−cyanobacteria associations (Davey and Marchant, 1983).

5. Physiology and Adaptation of Cyanobacteria to Environmental Conditions of the Meltwater Pond Ecosystems on the McMurdo Ice Shelf

5.1. ADAPTATION TO LOW TEMPERATURE, OSMOTIC STRESS AND DESICCATION

Cyanobacteria in microbial mats of the McMurdo Ice Shelf tend to be psychrotrophs, with suboptimal growth in low temperatures (Tang et al., 1997), which possibly allows a faster adaptation to the rapidly changing temperature conditions of the meltwater ponds. A number of mechanisms permit them to tolerate low temperatures and continue to grow, though mostly at slow rates (Vincent, 2007). During freezing, extracellular compounds can be synthesized to reduce ice nucleation around the cells (Vincent, 1988). It was shown that desiccated microbial mats can recover and resume photosynthesis and respiration. However, differences in recovery times were identified between *Nostoc*- and *Phormidium*-dominated mats. *Nostoc*-dominated mats recovered within hours, whereas *Phormidium*-dominated mats had not completed their recovery after 10 days under these experimental conditions (Hawes et al., 1992). Polyunsaturated fatty acids are incorporated into membranes to retain fluidity at low temperatures (Laybourn-Parry, 2002). In addition, compatible solutes such as trehalose are produced to decrease the freezing point of intracellular fluids (Oren, 2000). Some organic osmolytes such as glycine betaine can also be used as a long-term strategy to balance extracellular ions in hypersaline conditions during brine accumulation due to ice formation, or in high conductivity ponds.

5.2. ADAPTATION TO UV RADIATION

High UV radiation is a major stress factor for microorganisms in Antarctic aquatic ecosystems, such as the meltwater ponds on the McMurdo Ice Shelf (Roos and Vincent, 1998). In cyanobacteria, it can lead to photo-inhibition, phycobiliprotein degradation and chlorophyll bleaching (Castenholz, 1992; Ehling-Schulz and Scherer, 1999). Furthermore, exposure of DNA to UVB and UVC radiation can lead to DNA lesions and mutagenesis, including dimerization of adjacent pyrimidine bases. However, cyanobacteria have evolved a variety of DNA repair mechanisms, such as excision repair and photo-reactivation, to cope with UV-induced DNA damage (Garcia-Pichel and Castenholz, 1991). Cyanobacteria are also able to synthesize a variety of pigments, such as carotenoids, scytonemin and mycosporine-like amino acids for protection against UV radiation (Garcia-Pichel and Castenholz, 1991; Vincent et al., 1993a). Another strategy observed in motile cyanobacteria is the migration to deeper layers within the microbial mats to avoid radiation (Vincent et al., 1993b; Quesada and Vincent, 1997).

5.3. LIGHT HARVESTING AND NUTRIENT SUPPLY

Phototrophs in polar and alpine regions must also contend with low irradiances caused by snow and ice cover. The cyanobacteria utlize highly efficient light capturing complexes, encompassing multiple phycobiliproteins (phycoerythrin, allophycocyanin and phycocyanin) with photosynthetic quantum yields close to the theoretical maximum (Hawes and Schwarz, 2001). In addition, cyanobacteria in frozen and liquid water on the McMurdo Ice Shelf may have prolonged seasonal dormancy phases, and freeze-dried cyanobacterial mats in Antarctica resume photosynthesis within minutes to hours after thawing (Vincent, 1988).

5.4. SECONDARY METABOLITE PRODUCTION

Cyanobacteria are known to produce a great variety of secondary metabolites, which are not necessary for survival or reproduction. To date, 600 cyanobacterial secondary metabolites have been described, most of which have been found in the orders Chroococcales, Oscillatoriales and Nostocales (Welker and von Döhren, 2006). Many of these compounds have antibacterial, antiviral, fungicide, immunosuppressive, enzyme inhibiting or cytotoxic properties (Carmichael et al., 1990). These properties could be of importance for survival within complex microbial communities and in extreme environments such as the McMurdo Ice Shelf.

One very well-studied group of cyanobacterial secondary metabolites are heptatotoxic microcystins. As with certain other secondary metabolites, microcystins are synthesised *via* a mixed pathway including non-ribosomal peptide synthetases and polyketide synthases (Tillet et al., 2000). So far, almost 80 different isoforms of microcystin have been identified; however, all are low molecular weight compounds (900–1,000 Da) that have a characteristic polyketide Adda ((2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) side chain. The ecological role of these compounds is a topic of much discussion. Possible putative functions include feeding deterrence, quorum sensing and iron scavenging, as reviewed by Kaebernick and Neilan (2001). They may also be an ancestral relict (Rantala et al., 2004).

Recent studies identified protein phosphatase inhibition activity and microcystins including microcystin-LR and [D-Asp^3] microcystin-LR by mass spectrometric analysis in microbial mats from the McMurdo Ice Shelf (Hitzfeld et al., 2000; Jungblut et al., 2006). *Microcystis* and *Planktothrix*, which are most often associated with hepatotoxic microcystin blooms, could not be identified in these Antarctic communities (Chorus and Bartram, 1999). Therefore, the most likely microcystin-producing genera in the mats are *Phormidium*, *Nostoc, Anabaena* and *Oscillatoria,* as these have been found to produce toxins in other benthic cyanobacterial communities (Mez et al., 1997; Izaguirre et al., 2007; Rouhiainen et al., 2004; Sivonen et al., 1992). A related microcystin synthetase gene cluster has not

been identified thus far in cyanobacterial communities on the McMurdo Ice Shelf, but the presence of cyanobacterial non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes related to *Nostoc* were found from the total genetic pool of these microbial communities (Jungblut et al., 2006). This shows that these Antarctic microbial communities have a high genetic potential and harbor secondary metabolites within the microbial mats of the McMurdo Ice Shelf cryosphere.

6. Conclusion and Future Perspectives

Cyanobacteria-dominated microbial mats represent most of the biomass in the meltwater ponds of the McMurdo Ice Shelf, Antarctica. Microbial mats are often stratified in oxic and anoxic layers with cyanobacteria as dominant primary producers in the upper layer of the mats. The cyanobacterial diversity includes morpho- and phylotypes belonging to Nostocales and Oscillatoriales with different biogeographic distribution patterns (Taton et al., 2003). Other bacterial groups in the mats are Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Bacteroidetes, Spirochaeta, Actinobacteria, Firmicutes, Chloroflexi and Crenarchaeota with functional diversity related to sulfate reduction and sulfur and iron oxidation. Eukaryotic diversity entails mainly protists including Chlorophyta, Dinophyta, Bacillariophyta, Haptophyta, Chrysophyta and Cryptophyta and metazoa (Rotifera, Nematoda and Tardigrada). N_2 fixation is an important source of dinitrogen for the microbial communities on the McMurdo Ice Shelf. *Nostoc* spp. were found to be the dominant contributors to the dinitrogen budget, but *nifH* phylotypes from the other bacterial groups Firmicutes, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Spirochaetes and Verrucomicrobiae have also been detected. To withstand the harsh conditions of these cryo-ecosystems, including frequent freeze−thaw cycles, short growth period, high UV radiation and variable salinities and nutrient availability, cyanobacteria have developed a variety of mechanisms. Furthermore, the communities have the potential to produce secondary metabolites including hepatotoxic microcystins.

Future molecular studies will be needed to fully elucidate the biodiversity of the microbial mats and their biogeographic distribution within the Antarctic and also in a global context. Integrated efforts combining physiological and molecular approaches, such as metagenomics, will allow us to examine the functional diversity of the microbial mats in relation to the present environmental conditions of the meltwater pond cryosphere and to potentially reveal novel taxa and functions within these unique Antarctic microbial communities.

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8. References

Affourtit, J., Zehr, J.P. and Paerl, H.W. (2001) Distribution of nitrogen-fixing microorganisms along the Neuse River Estuary, North Carolina. Microb. Ecol. **41**: 114–123.

Broady, P.A. (1989) Broadscale patterns in the distribution of aquatic and terrestrial vegetation at three ice-free regions on Ross Island, Antarctica. Hydrobiologia **172**: 77–95.

Carmichael, W.W., Mahmood, N.A. and Hyde, E.G. (1990) Natural toxins from cyanobacteria, In: S. Hall and G. Strichartz (eds.) *Marine Toxins, Origin, Structure, and Molecular Pharmacology*, ACS Symposium Series 418. American Chemical Society, Washington, DC, pp. 87–106.

Castenholz, R.W. (1992) Species usage, concept, and evolution in the cyanobacteria (blue-green algae). J. Phycol. **28**: 737–745.

Chorus, I. and Bartram, J. (1999) *Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring and Management.* World Health Organization, London.

Cowen, J.P., Giovannoni, S.J., Kenig, F., Johnson, H.P., Butterfield, D., Rappé, M.S., Hutnak, M. and Lam, P. (2003) Fluids from aging ocean crust that support microbial life. Science **299**: 120–123.

Davey, A. and Marchant, H.J. (1983) Seasonal variation in nitrogen fixation by *Nostoc commune* Vaucher at the Vestfold Hills, Antarctica. Phycology **22**: 377–385.

de Mora, S.J., Whitehead, R.F. and Gregory, M. (1991) Aqueous geochemistry of major constituents in the Alph River and tributaries in Walcott Bay, Victoria Land, Antarctica. Antarct. Sci. **3**: 73–86.

de Mora, S.J., Whitehead, R.F. and Gregory, M. (1994) The chemical composition of glacial melt water ponds and streams on the McMurdo Ice Shelf, Antarctica. Antarct. Sci. **6**: 17–27.

Ehling-Schulz, M. and Scherer, S. (1999) UV protection in cyanobacteria. Eur. J. Phycol. **34**: 329–338.

Fang, J., Chan, C., Joeckel, R.M., Huang, Y.Y., Wang, Y., Bazylinski, D.A., Moorman, T.B. and Clement, B.J.A. (2006) Biomarker analysis of microbial diversity in sediments of a saline groundwater seep of Salt Basin, Nebraska. Org. Geochem. **37**: 912–931.

Fernández-Valiente, E., Quesada, A., Howard-Williams, C. and Hawes, I. (2001) N_2-fixation in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Microb. Ecol. **42**: 338–349.

Fourçans, A., García de Oteyza, T., Wieland, A., Solé, A., Diestra, E., van Bleijswijk, J., Grimalt, J.O., Kühl, M., Esteve, I., Muyzer, G., Caumette, P. and Duran, R. (2004) Characterization of functional bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue, France). FEMS Microbiol. Ecol. **51**: 55–70.

Fritsch, F.E. (1912) *Freshwater Algae.* British Museum of Natural History, London.

Garcia-Pichel, F. and Castenholz, R.W. (1991) Characterization and biological implication of scytonemin, a cyanobacterial sheath pigment. J. Phycol. **27**: 395–409.

Golubic, S. and Seong-Joo, L. (1999) Early cyanobacterial fossil record: preservation, palaeoenvrionments and identification. Eur. J. Phycol. **34**: 339–348.

Grimalt, J.O., de Wit, R., Teixidor, P. and Albaigés, J. (1991) Lipid biogeochemistry of *Phormidium* and *Microcoleus* mats. Adv. Geochem. **19**: 509–530.

Hawes, I. and Schwarz, A.-M. (2001) Absorption and utilization of irradiance by cyanobacterial mats in two ice-covered Antarctic lakes with contrasting light climates. J. Phycol. **37**: 5–15.

Hawes, I., Howard-Williams, C. and Vincent, W.F. (1992) Desiccation and recovery of Antarctic cyanobacterial mats. Polar Biol. **12**: 587–594.

Hawes, I., Howard-Williams, C., Schwarz, A.-M.J. and Downes, M.T. (1997) Environment and microbial communities in a Tidal Lagoon at Bratina Island, McMurdo Ice Shelf, Antarctica, In: B. Battaglia, J. Valencia and D. Walton (eds.) *Antarctic Communities: Species, Structure and Survival.* Cambridge University Press, Cambridge, pp. 170–177.

Hawes, I., Howard-Williams, C. and Fountain, A.F. (2008) Ice-based freshwater ecosystems, In: W.F. Vincent and J. Laybourn-Perry (eds.) *Polar Lakes and Rivers.* Oxford University Press, Oxford, pp. 103–118.

Hitzfeld, B., Lampert, C.S., Spaeth, N., Mountfort, D., Kaspar, H. and Dietrich, D.R. (2000) Toxin production in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Toxicon **38**: 1731–1748.

Howard-Williams, C. and Hawes, I. (2007) Ecological processes in Antarctic inland waters: interactions between physical processes and nitrogen cycle. Antarct. Sci. **19**: 205–217.

Howard-Williams, C., Pridmore, R., Broady, P. and Vincent, W.F. (1990) Environmental and biological variability in the McMurdo Ice Shelf Ecosystem, In: K. Kerry and G. Hempel (eds.) *Antarctic Ecosystems. Ecological Change and Conservation.* Springer, Berlin, pp. 23–31.

Izaguirre, G., Jungblut, A.D. and Neilan, B.A. (2007) Benthic cyanobacteria (Oscillatoriaceae) that produce microcystin-LR, isolated from four reservoirs in southern California. Water Res. **41**: 492–498.

Jahnke, L.J., Embaye, T., Hope, J.M., Turk, K.A., Van Zuilen, M., Des Marais, D.J., Farmer, J.D. and Summons, R.E. (2004) Lipid biomarker and carbon isotopic signatures for stromatolite-forming, microbial mat communities and *Phormidium* cultures from Yellowstone National Park. Geobiology **2**: 31–47.

Jungblut, A.D. and Neilan, B.A. (unpublished) Nitrogen-fixing phylotypes in microbial mat communities on the McMurdo Ice Shelf, Antarctica.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ. Microbiol. **74**: 519–529.

Jungblut, A.D., Höger, S.J., Mountfort, D., Hitzfeld, B.C., Dietrich, D.R. and Neilan, B.A. (2006) Characterization of microcystin production in an Antarctic cyanobacterial mat community. Toxicon **47**: 271–278.

Jungblut, A.D., Allen, M.A., Burns, B.P. and Neilan, B.A. (2008) Lipid biomarker analysis of cyanobacterial dominated microbial mats in meltwater ponds on the McMurdo Ice Shelf, Antarctica. Org. Geochem. DOI: 10.1016/j.orggeochem.2008.10.002.

Kaebernick, M. and Neilan, B.A. (2001) Ecological and molecular investigations of cyanotoxin production. FEMS Microbiol. Ecol. **35**: 1–9.

Keys, J.R. and Williams, K. (1981) Origin of crystalline, cold desert salts in the McMurdo region, Antarctica. Geochim. Cosmochim. Acta **45**: 2299–2309.

Kirschvink, J.L., Gaidos, E.J., Bertani, L.E., Beukes, N.J., Gutzmer, J., Maepa, L.N. and Steinberger, R.E. (2000) Paleoproterozoic snowball Earth: extreme climatic and geochemical global change and its biological consequences. Proc. Natl. Acad. Sci. USA **97**: 1400–1405.

Laybourn-Parry, J. (2002) Survival mechanisms in Antarctica lakes. Philos. Trans. R. Soc. **357**: 863–869.

Londry, K.L., Jahnke, L.L. and Des Marais, D.J. (2004) Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria. Appl. Environ. Microbiol. **70**: 745–751.

Mez, K., Beattie, K., Codd, G., Hanselmann, K., Hauser, B., Naegli, H. and Preisig, H. (1997) Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. Eur. J. Phycol. **32**: 111–117.

Morgan-Kiss, R.M., Ivanov, A.G., Modla, S., Czymmek, K., Hüner, N.P.A., Priscu, J.C., Lisle, J.T. and Hanson, T.E. (2008) Identity and physiology of a new psychrophilic eukaryotic green alga, *Chlorella* sp., strain BI, isolated from a transitory pond near Bratina Island, Antarctica. Extremophiles **12**: 701–711.

Mountfort, D., Rainey, F., Burghardt, J., Kaspar, H. and Stackebrandt, E. (1997) *Clostridium vincentii* sp. nov., a new obligately anaerobic, saccharolytic, psychrophilic bacterium isolated form low-salinity pond sediment of the McMurdo Ice Shelf, Antarctica. Arch. Microbiol. **167**: 54–60.

Mountfort, D., Kaspar, H., Downes, M. and Asher, R. (1999) Partitioning effects during terminal carbon and electron flow in sediments of a low-salinity meltwater pond near Bratina Island, McMurdo Ice Shelf, Antarctica. Appl. Environ. Microbiol. **65**: 5493–5499.

Mountfort, D., Kaspar, H., Asher, R.A. and Sutherland, D. (2003) Influence of pond geochemistry, temperature and freeze-thaw on terminal anaerobic processes occurring in sediments of six ponds of the McMurdo Ice Shelf, near Bratina Island, Antarctica. Appl. Environ. Microbiol. **69**: 582–592.

Nadeau, T.L. and Castenholz, R.W. (2000) Characterization of psychrophilic oscillatorians (Cyanobacteria) from Antarctic meltwater ponds. J. Phycol. **36**: 914–923.

Nadeau, T.L., Milbrandt, E.C. and Castenholz, R.W. (2001) Evolutionary relationships of cultivated Antarctic oscillatorians (Cyanobacteria). J. Phycol. **37**: 650–654.

Olson, J., Steppe, T., Litaker, R. and Paerl, H.W. (1998) N_2-fixing microbial consortia associated with the ice cover of Lake Bonney, Antarctica. Microb. Ecol. **36**: 231–238.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004) Determination of nitrogen-fixing phylotypes in *Lyngbya* and *Micrcocoleus chthonoplastes* cyanobacterial mats from Guerrero Negro, Baja California, Mexico. Appl. Environ. Microbiol. **70**: 2119–2128.

Oren, A. (2000) Salt and brines, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria. Their Diversity in Time and Space.* Kluwer, Dordrecht, pp. 281–306.

Priscu, J.C., Fritsen, C.H., Adams, E.E., Giovannoni, S.J., Paerl, H.W., McKay, C.P., Doran, P.T., Gordon, D.A., Lanoil, B.D. and Pinckney, J.L. (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science **280**: 2095–2098.

Quesada, A. and Vincent, W.F. (1997) Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. Eur. J. Phycol. **32**: 335–342.

Rantala, A., Fewer, D., Hisburges, M., Rouhiainen, L., Vaitomaa, J. and Börner, T. (2004) Phylogenetic evidence for the early evolution of the microcystin synthesis. Proc. Natl. Acad. Sci. USA **101**: 568–573.

Roos, J.C. and Vincent, W.F. (1998) Temperature dependence of UV radiation effects on Antarctic cyanobacteria. J. Phycol. **34**: 118–125.

Rouhiainen, L., Vakkilainen, T., Siemer, B.L., Buierna, W., Haselkorn, R. and Sivonen, K. (2004) Genes coding for hepatotoxic heptapeptides (microcystins) in the cyanobacterium *Anabaena* strain 90. Appl. Environ. Microbiol. **70**: 686–692.

Scott, R.F. (1905) *The Voyage of the Discovery*, Vols. 1 and 2. Scibner's, New York.

Sivonen, K., Namikoshi, M., Evans, W.R., Fardig, M., Carmichael, W.W. and Rinehart, K.L. (1992) Three new microcystins, cyclic heptapeptide hepatotoxins, from *Nostoc* sp. strain 152. Chem. Res. Toxicol. **5**: 464–469.

Sjöling, S. and Cowan, D.A. (2003) High 16S rDNA bacterial diversity in glacial meltwater lake sediment, Bratina Island, Antarctica. Extremophiles **7**: 275–282.

Skirnisdottir, S., Hreggvidsson, G.O., Hjörleifsdottir, S., Marteinsson, V.T., Petursdottir, S.K., Holst, O. and Kristjansson, J.K. (2000) Influence of sulfide and temperature on species compositions and community structure of hot spring microbial mats. Appl. Environ. Microbiol. **66**: 2835–2841.

Stal, L.J. (2000) Cyanobacterial mats and stromatolites, In: B.A. Whitton and M. Potts (eds.) *The Ecology of Cyanobacteria. Their Diversity in Time and Space.* Kluwer Academic, Dordrecht, pp. 281–306.

Stal, L.J., van Gemerden, H. and Krumbein, W.E. (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol. Ecol. **45**: 111–125.

Steppe, T.F., Olson, J.B., Paerl, H.W., Litaker, R.W. and Belnap, J. (1996) Consortial N_2 fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol. Ecol. **21**: 149–156.

Suren, A. (1988) Microfauna associated with algal mats in melt ponds of the Ross Ice Shelf. Polar Biol. **10**: 329–335.

Swithenbank, C.W.M. (1970) Ice movement in the McMurdo Sound Area of Antarctica, In: *International Symposium on Antarctic Glaciological Exploration.* International Association of the Science of Hydrology, Hanover, NH, pp. 472–487.

Tang, E.P.Y., Tremblay, R.F. and Vincent, W.F. (1997) Cyanobacterial dominance of polar freshwater ecosystems: are high-latitude mat-formers adapted to low temperatures. J. Phycol. **33**: 171–181.

Taton, A., Grubisic, S., Brambilla, E., de Wit, R. and Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl. Environ. Microbiol. **69**: 5157–5169.

Taton, A., Grubisic, S., Balthasart, P., Hodgson, D.A., Laybourn-Parry, J. and Wilmotte, A. (2006a) Biogeographical distribution and ecological range of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiol. Ecol. **57**: 272–289.

Taton, A., Grubisic, S., Ertz, D., Hodgson, D.A., Piccardi, R., Biondi, N., Tredici, M.R., Mainini, M., Losi, D., Marinelli, F. and Wilmotte, A. (2006b) Polyphasic study of Antarctic cyanobacterial strains. J. Phycol. **42**: 1257–1270.

Thiel, V., Merz-Preiss, M., Reitner, J. and Michaelis, W. (1997) Biomarker studies on microbial carbonates: extractable lipids of a calcifying cyanobacterial mat (Everglades, USA). Facies **36**: 163–172.

Tillet, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T. and Neilan, B.A. (2000) Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system. Chem. Biol. **7**: 753–764.

Velázquez, D., Rochera, C., Justel, A., Toro, M. and Quesada, A. (2006) Temperature dependency of photosynthesis and N_2-fixation in microbial mats from two mats from different Antarctic latitudes. International Symposium on Alpine and Polar Microbiology, Innsbruck.

Vestal, J.R. and White, D.C. (1989) Lipid analysis in microbial ecology. Bioscience **39**: 535–540.

Vincent, W.F. (1988) *Microbial Ecosystems of Antarctica.* Cambridge University Press, Cambridge.

Vincent, W.F. (2007) Cold tolerance in cyanobacteria and life in the cryosphere, In: J. Seckbach (ed.) *Algae and Cyanobacteria in Extreme Environments.* Springer, Dordrecht, pp. 289–301.

Vincent, W.F. and Howard-Williams, C. (2000) Life on snowball Earth. Science **287**: 2421.

Vincent, W.F., Downes, M.T., Castenholz, R.W. and Howard-Williams, C. (1993a) Community structure and pigment organisation of cyanobacteria-dominated microbial mats in Antarctica. Eur. J. Phycol. **28**: 213–221.

Vincent, W.F., Castenholz, R.W., Downes, M.T. and Howard-Williams, C. (1993b) Antarctic cyanobacteria: light, nutrients, and photosynthesis on the microbial mat environments. J. Phycol. **29**: 745–755.

Vincent, W.F., Mueller, D.R. and Bonilla, S. (2004) Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the High Arctic. Cryobiolology **48**: 103–112.

Vincent, W.F., Mueller, D., Van Hove, P. and Howard-Williams, C. (2004) Glacial periods on Early Earth and implications for the evolution of life, In: J. Seckbach (ed.) *Origins: Genesis, Evolution and Diversity of Life*. Kluwer Academic, Dordrecht, pp. 483–501.

Volkman, J.K. (2003) Sterols in microorganisms. Appl. Microbiol. Biotechnol. **60**: 495–506.

Wait, B.R., Webster-Brown, J.G., Brown, K.L., Healy, M. and Hawes, I. (2006) Chemistry and stratification of Antarctic meltwater ponds I: coastal ponds near Bratina Island, McMurdo Ice Shelf. Antarct. Sci. **18**: 515–523.

Welker, M. and von Döhren, H. (2006) Cyanobacterial peptides – Nature's own combinatorial biosynthesis. FEMS Microbiol. Ecol. **30**: 530–563.

West, W. and West, G.S. (1911) Freshwater algae, In: *Reports on the Scientific Investigations: Biology, by the British Antarctic Expedition 1907–1909,* Vol. 1, pp. 263–298.

Zehr, J.P., Jenkins, B.D., Short, S.M. and Steward, G.F. (2003) Nitrogenase gene diversity of microbial community structure: a cross-system comparison. Environ. Microbiol. **5**: 539–554.

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DIVERSITY AND ECOLOGY OF CYANOBACTERIAL MICROFLORA OF ANTARCTIC SEEPAGE HABITATS: COMPARISON OF KING GEORGE ISLAND, SHETLAND ISLANDS, AND JAMES ROSS ISLAND, NW WEDDELL SEA, ANTARCTICA

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1. Introduction – The Freshwater Microflora in Coastal Antarctica

The oxyphototrophic microflora plays a substantial role in freshwater and terrestrial ecosystems of maritime Antarctica. Short life-cycles and high turnover rates characterize the cyanoprokaryotes and microscopic algae among the main primary producers of organic matter in coastal regions of subantarctic islands and the Antarctic coasts. The phototrophic microflora is particularly important in the succession processes in deglaciated regions and in ornithogenic soils, in periodical water biotopes, and in marginal parts of snow fields and glaciers. The microflora of deglaciated maritime areas of the Antarctic Peninsula region is mostly limited to the periphyton, epilithon, and mats in different types of microhabitats. Phototrophic microorganisms grow in shallow depths below the soil surface and in periodic water biotopes.

The diversity of the Antarctic microflora is still little known (Olech, 1993). Old studies describing Antarctic taxa exist (West and West, 1911; Fritsch, 1912, 1917; Carlson, 1913), and numerous valuable taxonomic papers by Broady (1986, 1989a, b, 1996, 2005), and by Japanese (e.g., Ohtani, 1986; Ohtani et al., 1991) and Argentinian authors (e.g., Vinocur and Pizzaro, 1995; Mataloni et al., 2000; Mataloni and Pose 2001) from the second half of the 20th century are particularly important. The problem is that the modern investigations, oriented to molecular analyses of genotypes from Antarctic ecosystems, cannot always be related to known phenotypes described on the basis of morphological characters. Therefore, on the one hand, we know numerous traditional morphospecies, which are characterized only by morphological criteria and whose ecology can be studied, but these cannot be identified using a molecular approach. On the other hand, we have records of various genotypes, the molecular data of which are precisely determined, but whose morphological and ecological properties are largely unknown.

There is no good identification literature for freshwater Antarctic algae and cyanobacteria, and the identification of Antarctic species based on populations from temperate or tropical zones is questionable. The number of algal species in the Antarctic literature, originally known from other niches, is large (cf. Prescott, 1979; Komárek and Anagnostidis, 2005), but data about different species from various papers are not comparable without precise documentation. Ubiquitous and cosmopolitan cyanobacterial species possibly exist, but the genotypic identity of morphologically similar but ecologically different and geographically distant populations was never proved.

The diversity of cyanobacteria in various Antarctic areas was recently evaluated using molecular methods; however, the results were not linked to previous data, and no information was collected on the ecologically distinct taxonomic units. It is difficult to identify various ecotypes in different Antarctic microbiotopes from such studies. For example, in the case of South Shetland Islands and similar coastal Antarctic areas, several taxonomic groups of algae were studied only by traditional morphological methods (Beljakova, 1987; Kawecka and Olech, 1993; Luścinska and Kyć, 1993; Broady, 1996; Komárek, 1999), and ecological data were related to the concept of diversity (Ohtani et al., 1991; Vinocur and Pizzaro, 1995). Therefore, we use for our review mainly taxonomic data derived from traditional, phenotypic identifications.

A biological program of Czech phycologists in Antarctica oriented to microvegetation ecology was initiated at King George Island, South Shetland Islands, in 1996. The main studied localities were the deglaciated areas on the coasts of the Admiralty Bay up to the Cape Demay, and partly also in the Fildes Peninsula. The study was realized in a few summer seasons from 1996 to 2005. In 2006, the Czech J.G. Mendel Station was constructed on the north coast of James Ross Island, NW part of the Weddell Sea, which enabled to continue our studies also in this, almost 100 km² deglaciated area during the 2006–2009 seasons.

The main tasks for our studies of microflora at the Antarctica were:

1. To prepare a review of cyanoprokaryotic and algal diversity in the deglaciated region of King George Island and James Ross Island
2. To perform a taxonomic (genotypic) characterization of important (dominant and characteristic) species, and compare these with taxonomically similar and convergent populations from other geographic regions and different ecological situations
3. To study the autecology and ecophysiology of dominant and characteristic species
4. To characterize the composition, structure, seasonal changes, and production parameters of various cyanobacterial and algal communities in various terrestrial and freshwater microbiotopes
5. To describe the contribution of freshwater and terrestrial algae in the life of the ecosystem in maritime Antarctica

Our studies focused on the detailed recognition of the species diversity of freshwater and terrestrial cyanobacteria, diatoms, and green algae, which along with mosses and lichens belong to the most abundant components of photoautotrophic vegetation in the whole ecosystem of maritime Antarctica. The ecological studies of important microhabitats in the coastal Antarctic ecosystem were started together with this investigation.

Five main habitats with characteristically developed cyanobacteria assemblages were recognized in the coastal areas of the studied islands: soils (S), seepages (P), streams (R), wetted rocky walls (W), and lakes (L). Our first detailed ecological studies were focused on microvegetation of creeks (Elster, 2002; Elster and Komárek, 2003; Elster and Benson, 2004) and seepages (Komárek and Komárek, 2003). The main ecological parameters (pH, temperature, intensity of global radiation, conductivity, nutrients) were measured and the dominant species, characteristic for different habitats, were identified. The basic ecological factors are similar in various microhabitats, but certain differences in the species composition are quite distinct. In this review, we compare the biotopes of seepages from maritime Antarctica (King George Island, about 62°23′ S 58°27′ W) and James Ross Island (about 63°48′ S, 57°52′ W) in the NW part of the Weddell Sea. Both localities belong to the coastal Antarctica at the vicinity of the Antarctic Peninsula with colonized seepages, but differ in macroclimatic conditions. The South Shetland Islands are exposed to western more humid winds from southern Pacific, whereas the more arid James Ross Island is shaded against the western winds by the Antarctic Peninsula (Fig. 1).

Seepages represent characteristic habitats of coastal polar regions, with shallow wetland ecosystems on soil surface (with permafrost in the lower soil layers). A wide scale of wetland biotopes and microbiotopes are continually supplied by melting water for a longer period of the Antarctic summer season, from permafrost, snow fields, and glaciers. A system of very shallow pools (only a few centimeters deep), intensely wetted soils, and small streams is characterized by stagnant rather than by continually running water.

The developed cyanobacterial communities form mats with a special structure (microzonation) and species composition (Vincent et al., 1993; Komárek and Komárek, 1999, 2003). The structure of mats regularly changes during the summer season with a climax stage at the end of February. These communities are characterized by characteristic distinct dominant species. The majority of species in seepages were found endemic for Antarctica, and only 20% of the taxa were morphologically or genetically identical with the types of lower geographic widths. Many of these species are connected with the special Antarctic habitat. Certain differences were found between seepages from King George Island and James Ross Island, where several morphospecies appear, known rather from continental Antarctica. Forty-four traditional morphospecies were found in the mats. The dominant species, characteristic only for seepages (one habitat) form especially structured communities with a distinct seasonality.

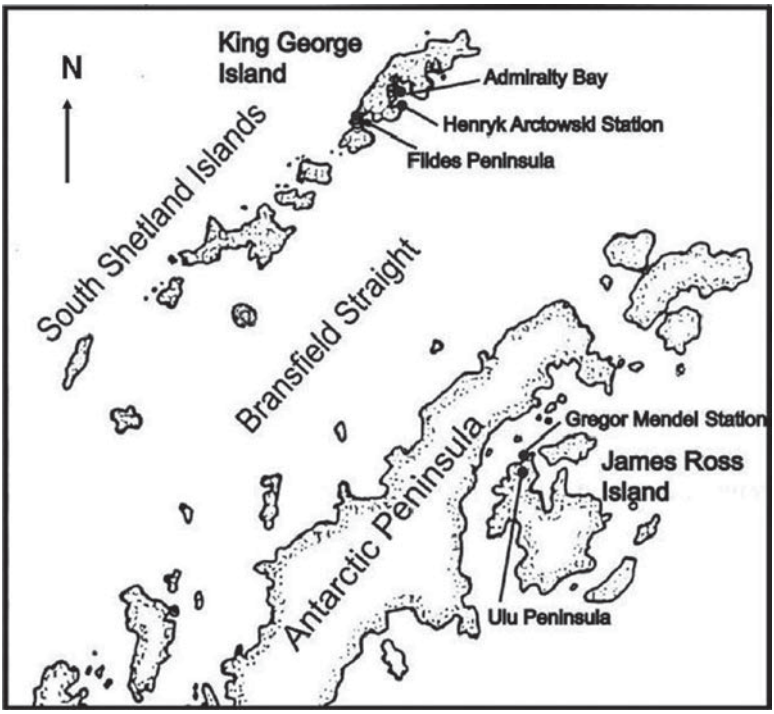
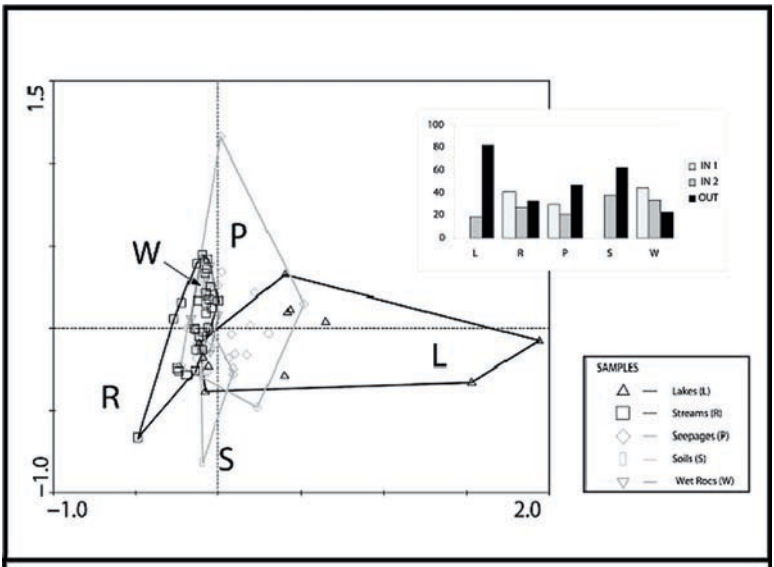


Figure 1. Map of the northern part of Antarctic Peninsula with designated areas of interest.

However, several cyanobacterial species correspond also to types known from other habitats of the deglaciated Antarctic area (mainly from maritime Antarctica and Antarctic oases).

The species composition and diversity in the communities was evaluated by the TWINSPAN clustering method and by Canonical Correspondence Analysis (CANOCO) according to Ter Braak and Prentice (1988). We used Detrended Correspondence Analysis (DCA) for the description of the species/localities relationships and Redundancy Analysis (RDA) for the species + factors versus localities interaction model.

In this review, we show the major differences of the phototrophic flora in seepages of the coastal deglaciated parts of King George Island and James Ross Island with aspects of geomorphology, geography, climate, and nutrient availability (Fig. 2). The cyanobacterial mats occur here in specific and very special habitats, highly sensitive to climatic changes. The maintenance of the habitats specific for cyanobacterial mats depends on the ambient climatic conditions. The exceptions are cryosestic species, soil algae, and hypertrophic (or saline) biotopes around the coast in Arctowski (King George Island) area, which do not occur at James Ross Island.



Distribution of cyanoprokaryotic species in main biotopes:

		mass development
Creeks	9	5
Seepages	31	5
Coastal swamps (partly polluted)	9	4
Seepages with mosses	4	2
Hypertrophic coastal pools	5	4
Cryoseston	2	2
Soils	6	2
Wet rocks (subaerophytic)	9	7

Figure 2. The distribution of the localities according to species composition at James Ross Island by Detrended Correspondence Analysis (DCA). The different species composition in various microhabitats is recognizable from the number of species in various habitats and also follows from the analyses in King George Island (cf. the table at the bottom of the picture). (Modified from Komárek et al., 2008; Komárek and Komárek, 2003, reproduced with permission from Polish Polar Research.)

2. Seepages on King George Island

King George Island is the largest island of the South Shetland Islands, which belong already to the inner coastal Antarctic ecosystem, devoid of higher vegetation (in comparison with subantarctic islands; only two species of vascular plants occur in this region). It is composed mostly from volcanic rocks. The tertiary volcanites are relatively soft materials, easily modified by glacial processes. The deglaciated area around Arctowski station is relatively young and although the glacier layer is not very high, it is maintained because of the high amount of precipitation during the whole year. Our studies were concentrated mainly in the vicinity of Polish Antarctic station H. Arctowski in Admiralty Bay (Fig. 3).

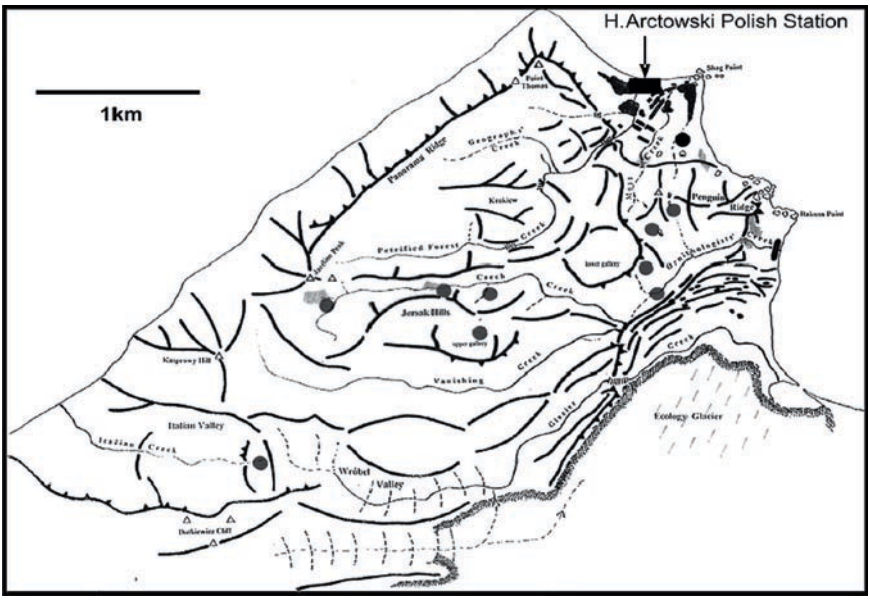


Figure 3. Map of the vicinity of the Polish H. Arctowski Station, indicating localities with studied seepages (*black dots*), King George Island.

The area was described in detail in several papers (Myrcha et al., 1991; Kawecka and Olech, 1993; Rakusa-Suszczewski, 1993; Tatur and Myrcha, 1993).

The water temperature during the summer season usually changes from 2.2 to 7.3 (8.2)°C, but on sunny days it can occasionally increase up to 18°C and exceptionally to about 20°C. However, such extreme values are rare and occur only several times during the peak of the summer for only short periods (1–3 h). All the species are probably adapted to survive such increase in temperature. Higher temperature values commonly occur on the surface of the mat, under direct irradiation. The conductivity in the seepages near Arctowski station was 60–180 μS; pH ranged from (7.7) 8.1 to 9.6 (10.7).

The structure of mats (Fig. 4) changes during the vegetation season, and the communities develop into the climax state in the second part of summer season showing a very characteristic zonation (Fig. 5).

1. Just after temperature increases above 0°C in meltwater, the first aspect of benthic diatoms develops. Small *Luticola*, *Achnanthes*, and *Nitzschia* species were dominant. The visible mats appeared in water in temperature over 2°C. In this first stage, solitary trichomes of *Phormidium pseudopriestleyi* and *Leptolyngbya fritschiana* started to grow.
2. In the second period, *Leptolyngbya fritschiana* started to be dominant and formed fine mats on the substrate. The accessory species are the cyanoprokaryotes *Phormidium pseudopriestleyi* and *Leptolyngbya borchgrevinkii*, and the production of diatoms decreased. The water temperature slightly increased to the average values of about 4–6°C.

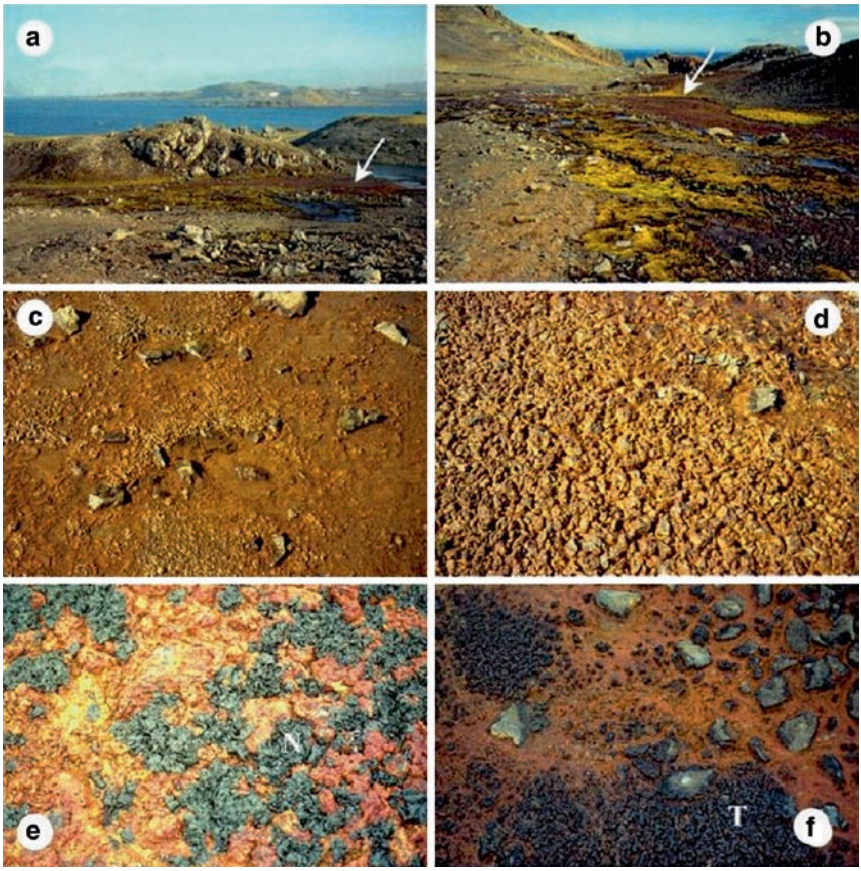


Figure 4. Photograph of the King George Island seepages: (**a**–**b**) = seepages from the south of Fildes Peninsula, (**c**–**d**) = mats with dominant *Leptolyngbya vincentii* on the surface, (**e**–**f**) = climax stages with blackish colonies of *Nostoc commune* (N) and *Tolypothrix* sp. (T). (Modified from Komárek and Komárek, 2003; reproduced with permission from Backhuys Publishers, Leiden.)

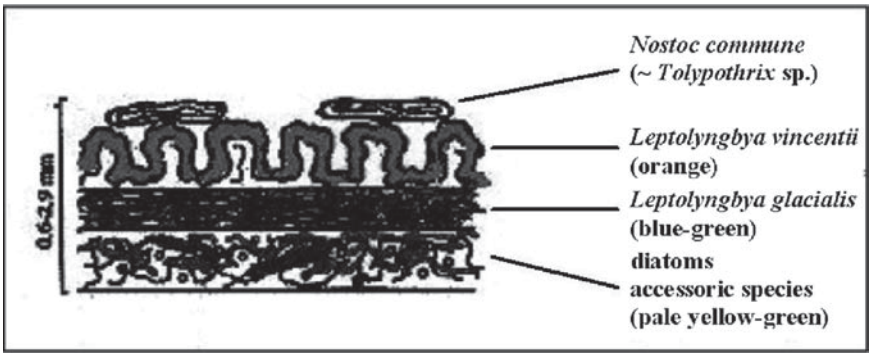


Figure 5. The zonation of cyanobacterial mats from the climax stage seepage at King George Island. (Modified from Komárek and Komárek, 2003; reproduced with permission from Backhuys Publishers, Leiden.)

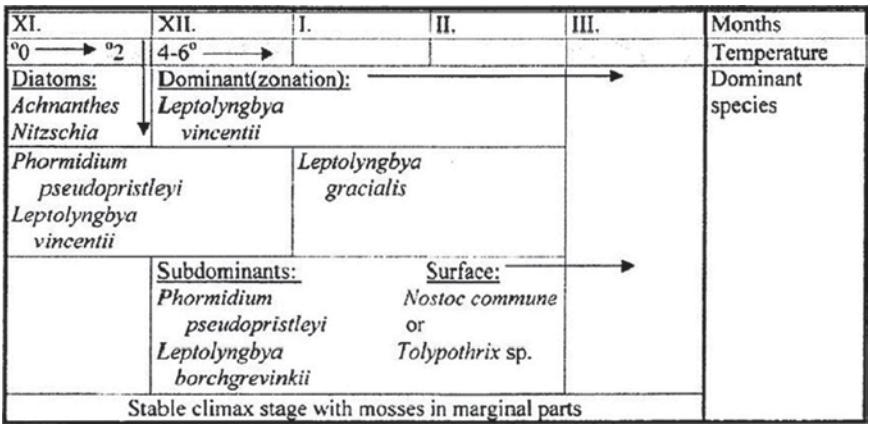


Figure 6. Seasonal changes of seepages mats at King George Island. (Modified from Komárek and Komárek, 2003; reproduced with permission from Backhuys Publishers, Leiden.)

3. By the end of December, thick mats developed at these localities, forming a very characteristic zonation. The upper layer was rusty reddish, compact, wavy on the surface, and up to 2–3 mm thick. It was formed by approximately parallel, heavily agglomerated filaments of the dominant *Leptolyngbya vincentii*. A bright blue-green zone of slightly morphologically different *Leptolyngbya glacialis* with scattered diatoms (e.g., *Fragilaria*) and small clusters of filaments of *Phormidium pseudopriestleyi* was situated below this layer (Fig. 6). This last species developed sometimes more intensely at the edge of *Leptolyngbya* mats or in places with some water flow. The deepest zone was without distinct dominants and it was composed of several species of diatoms and cyanobacteria. The species composition (cf. Table 1) can vary and some of the main representatives can occur locally and occasionally in higher quantity.
4. At the end of the season (usually end of January and February till the frost), the brownish-olive-green colonies of *Nostoc commune* or black *Tolypothrix* sp. started to develop on the upper surface of the mats, initially in small colonies. Rarely, *Nostoc commune* but more frequently *Tolypothrix* can develop large colonies. Occasionally, monospecific communities developing outside the zonated mats can be formed (Fig. 7).

The special modification of seepages represents dominant mosses, which develop at the edges of seepage on the stony substrate. They form a characteristic pattern around algal and cyanoprokaryotic populations at places with a relatively deep source of water (continual carpets of mosses are dominant usually on less wetted zones). They have a clear role in the last period of the development of the

Table 1. Traditional cyanobacterial morphospecies found in seepages at King George Island (KJI) and James Ross Island (JRI). If the species occur also in other microhabitats, they are marked in the column "other biotopes." The category "probably endemic" indicates species not found outside of Antarctica.

Species	KJI	JRI	Other biotopes	Probably endemic
Aphanocapsa sp.	I	I		?
Aphanothece sp.		I		?
Chlorogloea sp.	I	I	(I)	X
Coelomoron chroococcoideum	I			X
Chroococcus sp.	(I)	I	(I)	X
Cyanothece cf. *aeruginosa*	I	I	I	X
Eucapsis sp.	I	I	I	X
Gloeocapsa sp.		I	?	?
Gloeocapsopsis aurea	I	(I)	I	X
Gomphosphaeria antarctica	I			X
Synechococcus sp.		I		?
Blennothrix lauterbachii	I	I	(I)	?
Geitlerinema deflexum	I	I	I	X
Leptolyngbya borchgrevinkii	D	I	I	X
Leptolyngbya cf. *borchgrevinkii*		I	I	X
Leptolyngbya erebii	(I)	(I)	I	X
Leptolyngbya fritschiana	(I)	I	I	?
Leptolyngbya glacialis	D	D	I	
Leptolyngbya nigrescens		(I)	I	X
Leptolyngbya vincentii	D	I	I	X
Microcoleus antarcticus		(I)	I	X
Microcoleus sp. 1	I	I	I	
Microcoleus sp. 2		(I)	I	
Oscillatoria fracta		(I)	I	?
Oscillatoria koettlitzii		I	I	X
Oscillatoria subproboscidea	I	(I)	I	?
Phormidium autumnale		I	I	
Phormidium cf. autumnale	I	D	I	
Phormidium murrayi		(I)	I	?
Phormidium pseudopriestleyi	D	I	(I)	X
Phormidium sp.	(I)		I	?
Plectolyngbya hodgsonii		(I)		X
Pseudanabaena frigida	(I)	(I)	I	?
Romeria nivicola	(I)		I	
Schizothrix antarctica		(I)	I	?
Trichocoleus sp.		(I)	I	?
Anabaena sp.	(I)	(I)	I	?
Calothrix sp.		I	I	?
Hassallia sp.		I		?
Hydrocoryne sp.		(I)	(I)	X
Nodularia quadrata		I	I	X
Nostoc cf. *commune* 1	D	(I)		
Nostoc cf. *commune* 2	(I)	D	I	?
Nostoc cf. *commune 3*		I	I	?
Tolypothrix sp. 1	D		I	?
Tolypothrix sp. 2		I	I	X

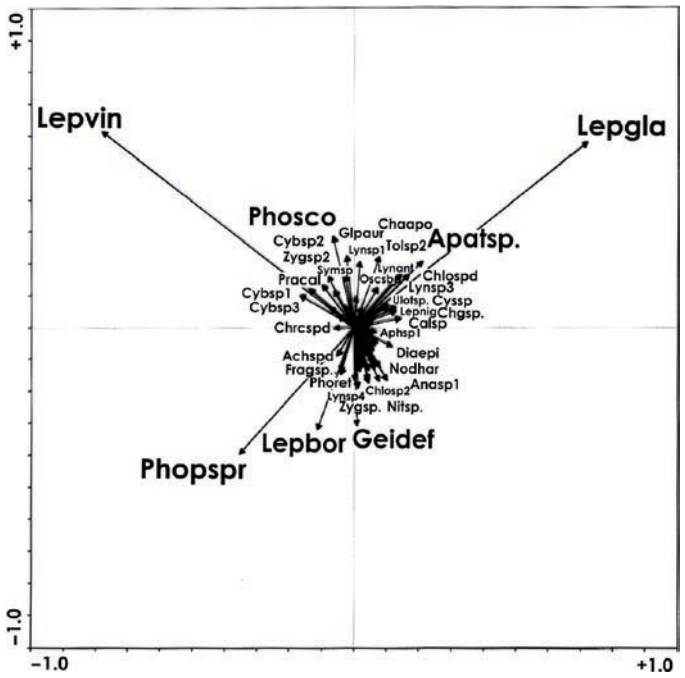


Figure 7. Species distribution at the ordination space of first and second axis of Detrended Correspondence Analysis of the King George Island seepage. (Modified from Komárek and Komárek, 2003; reproduced with permission from Polish Polar Research.)

seepages. They bring to the habitat, biomass with a high content of organic material based on cellulose. The water temperatures in localities with dominant mosses did not exceed +7.5°C, and pH varied from 6.5 to 9.6 in the Arctowski station region. Cyanoprokaryotes and algae close to mosses were scattered in small microbiotopes, pools, and depressions with water, but they did not form distinct communities. Several species were found only in this biotope, e.g., the cyanoprokaryotes *Coelomoron chroococcoideum*, *Gomphosphaeria antarctica*, *Phormidium* and *Leptolyngbya* spp., two *Anabaena* species, and several diatoms and desmids, which are highly specific for this habitat.

3. Seepages in James Ross Island

The northern deglaciated part of James Ross Island in the NW part of the Weddell Sea belongs to the largest areas without glaciers and snow fields in summer season in Antarctica (almost 100 km²). It is located east from the Antarctic Peninsula

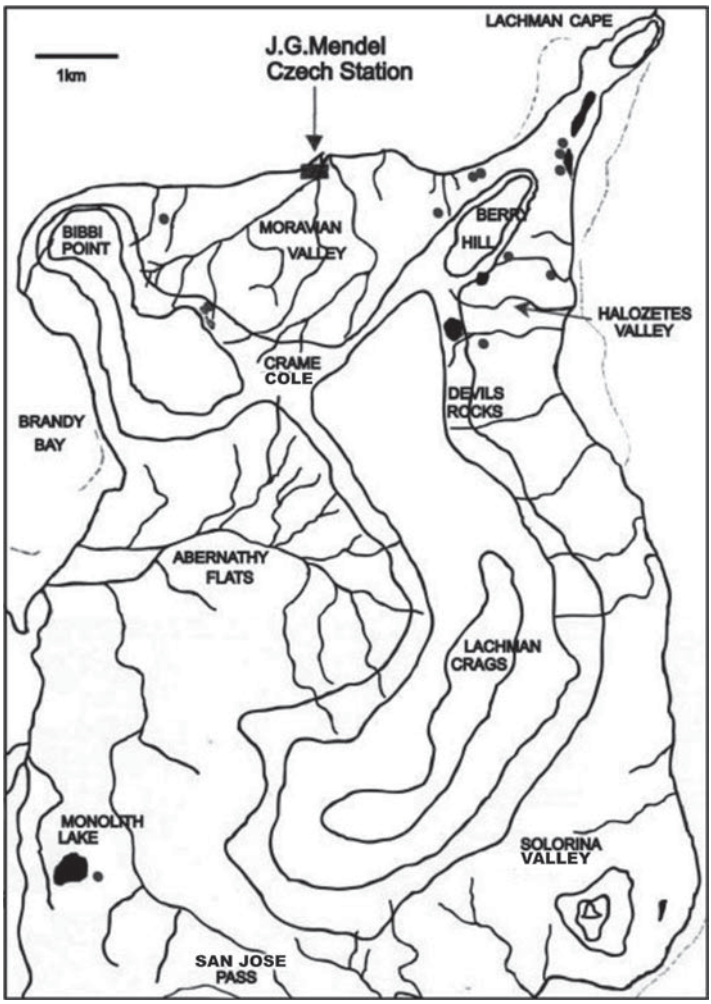


Figure 8. Map of the studied deglaciated area at the vicinity of the Czech J.G. Mendel Station at James Ross Island (Ulu peninsula). The studied localities (seepages) are marked by dots.

(Fig. 1). The mountain range on the peninsula prevents exposure of this area to the western winds prevailing in the southern Pacific Ocean. This locality is therefore relatively arid with extremely rare precipitation during the summer season. It is broken by numerous ridges, mesetas, and moraines, and the highest points reach over 400 m (Lachman Crags, Davis Dome) (Fig. 8). In the whole area, a permafrost layer exists below the surface of soils and gravel-soils. The glacial streams flow mainly from melting snow fields and ice cores in morains, and some of these are up to several km long. They flow continually from mid-November to mid-February, and

this time is convenient for the development of a benthic microvegetation. A similar situation is encountered in seepages, which can be developed along the streams or in depressions. The northern part of James Ross Island (Ulu peninsula) is composed of Quaternary sediments and rocks, through which the Tertiary volcanites pass with connected intrusive rocks. Large parts are covered with Cretaceous sediments (Nývlt and Mixa, 2003). The geomorphology is relatively more complicated than at King George Island (higher altitudes and more broken terrain).

The monthly mean air temperature exceeds 0°C only in the summer season from November to February, but is never higher than 3°C according to recent measurements (Komárek et al., 2008). The maximal air temperature was measured in January and February (over 10°C). The minimal air temperatures below 0°C occur during eight months per year from March to October. The daily average and extreme temperatures in soil (5 cm below surface) at the J.G. Mendel Station are presented in Fig. 9. From the diagrams, it follows that January is distinctly warmer than other summer months in the studied area. The temperature of the water in seepages during our studies of cyanobacterial microflora is presented in Fig. 10. The water habitats (streams, lakes, seepages) keep their temperature almost over the whole second part of the summer season above 0°C, and thus enable rapid development of cyanobacterial and algal communities during this season.

The main physico-chemical parameters of waters and soils (temperature, pH, conductivity, total N and P) were measured during the summer seasons (I–II) in 2004 and 2006. The pH values in aquatic habitats were slightly alkaline and varied from 7.3 to 8.6 in streaming waters, and from 6.8 to 9.4 in lakes and pools. Values below 7 and over 8 were found only in small lakes and pools with

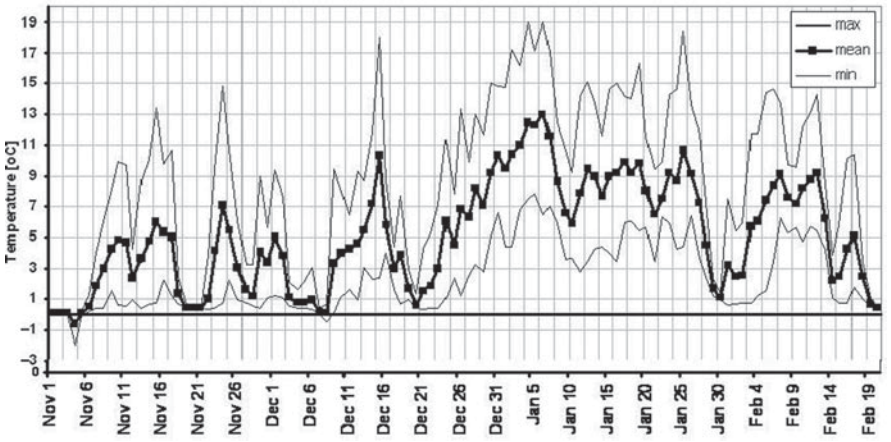


Figure 9. Mean and extreme daily temperatures in soils (5 cm below surface) during the Antarctic summer season (Nov. 1, 2005–Feb. 20, 2006) on the J.G. Mendel station (J. Ross Island). (From Komárek et al., 2008; reproduced with permission from Polar Biology.)

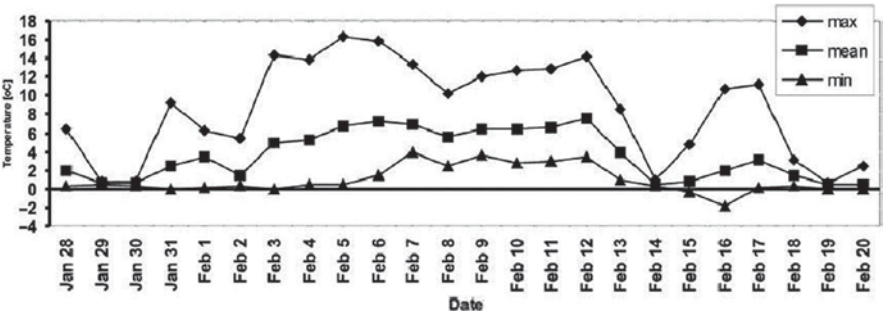
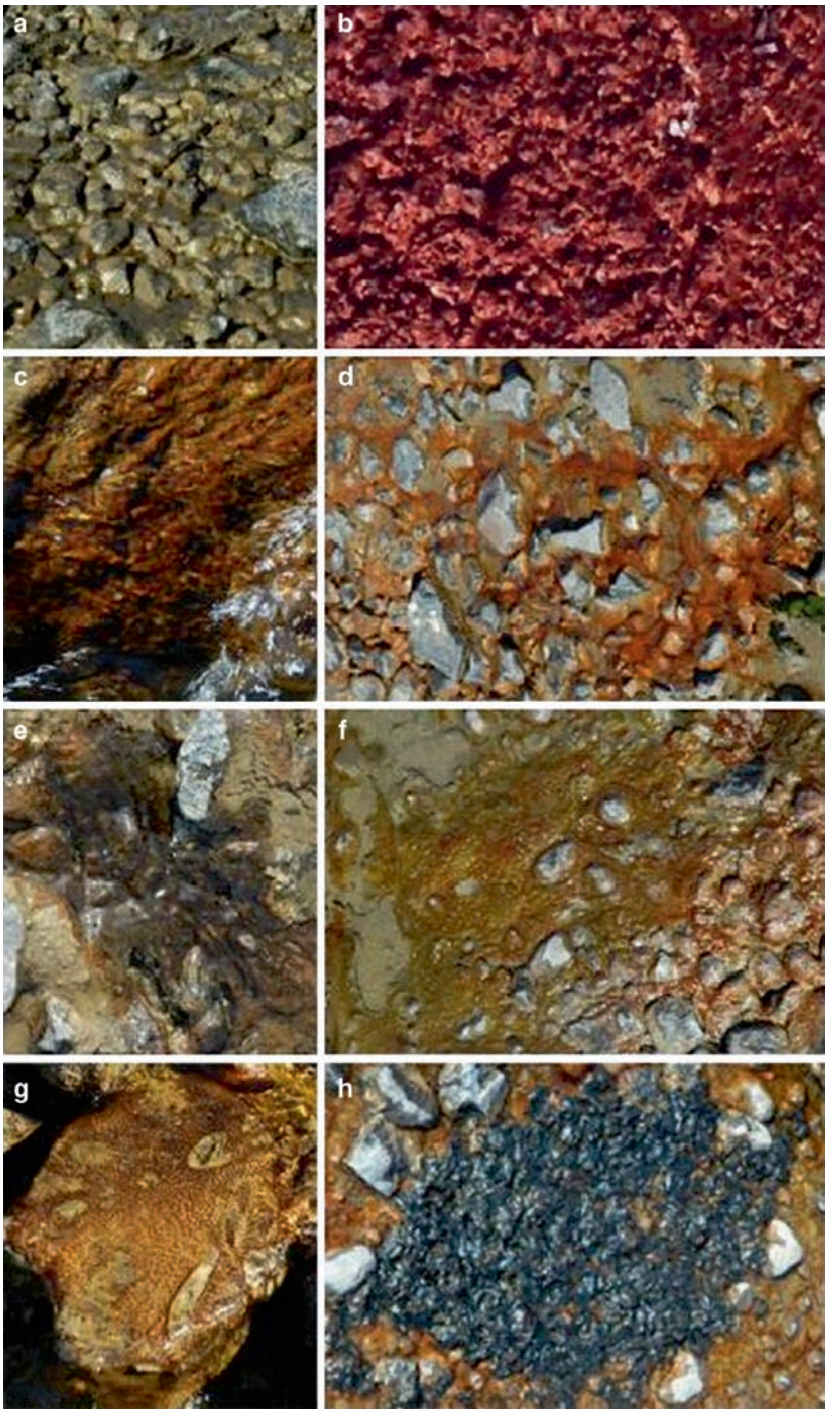


Figure 10. Temperature values of water in seepages (northern slopes below Berry Hill in James Ross Island) observed in the period of our studies (Jan. 28–Feb. 20, 2006). Values were selected from four parallel measured locations. (From Komárek et al., 2008; reproduced with permission from Polar Biology.)

developed and diversified cyanobacteria and algae. Conductivity of inland waters is usually low (90–350 μS). The natural samples were studied in the living state by optical microscopy, measured and documented by drawing and microphoto techniques. The dominant types were cultured on BG11 agar medium under moderate illumination at temperatures between 10 and 20ºC, but only few were able to grow under such conditions. Sixty-five traditional cyanobacterial morphospecies were found in habitats of the northern part of the James Ross Island during the period from January to February 2006, of which 14 (22%) were coccoid or colonial, 37 (56%) filamentous without heterocysts, and 14 (22%) with heterocysts. Moreover, ten ecological or morphological modifications of uncertain taxonomic status of previous typical populations were registered. However, only 41 morphospecies were registered in seepages (Table 1).

The structure of the cyanobacterial communities and species composition were in principle the same like in King George Island, but the mats developed were usually more patchy, forming a mosaic of colonies on the substrate (Fig. 11). The zonation was not distinct and the mats were composed of different species, which formed separated colonies, transient from one to another, and occasionally forming layers. The mats were formed mostly by *Leptolyngbya fritschiana*, *L. borchgrevinkii* (a special morphotype occurs commonly in James Ross Island), *Phormidium autumnale* (two morphotypes), *Phormidium pseudopriestleyi*, and *Nostoc* cf. *commune*. The strata with *Leptolyngbya vincentii* and *L. glacialis* occur only sporadically on places with continual supply of water without strong flow. The rich diversity follows also from the list of other accessoric species, which do not occur in higher biomass. Coccoid morphospecies also belong to such type, which occur only sporadically and in restricted areas (*Coelomoron chroococcoideum*, *Eucapsis* sp., *Chroococcus* sp., and others). The climax stages with mosses are developed usually only near streams.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4. The Species Composition of Antarctic Cyanobacterial Communities

The origin of the modern cyanobacterial microflora of Antarctica can be in principle secondary. The possible transport of diaspores by wind was precisely analyzed by Wynn-Williams (1991). However, the present continual input of new spores cannot explain the special diversity of Antarctic cyanobacteria. Cyanobacteria are ecologically very plastic and adaptable, and ecotypes and physiological modifications can change very rapidly (Hagemann, 2002; Huckauf et al., 2000). The dominant morphospecies in various Antarctic habitats (benthos of frozen lakes, endolithic communities, characteristic mats in seepages and in creeks, special types on dripping rocks, etc.) are very stable and characteristic and cannot be occasionally transported (every year in enormous quantity) from other continents. The dominant forms are represented by the special morphotypes, not commonly known from habitats in other regions from which the transport of diaspores is considered. Moreover, the typical assemblages develop with the same dominants and structure of communities every year (cf. Komárek and Komárek, 1999) and therefore indicate the existence of stable adapted, domestic geno- and morphotypes.

When comparing the list of cyanobacteria from James Ross Island with species reviews from typical maritime Antarctica (Komárek, 1999; Komárek and Komárek, 1999), numerous up to now registered "endemic Antarctic morphospecies" are identical and characteristic in both studied regions. Also in accessory species, a wide spectrum of types exists that evidently originate from other Antarctic biotopes. However, in special habitats (lakes, dripping rocks, seepages) several morphospecies were found, which evidently differ from similar habitats in King George Island, and are unknown also from any other ecosystems (particularly the morphotypes with heterocysts from the form-genera *Calothrix*, *Coleodesmium*, *Dichothrix*, *Microchaete*, and *Tolypothrix*). Analyses of morphospecies from seepages indicate the endemic character of numerous stabilized cyanobacterial morphotypes, which play an important role in the formation of characteristic communities. Certain differences in species composition also exist in various, geographically distant seepages. The ecology of similar mats from the continental Antarctic coast (McMurdo Sound region) was well described, e.g., by Vincent et al. (1993). The authors distinguished five types of mats, characterized according to the color of their microlayers. The species diversity in mats of continually wetted localities is

Figure 11. Mats of dominant cyanobacterial species from seepages and creeks below Berry Hill (James Ross Island): (**a**) grayish mats of *Leptolyngbya fritschiana* covering stones in upper part of Algal Creek, (**b**) orange surface layer of *Leptolyngbya vincentii* from mats in seepages near upper part of Elster Creek, (**c**) dark orange-red colonies of *Phormidium priestleyi* on the edge of stones in rapid lower part of Tern Creek, (**d**) rusty-brownish mats of *Leptolyngbya borchgrevinkii* in shallow water among stones of seepages below Berry Hill, (**e**) dark blackish mats of *Phormidium autumnale* (typical form) in the middle part of Tern Creek, (**f**) mats of *Phormidium autumnale* (narrow form) from littoral part of Tern Creek, (**g**) typical structured (*lamellated*) mats of *Phormidium pseudopriestleyi* from seepages below Berry Hill, (**h**) subaerophytic colonies of *Nostoc commune* from seepages below Berry Hill. (From Komárek et al., 2008; reproduced with permission from Polar Biology.)

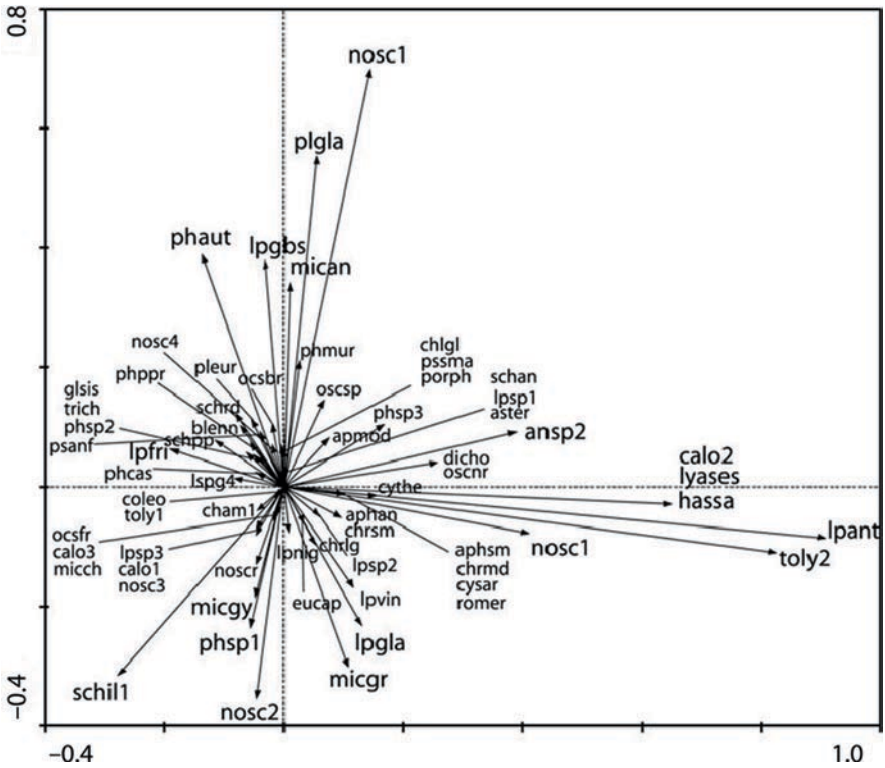

Figure 12. Species distribution at the first and second ordination axes of DCA in seepages at James Ross Island. (From Komárek et al., 2008; reproduced with permission from Polar Biology.)

relatively high and the majority of cyanoprokaryotic and algal species seems to be endemic, ecologically very distinct, and special also for various subantarctic regions (Wharton et al., 1983; Broady, 1989a, b; Vincent et al., 1993). The species dominance distribution in seepages from King George Island and James Ross Island are illustrated in Figs. 7 and 12.

Summarizing, strongly distinct species occur in various special Antarctic microbiotopes (streams, soils, various lakes, and wetted rocks) and are specific only for such types of localities and absent in seepages. The species differences between microhabitats follow clearly from the statistical evaluation of the species composition in various ecological situations (Fig. 13).

Up to now, the majority of results concerning the cyanoprokaryotic diversity is based on studies using traditional taxonomy and nomenclature (Prescott, 1979; Broady, 1986, 1996; Broady and Ohtani, 1990; Broady and Kibblewhite, 1991; Cavacini, 2001; De los Rios A et al., 2004). However, the modern study of cyanobacterial diversity should be based on the molecular background. The first reports on the genetic study of the Antarctic cyanobacterial microflora were already

published (Priscu et al., 1998; Gordon et al., 2000; Nadeau and Castenholz, 2001; Taton et al., 2003; Casamatta et al., 2005; Jungblut et al., 2005; Comte et al., 2007). Genetic analyses can finally yield information on the distribution of certain

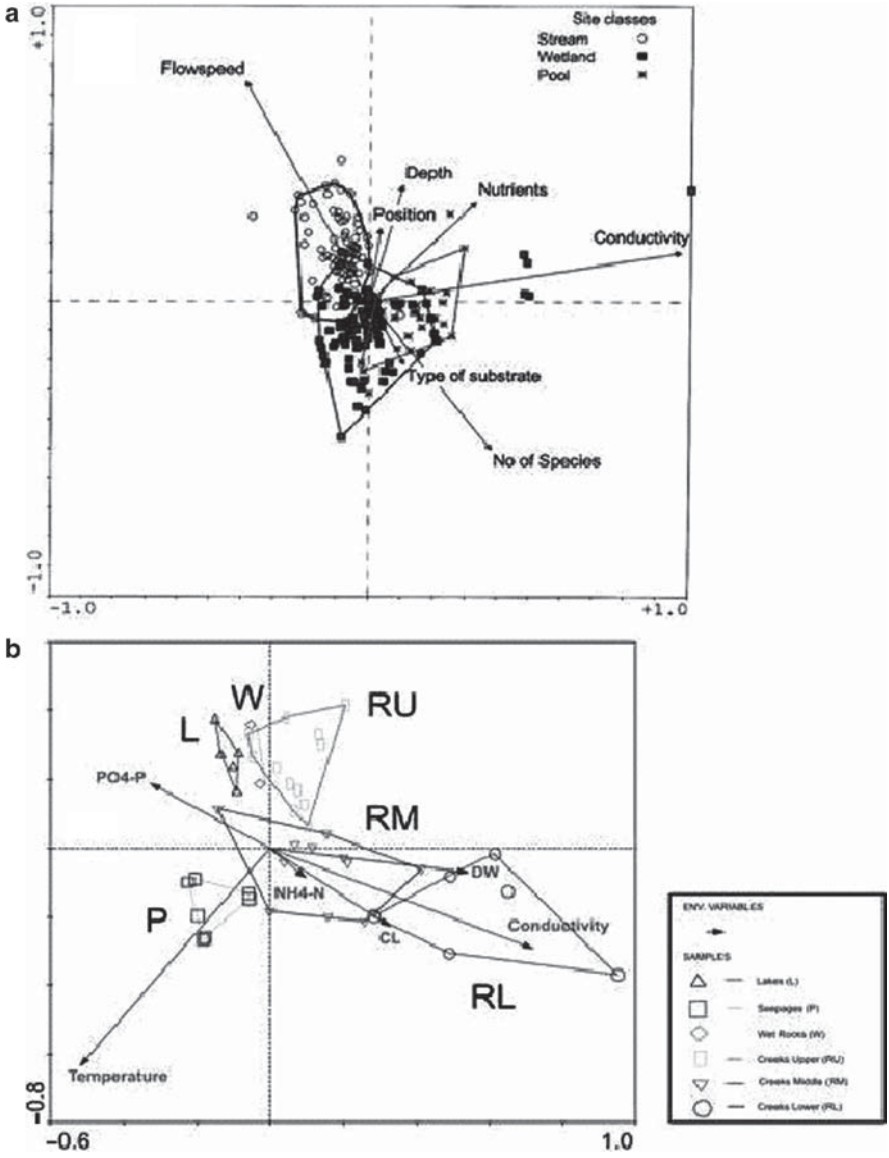

Figure 13. The distribution of localities from different microhabitats owing to the species composition and factor conditions at the first and second axis of Redundancy Analysis (RDA) at King George Island (**a**) and James Ross Island (**b**). (From Komárek and Komárek, 1999; Komárek et al., 2008; reproduced with permission from Polar Biology.)

genotype clusters over Antarctica, but their importance for detailed ecological studies is still limited. Such studies usually compare randomly selected strains with unknown morphology within clusters, designated by arbitrary selected names, often designated only by generic name ("*Leptolyngbya* sp.,", etc.), or even as "uncultured Antarctic cyanobacterium," "Environmental clone," etc. Morphological analysis according to phenotype taxonomy is often underestimated and rejected in advanced science. However, not even the most modern studies can replace the study of natural populations and the assigning of scientific names.

The study of seepages must be followed particularly by special genetic analyses, in which the recognition of different genotypes is particularly important (mainly for the genera *Leptolyngbya*, *Phormidium*, and *Nostoc*). An example is *Leptolyngbya antarctica*, which is the dominant cyanobacterium from benthic communities of Antarctic lakes, or *L. glacialis*, one from the dominant morphospecies in characteristic mats in seepages. These ecotypes appear in the literature under very different designations, but the real distribution of the particular genotypes and corresponding morphotypes in space does not follow from modern studies. Taton et al. (2006) analyzed about 40 "thin *Oscillatoria*" strains mostly belonging to the vicinity of form-genera *Leptolyngbya* and *Pseudophormidium* by combined sequencing and morphological evaluation. They were classified into several clusters, but it is difficult to identify which clusters can represent distinct morpho- and ecotypes. The comparison of morphology of simple types like *Leptolyngbya* from natural localities and from cultures is difficult (particularly, if the ecological differences in situ are quite special on the level of microhabitats).

An example of a special problematic genus is *Nostoc* (corresponding traditionally mostly with the widely conceived *N. commune*), which commonly occurs in Antarctic deglaciated areas in several eco- and morphotypes. The question, whether it is a widely adapted single genotype or whether different genotypes appear in distinct soil and seepages locations is not yet solved. Three different clusters of *Nostoc* from microbial mats from McMurdo Dry Valleys was identified by Taton et al. (2003), but it is necessary to determine their ecological variability and relationships to the life forms of natural habitats. Novis and Smissen (2006) found two ecologically and genetically distinct *Nostoc commune* types in Victoria Land. An understanding of the genetic and phenotypic diversity in various Antarctic ecosystems is therefore very desirable.

5. Conclusions

In the seepages of maritime Antarctica, special and characteristic biocenoses of microphytes develop, with a characteristic succession, seasonality, ecological characteristics and structure of communities, and species composition. Thus, the microphyte communities of Antarctic seepages represent a unique endemic biotope. The biological and ecological importance is the same as of all specialized

polar endemic organisms. From the ecological points of view, these types belong to the first colonizers of sites where water appears in the soil. They are followed by the succession of other plants, which are not able to inhabit or settle originally dry substrates (mosses and possibly higher plants).

Seepages arise on places with deglaciated substrate, where the water regime supplies the minimum necessary inflow of nutrients. In maritime Antarctica, communities with special species composition and structure in the form of mats develop in seepages (Komárek and Komárek, 2003). According to our results, similar mats in the Arctic region have a different morphospecies composition. The richness of cyanobacterial vegetation is controlled particularly by the continual supply of water over the vegetation season (in periodically drying seepages, the typical communities never develop), by exposure to light in flat and open localities, and by a range of temperatures from 0 up to 17–18°C in sunny days. pH was found to range from 6.8 to 7.4, and conductivity from less than 100 to about 360 μS at seepages localities on James Ross Island. In typical cases in coastal Antarctica (King George Island), the upper intensely orange layer is formed by a special morphospecies *Leptolyngbya vincentii*, condominated by green *Leptolyngbya glacialis* in the lower layers. While *L. vincentii* seems to be very specific just for the upper layers of mats in seepages, *L. glacialis* sporadically occurs also on edges of streams and in soils (Komárek, 2007). The lowest parts of mats are colonized by more species, sometimes also of coccoid types. In each case, seepages are the richest habitats of cyanobacterial morphotypes in the whole area. In the James Ross Island, over 40 morphospecies were found (out of a total of 65 species registered), with seven dominating types, which occur in different situations in high quantity and form characteristic assemblages (*Geitlerinema* cf. *deflexum*, *Leptolyngbya borchgrevinkii*, *Phormidium autumnale* "typical form", *P. pseudopriestleyi*, *Oscillatoria subproboscidea*, *Nodularia* sp. etc.). It is possible to designate at least 18 species as characteristic for this habitat. At the end of the season, few other species participate on the climax of developed mats (*Nostoc commune* sensu lato, *Tolypothrix* sp., etc.), but only *Nostoc commune* (type "2") was found regularly and obligatory as most common in seepages of James Ross Island. Moss communities sometimes develop at the margin in stabilized, old seepages as a climax-stadium.

The connection of the different types of habitats at the studied area is an important feature for the stability of the whole ecosystem. The major differences between King George Island and James Ross Island in habitat interconnection and fractionation is caused by the highly humid character of the King George Island area, where frequent precipitation and ample water supply occur and temperature maintenance by the ocean keeps the landscape in continual mild weather throughout the year. The James Ross Island low precipitations are combined with only occasional flash floods of melting water, connected with a seasonally changing temperature (a more continental character). The high altitudes and slopes of the landscape together with the specific climate lead to a rise of the breaks between freshwater habitats and individual disconnected water systems. This increase in arid character

of the ecosystem causes irregular water fluctuations and finally fractionation of individual habitats. The communities in seepages of King George Island and James Ross Island have a similar species composition, but the structure at James Ross Island is patchier and not fully developed in comparison with maritime areas. James Ross Island represents therefore rather transitions to communities of deglaciated parts of continental Antarctica highly limited by climate.

Detrended Correspondence Analysis (DCA; cf. Fig. 2) divided the localities according to their species composition. Several gradients of conditions influence very specifically each biotope. To follow the influence of factors on species occurrence and diversity, we used Redundancy Analysis (RDA). The samples from pools, wetlands, and streams at King George Island (cf. Fig. 7) have mixed environmental conditions, and the main influence causing changes in this community is conductivity and flow speed (based on the Monte Carlo permutation test $P < 0.01$ for both factors). At James Ross Islands, it is conductivity and temperature, but the major set of factors guiding the structure of community is chemistry of water determined by geomorphology.

The cyanoprokaryotic and algal microflora in maritime Antarctica contains many endemic species (about 60% in cyanobacteria; Komárek 1999), and several characteristic and very specific algal communities, which need special protection like other (more prominent) Antarctic plants and animals. Global climatic changes will lead to the desertification of the area and damage the unique habitats by unpredictable and harmful climatic and geomorphological events.

6. Acknowledgments

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7. References

Beljakova, R.N. (1987) Cyanophyta zonae litoralis insulae King-George (Antarctis). Novit. Syst. Plant. Non Vasc. **24**: 26–30.

Broady, P.A. (1986) Ecology and taxonomy of the terrestrial algae of the Vestfold Hills. In: J. Pickard (ed.) *Antarctic Oasis*. Academic, Australia, pp. 165–202.

Broady, P.A. (1989a) Broadscale patterns in the distribution of aquatic and terrestrial vegetation at three ice-free regions on Ross Island, Antarctica. Hydrobiologia **172**: 77–95.

Broady, P.A. (1989b) Survey of algae and other terrestrial biota at Edward VII Peninsula, Marie Byrd Land. Antarct. Sci. **1**: 215–224.

Broady, P.A. (1996) Diversity, distribution and dispersal of Antarctic terrestrial algae. Biodivers. Conserv. **5**: 1307–1335.

Broady, P.A. (2005) The distribution of terrestrial and hydro-terrestrial algal associations at the three contrasting locations in southern Victoria land, Antarctica. Arch. Hydrobiol./Algolog. Stud. **118**: 95–112.

Broady, P.A. and Kibblewhite A.L. (1991) Morphological characterisation of oscillatoriales (cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. Antarct. Sci. **3**: 35–45.

Broady, P.A. and Ohtani, S. (1990) Joint New Zealand – Japanese studies on the taxonomy of terrestrial Antarctic algae. N Z Antarct. Rec. **10**: 22–27.

Carlson, G.W.F. (1913) Süsswasseralgen aus der Antarktis, Südgeorgien, und den Falkland Inseln. Wiss. Ergebn. Schwed. Südpolar-Exped. 1901–1903, **4**: 1–94.

Casamatta, D.A., Johansen, J.R., Vis, M.L. and Broadwater, S.T. (2005) Molecular and morphological characterization of ten polar and near-polar strains within the oscillatoriales (cyanobacteria). J. Phycol. **41**: 421–438.

Cavacini, P. (2001) Soil algae from northern Victoria Land (Antarctica). Polar Biosci. **14**: 45–60.

Comte, K., Šabacká, M., Carre-Mlouka, A., Elster, J. and Komárek, J. (2007) Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. FEMS Microbiol. Ecol. **59**: 366–376.

Rios, A., Ascaso, C., Wierzchoz, J., Fernandez-Valiente, E. and Quesada, A. (2004) Microstructural characterization of cyanobacterial mats from the McMurdo Ice Shelf, Antarctica. Appl. Environ. Microbiol. **70**: 569–580.

Elster, J. (2002) Ecological classification of terrestrial algal communities of polar environment, In: Beyer, L. and Boelter, M. (eds.) *Ecological Studies.* Springer, Berlin, pp. 303–319.

Elster, J. and Benson, E. (2004) Life in the polar terrestrial environment with a focus on algae and cyanobacteria, In: B.J. Fuller, N. Lane and E.E. Benson (eds.) *Life in Frozen State.* CRC Press, Boca Raton, FL, pp. 111–150.

Elster, J. and Komárek, O. (2003) Ecology of periphyton in a meltwater stream ecosystem in the maritime Antarctic. Antarct. Sci. UK **15**: 189–201.

Fritsch, F.E. (1912) Freshwater algae. National Antarctic Discovery Expedition, 1901–1904. Br. Mus. Nat. Hist. **6**: 1–66.

Fritsch, F.E. (1917) Freshwater algae. British Antarctic (Terra Nova) Expedition. Nat. Hist. Rep. Part I. London.

Gordon, D.A., Priscu, J.C. and Giovannoni, S. (2000) Origin and phylogeny of microbes living in permanent Antarctic lake ice. Microb. Ecol. **39**: 197–202.

Hagemann, M. (2002) Environmental stress, signalling and basic acclimation reactions, In: R. Solheim, S. Ventura and A. Wilmotte (eds.) *Cyanobacteria and Nitrogen Fixation in Extreme Environments*. European Science Foundation CYANOFIX, Longyearbyen, Svalbard, p. 24.

Huckauf, J., Nomura, C., Forchhammer, K. and Hagemann, M. (2000) Stress responses of *Synechocystis* sp. Strain PCC 6803 mutants impaired in genes encoding putative alternative sigma factors. Microbiology **146**: 2877–2889.

Jungblut, A.-D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ. Microbiol. **7**: 519–529.

Kawecka, B. and Olech, M. (1993) Diatom communities in the Vanishing and Ornithologist Creek, King George Island, South Shetlands, Antarctica. Hydrobiologia **260/270**: 327–333.

Komárek, J. (1999) Diversity of cyanoprokaryotes (cyanobacteria) of King George Island, maritime Antarctica – a survey. Arch. Hydrobiol./Algolog. Studies **94**: 181–193.

Komárek, J. (2007) Phenotype diversity of the cyanobacterial genus *Leptolyngbya* in maritime Antarctica. Polish Polar Res. **28**: 211–231.

Komárek, J. and Anagnostidis, K. (2005) Cyanoprokaryota 19 Teil/2nd Part: Oscillatoriales, In: B. Büdel, L. Krienitz, G. Gärtner and M. Schagerl (eds.) *Süsswasserflora von Mitteleuropa 19/2.* Elsevier/Spektrum, Heidelberg, 759 pp.

Komárek, J. and Komárek, O. (2003) Diversity of cyanobacteria in Seepages of King George Island, Maritime Antarctica, In: A.H.L. Huiskes, W.W.C. Gieskes, J. Rozema, R.M.L. Schorno, S.M. van der Vies and W.J. Wolff (eds.) *Antarctic Biology in a Global Context.* Backhuys Publishers, Leiden, Proceedings VIIIth SCAR Internat. Symp. 2001, Amsterdam, pp. 244–250.

Komárek, O. and Komárek, J. (1999) Diversity of freshwater and terrestrial habitats and their oxyphototroph microflora in the Arctowski Station region, South Shetlands Islands. Polish Polar Res. **20**: 259–282.

Komárek, J., Elster, J. and Komárek, O. (2008) Diversity of the cyanobacterial microflora of the northern part of James Ross Island, NW Weddell Sea, Antarctica. Polar Biol. **31**: 853–865.

Luścinska, M. and Kyć, A. (1993) Algae inhabiting creeks of the region "H. Arctowski" Polish Antarctic Station, King George Is., South Shetlands. Polish Polar Res. **14**: 393–405.

Mataloni, G. and Pose, M. (2001) Non-marine algae from islands near Cierva Point, Antarctic Peninsula. Cryptog./Algolog. **22**: 41–64.

Mataloni, G., Tell, G. and Wynn-Williams, D.D. (2000) Structure and diversity of soil algal communities from Cierva Point (Antarctic Peninsula). Polar Biol. **23**: 205–211.

Myrcha, A., Ochyra, R. and Tatur, A. (1991) Site of Special Scientific Interest no. 8 – western shores of Admiralty Bay, King George Island, South Shetland Islands. – First Polish – Soviet Antarct. Symp. "ARCTOWSKI '85", PAS, II Div. Biol. Sci., pp. 157–168.

Nadeau, T. and Castenholz, R.W. (2001) Evolutionary relationships of cultivated Antarctic oscillarians (cyanobacteria). J. Phycol. **37**: 650–654.

Novis, P.M. and Smissen, R.D. (2006) Two generic and ecological groups of *Nostoc commune* in Victoria Land, Antarctica, revealed by AFLP analysis. Antarct. Sci. **18**: 573–581.

Nývlt, D. and Mixa, P. (2003) [Paleogeographical development of the Antarctic Peninsula during the late Cainozoic.] Geografie – Sborn. ČGS **108**: 245–260 (In Czech).

Ohtani, S. (1986) Epiphytic algae on mosses in the vicinity of Syowa Station, Antarctica. Mem. Natl. Inst. Polar. Res., Special Issue **44**: 209–219.

Ohtani, S., Akiyama, M. and Kanda, H. (1991) Analysis of Antarctic soil algae by the direct observation using the contact slide method. Antarct. Rec. **35**: 285–295.

Olech, M. (1993) Lower plants, In: S. Rakusa-Suszczewski (ed.) *The Maritime Antarctic Coastal Ecosystem of Admiralty Bay.* Polish Academy of Sciences, Warsaw, pp. 173–179.

Prescott, G.W. (1979) A contribution to a bibliography of Antarctic and subantarctic algae together with a checklist of freshwater taxa report to 1979. Bibliotheca Phycol. **45**: 1–312.

Priscu, J.C., Fritsen, C.H., Adams, E.E., Giovannoni, S.J., Paerl, H.W., McKay, C.P., Doran, P.T., Gordon, D.A., Lanoil, B.D. and Pinckney, J.L. (1998) Perennial Antarctic lake ice: an oasis for life in polar desert. Science **280**: 2095–2098.

Rakusa-Suszczewski, S. (ed.) (1993) *The Maritime Antarctic Coastal Ecosystem of Admiralty Bay.* Department of Antarctic Biology, Polish Academy of Sciences, Warsaw, 216 pp.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R. and Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl. Environ. Microbiol. **69**: 5157–5169.

Taton, A., Grubisic, S., Ertz, D., Hodgson, D.A., Piccardi, R., Biondi, N., Tredici, M.R., Mainini, M., Losi, D., Marinelli, F. and Wilmotte, A. (2006) On of Antarctic oscillatorian strains: molecular results vs microscopical results. J. Phycol. **42**: 1257–1270.

Tatur, A. and Myrcha, A. (1993) Ornithogenic soils, In: S. Rakusa-Suszczewski (ed.) *The Maritime Antarctic Coastal Ecosystem of Admiralty Bay.* Department of Antarctic Biology, Polish Academy of Sciences, Warsaw, pp. 161–165.

Ter Braak, C.J.F. and Prentice, I.C. (1988) A theory of gradient analysis. Adv. Ecol. Res. **18**: 271–317.

Vincent, W.F., Downes, M.T., Castenholz, R.W. and Howard-Williams, C. (1993) Community structure and pigment organization of cyanobacteria-dominated microbial mats in Antarctica. J. Phycol **28**: 213–221.

Vinocur, A. and Pizzaro, H. (1995) Periphyton flora of some lotic and lentic environments of Hope Bay (Antarctic Peninsula). Polar Biol. **15**: 401–414.

West, W. and West, G.S. (1911) Freshwater algae, In: J. Murray (ed.) *British Antarctic Expedition 1907–1909, Reports on the Scientific Investigations; Biology*, part 7, Vol. **1**, pp. 263–298.

Wharton, R.A., Parker, B.C. and Simmons, G.M., Jr. (1983) Distribution, species composition and morphology of algal mats in Antarctic dry valley lakes. Phycologia **22**: 355–365.

Wynn-Williams, D.D. (1991) Aerobiology and colonization in Antarctica, In: M. Hjelmroos, S. Nilsson and G. El-Ghazaly (eds.) *Proceedings of 4th International Conference of Aerobiology.* Stockholm, 1990, **30**, pp. 380–393.

PART 5:
MICROBIAL MATS AND ASTROBIOLOGY

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MICROBIAL MATS IN ANTARCTICA AS MODELS FOR THE SEARCH OF LIFE ON THE JOVIAN MOON EUROPA

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1. Introduction

The discovery of extreme environments with organisms adapted to these conditions has made them useful analogies for the presence of life beyond the Earth, whether autochthonous, or by the transport of microorganisms between different bodies of the solar system. This latter possibility has been known as the hypothesis of panspermia.

These microorganisms not only tolerate harsh environmental conditions, but even thrive on them. For this reason, such organisms are called "extremophiles." The occurrence of oxygenic phototrophs in extreme environments has been reviewed extensively (Seckbach and Oren, 2007), including phototrophic thermophiles (adapted to higher temperatures) that are able to survive up to 74°C. Certain archaea may survive in environments up to 114°C. Halophiles (high salt-loving microorganisms) tolerate salt concentration up to saturation. Acidophiles thrive at pH values as low as 0.5. Alkaliphiles living at high pH may survive up to pH 10–13.

The extreme environments and their microbes can thus act as models for extraterrestrial life (Seckbach and Chela-Flores, 2007). Active photosynthetic microbial communities (discussed in Section 2) are found in Antarctica, both in and on ice, in freshwater, in saline lakes and streams, and within rocks. In the dry valley lakes of Antarctica close to the McMurdo Base, microbial mats (discussed in Sections 2 and 3) are known to selectively remove a huge quantity of sulfur (Parker et al., 1982). Lake Vostok in Antarctica (discussed in Section 4) possesses a perennially thick (3–4 km) ice cover that precludes photosynthesis below, thus making it a good model system for determining how a potential Europan biota might survive (Stone, 1999). The presence of liquid water is a prerequisite for life (Oren, 2008).

Jupiter's moon Europa may harbor a subsurface water ocean. This putative ocean lies beneath an ice layer that is too thick to allow photosynthesis. However, that disequilibrium chemistry in the icy surface, driven by charged particles that are accelerated in Jupiter's magnetosphere could produce sufficient organic and oxidant molecules on a Europan biosphere (Chyba, 2000).

We restrict our attention to microbial mats that could be thriving under extreme conditions of radiation on Europa. We are especially concerned by the presence of the sulfur patches discovered by the Galileo mission.

2. Astrobiological Implications of the Microbial Mats in the Dry Valley Lakes

Microbial mats are stratified microbial communities that develop in the environmental microgradients established at the interfaces of water and solid substrates. The organic laminated multilayered biofilm has hydrated exocellular polymeric substances (EPS) secreted by microorganisms embedded within it (Davey and O'Toole, 2000; Konhauser, 2007) and largely alters the environmental microgradients in the interface as a result of their metabolism.

These microbial mats develop in a wide variety of environments (Cohen 1984; Cohen and Rosenberg, 1989; Stal, 1995). Therefore, we find a wide variation of communities living in them. The Antarctic dry valley lakes are among these ecological sites (Parker et al., 1982). These lakes are unique in that they consistently maintain a thick year-round ice cover (2.8–6.0 m) over liquid water. The persistent ice cover minimizes wind-generated currents and reduces light penetration, restricting sediment deposition into a lake and exchange of atmospheric gases between the water column and the atmosphere. The present lakes are mostly remnants of larger glacial lakes perhaps some 4.6 million years (Ma) before the present (Doran et al., 1994).

The microbial mats found in these environments are composed primarily of cyanobacteria, heterotrophic bacteria, protozoan cysts, eukaryotic algal cells, and minerals associated with microbial activity occurring throughout much of the benthic regions of the dry valley lakes (Wharton et al., 1983; Mikell et al., 1984; Vincent, 1988).

There are differences in relative abundances of species that make up the microbial mats (Wharton et al., 1983; Parker and Wharton, 1985). The most remarkable ones concern the distribution of mat morphologies within a lake and between the lakes. The four major categories are prostrate, lift-off, columnar, and pinnacle morphologies. We will discuss further mats that are referred to by their unusual properties simply as "lift-off" mats.

They result from an interesting combination of physical and biological processes. The phenomenon of literally lifting off mats has been observed in

every lake that has been studied so far (Parker et al., 1982), with the exception of Lake Vanda (Wharton et al., 1983; Parker and Wharton, 1985).

3. Microbial Mats: Transport Agents in Subglacial Lakes That Are Found in Antarctica

A lift-off mat is produced when the pressure of dissolved gases inside the prostrate (i.e., flattened) microbial mat exceeds the local hydrostatic pressure at that depth and bubbles from inside the mat causing it to 'lift-off' the lake bottom.

In some cases, lift-off mats tear loose from the lake bottom and float to the undersurface of the ice cover. Once at the bottom of ice cover, lift-off mats freeze into the ice and through the ablation of surface eventually make their way to the top.

3.1. THE ESCAPE MECHANISM OF LIFT-OFF MATS

The escape mechanism is important in the distribution of microbes between the lakes and other environments in the region (Wharton et al., 1983). The process of lift-off mats plays a role in removing nutrients and salts from these lakes (Parker et al., 1982). Areas of the lake bottom in Lake Hoare, a dry-valley lake in Antarctica, where lift-off was occurring, received at their surface a range from 0.4 to 1.2 $\mu mol\ m^{-2}\ s^{-1}$, as the amount of photosynthetic available radiation (PAR).

Even at these low intensities sufficient oxygen was generated by photosynthesis to cause bubble formation and mat lift-off (Parker et al., 1982). At greater depths where intensities were lower, usually beyond 5 m below the lower ice surface, only flattened prostrate mats occurred. These areas received intensities of PAR smaller than 0.10% of that striking the surface (Parker et al., 1980, 1981; Simmons et al., 1979).

Microscopic examination of mat samples reveal that from benthic regions below the ice covers, we always find the *Phormdium frigidum* Fritsch associated with the mats as dominant taxon. In addition, pennate diatoms may be present. Entrapped sediment and precipitated minerals were also abundant in algal mats. This implies that microbial communities are living together and surviving on some synergistic interactions in this microbial world (Schink, 2002) and forming a mutualistic microbial community. If this is happening, we can raise the following question: Can these communities contribute towards the transport of sulfur in various subglacial lakes to their surfaces? Indeed this is the case. Annual removal of sulfur by escaping algal mats in the Antarctic lakes Chad, Hoare, and Fryxell, are reported as 104, 56, and 40 kg, respectively (Parker et al., 1982).

3.2. MICROBIAL MATS THAT CONTAIN SULFATE-REDUCING BACTERIA

Versatility of sulfate-reducing bacteria (SRB) at two extremes in anoxic and oxic settings place sulfate respirers to be potential biomarkers in extreme conditions such as Europa. A central role is played by these bacteriae in the biogeochemistry of chemically stratified marine habitats. Such a significant role has been documented both in anaerobic conditions (Jørgensen, 1982a, b), as well as aerobic conditions (Cohen, 1984).

Earlier SBR have been recognized as obligate anaerobes in anaerobic marine and terrestrial environments (Widdel, 1988), though they may survive temporary exposure to oxygen and again become active under anaerobic conditions (Canfield and Des Marais, 1991). SRBs can live near the surface region of a cyanobacterial microbial mat (Minz et al., 1999), as well as in depth in syntrophic association with methane producing bacteria, For example, microbial mutualism, namely a syntrophic association has been found in *Desulfovibrio vulgaris* and in *Methanococcus* species (Stoylar et al., 2007). Syntrophy is one form of microbial mutualism that is commonly involved in the degradation of organic substrates by microbial communities (Pernthaler et al., 2008). In syntrophic interactions, the transfer of metabolites between species is essential for growth (Schink, 1997, 2002; Schink and Stams, 2002). Therefore, a contribution of SRB to biogeochemical cycling seems to be significant.

The bacterial sulfur cycle has been extensively reviewed (Pfennig and Widdel, 1982). Under aerobic conditions the reduction of sulfate is assimilatory (e.g., in green plants), whereas the oxidation of reduced sulfur compounds (e.g., sulfide minerals) is dissimilatory in many bacteria (as in the case of the colorless sulfur bacteria). Dissimilatory reduction of sulfate is equivalent to the oxidation of organic compounds for energy conserving reactions. Under anaerobic conditions, both oxidized and reduced sulfur compounds are substrates only for metabolic processes of bacteria. Oxidized sulfur compounds, including elemental sulfur, represent counterparts of oxygen as electron acceptors in the terminal oxidation of organic substances and hydrogen; this is true for strictly anaerobic, dissimilatory SRB and sulfur-reducing bacteria, which form hydrogen sulfide as a product. The SRB thereby drive an important dissimilatory sulfur cycle in which inorganic sulfur compounds serve as extra cellular electron carriers. Microbial sulfate reduction is an energy-yielding process during which sulfate is reduced and sulfide is released, coupled with the oxidation of organic matter or molecular hydrogen (Postgate, 1984). Such a sulfur cycle was even more important during the early Precambarian evolution of the biosphere before molecular oxygen was evolved by oxygenic phototrophs and began to accumulate on Earth. Dissimilatory sulfate reduction (DSR) is a process by means of which SRB and Archaea are able to use sulfate ions as electron acceptors for anaerobic respiration. This process, DSR, releases large amounts of free sulfides as the sole final product (Widdel, 1988). The turnover rates of sulfur in dissimilatory processes exceed assimilatory reduction by several orders of magnitude (Shen and Buick, 2004; Rabus et al., 2006). These characteristics of SRB suggest that they are possible candidates for life on

other extreme environment on Earth as in Lake Vostok, as well as elsewhere in the solar system such as Europa. In the next section we first consider Lake Vostok as a second environment where some hints may be gained on the possible presence of extremophiles beyond the Earth, especially SRB.

4. Lake Vostok as a Model for the Emergence of Life on Europa

We assume that the process of lift-off discussed in Section 3.2 in the dry valley lakes may be rehearsed firstly on Earth, in an Europa-like environment. For example, a good location is Lake Vostok that lies underneath the Vostok station of the Russian Antarctic base. This lake is at about 1,000 km from the south pole and is beneath 4 km of ice. In the southern region of Antarctica many bacterial species have been found in zones of accreted ice, about 120 m above the water-ice surface (Christner et al., 2006).

4.1. SULFATE-REDUCING BIOGENIC ACTIVITY IN LAKE VOSTOK

Bacterial density is found to be twofold to sevenfold higher in accretion ice than in the overlying glacial ice. This implies that Lake Vostok is a source of bacterial carbon beneath the ice sheath. Phylogenetic analysis of the amplified small subunit ribosomal ribonucleic acid (rRNA) gene sequences in this accretion ice has revealed the presence of *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* (Christner et al., 2006). With few exceptions, all other characterized species of the *Deltaproteobacteria* are strict anaerobes that respire via the reduction of electron acceptors, such as sulfate, elemental sulfur, iron (III), and Mn (IV) (Lovley et al., 1995). These bacterial communities are diverse and physically associated. Such ecosystems lead to fundamental questions regarding the physiology and metabolism of several microorganisms. Prominent among these microbes are *Deltaproteobacteria* and *Betaproteobacteria*. *Deltaproteobacteria* are phylogenetically linked with SBR (Shen and Buick, 2004). A detailed analysis on accreted ice has shown that bacterial cells are often associated with organic and inorganic particles (Priscu et al., 1999), implying that a portion of cells within the lake water are not free living. Similar results have been reported for the permanently ice-covered lakes in the dry valleys (Lisle and Priscu, 2004).

4.2. THE ICY SURFACE OF LAKE VOSTOK

Lake Vostok and its relevance for astrobiology has been extensively reviewed (Christner et al., 2006; Priscu et al., 2003). It has been estimated that the youngest water is at least 400,000 years old. It is a window into life forms and climates of primordial eras. Lake Vostok is the largest of more than 140 subglacial lakes (Siegert et al., 2005). The zone of ice layer up to 3,309 m (referred to as I), and the

layer between 3,310 and 3,509 m (zone II) provide detailed information about the paleoclimate record spanning during the last 420,000 years.

The basal portion of the ice core from 3,539 to 3,623 m having many features differing from overlying glacial ice, and its geochemical composition indicates that it represents actual lake water that has accreted (i.e., frozen) underneath the ice sheet. Despite extremely cold air temperatures above the ice (an average of −55°C), liquid water is stable in the lake owing to the combined effect of background geothermal heating, the insulating properties of the overlying icy sheet, and adiabatic lowering of the freezing point (Siegert et al., 2003).

4.3. HYDROTHERMAL VENTS IN LAKE VOSTOK

Lake Vostok appears to be harboring hydrothermal vents beneath the water surface. This is suggestive of what may be occurring on Europa. The circulation of pure water in Lake Vostok will be driven by the differences between the density of meltwater and lake water. Geothermal heating will warm the bottom water to a temperature higher than that of the upper layers.

The water density will decrease with increasing temperature resulting in an unstable water column. This leads to vertical convective circulation in the lake, in which cold meltwater sinks down the water column and water warmed by geothermal heat ascends up the water column (Siegert et al., 2001). Similarly, Europa may also have geothermally heated warm water under its ice-crust. Processes of the type that occur in Lake Vostok may be taking place on Europa, where biogenic sulfur may be reaching the surface.

5. Europa and the LAPLACE/EJSM Mission

There is at present a possibility for returning to Europa initially discussed with the project LAPLACE (Blanc and the LAPLACE consortium, 2008), a mission to Europa and the Jupiter System for ESA's Cosmic Vision Programme. This initiative has been promoted to a worldwide collaboration named the Europa Jupiter System Mission (EJSM). In both projects, the earlier LAPLACE Mission and the subsequent EJSM the question of habitability is a major priority.

5.1. INSTRUMENTATION FOR PROBING THE HABITABILITY OF EUROPA

The options for approaching the question of selecting the right instrumentation for measuring the more abundant sulfur isotope have been discussed (Chela-Flores and Kumar, 2008). Early discussions, long before the proposed LAPLACE mission,

also considered the possibility of exploring Europa's habitability in the future with a submersible called a hydrobot (Horvath et al., 1997). This question is still relevant a decade later, in terms of new NASA autonomous underwater vehicle (AUV) called ENDURANCE for the Astrobiology Science and Technology for Exploring Planets (ASTEP) program (Doran et al., 2007).

Significant papers have more recently addressed the conditions for establishing a stable ecosystem. They include discussions of the biochemistry (Chyba, 2000; Schulze-Makuch and Irwin, 2002), as well as the relevance of sulfur in the biogeochemistry of Europa (Zolotov and Shock, 2003; Chela-Flores, 2006). The radiation of the Jovian magnetosphere may damage traces of biogenic sulfur deposited on the surface. The stopping depth for ionic radiation in the Jovian magnetosphere is expected not to exceed 1 cm (Greenberg, 2005; Baumstark-Khan and Facius, 2002). Thus, organic molecules would not be destroyed below such a thin layer. Penetrators are instruments in the process of development that would impact planetary bodies such as the Moon and bury themselves into the surface. Based on to the preliminary results of the British Penetrator Consortium (Smith et al., 2008), a modest stopping depth of penetrators into the (icy) surface of Europa would be sufficient to obtain samples that can be used to interpret isotopic abundances of sulfur that in the presence of putative S-reducing microbes would show measurable anomalous deviations in the $\delta^{34}S$ parameter without radiation interference. The concept of "stopping depth" is discussed in Section 5.2 and in Appendix A.

5.2. SULFUR PHYSICS AND CHEMISTRY ON EUROPA

Finding elemental sulfur on Europa may be of special interest. The possibility of such traces of sulfur might have originated from the metabolism of extremophilic sulfur-reducing microorganisms. The relevance of the $\delta^{34}S$ parameter is discussed in some detail in Section A2. We have examined the influence of temperature and radiation on Europa's biosignatures, and compared with examples on Earth in Sections A2 and A3.

5.3. TESTING BIOGENICITY OF THE SULFUR PATCHES WITH PENETRATORS

With the forthcoming missions to explore Europa, a new technology innovation is the penetrator concept that is being developed for early trials on the Moon surface – the Moon-Lite mission (Smith et al., 2008). If the microbial mats that we understand well in the context of the dry valley lakes (cf. Section 2) for the expulsion of a large quantity of sulfur that may be used to introduce tests on the surface of Europa, it is pertinent to evaluate the stopping depths for the Europan surface. Our main result is shown in Fig. 1.

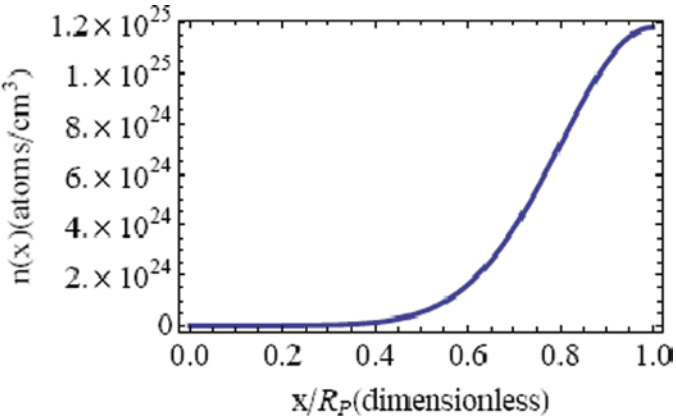

Figure 1. Density distribution of sulfur ions implanted from the Jovian atmosphere as a function of dimensional depth (x/R_p) for $t = 10^6$ years, $\phi = 9.0 \times 10^6$ (cm^2 s)$^{-1}$ and $R_p = 4.8 \times 10^{-5}$ cm. The maximum density is at the range $x = R_p$ The distribution is Gaussian. This figure is discussed in Section A3. The graph is based on the LSS theory (Lindhard-Scharff and Schiot) of ion implantation (Sze, 1988).

We may conclude from these estimates that a penetration of measuring instruments into the icy surface of Europa just beyond the few millimeters of the stopping depth would be sufficient for an accurate estimate of the δ^{34}S parameter (cf., Section A1). This would be a possible way for rejecting, or supporting, the hypothesis of biogenicity of the Europan sulfur patches. The fact that missions such as LAPLACE and others can envisage and undertake such measurements (in a relatively short period) is of utmost importance for astrobiology's most significant question: Are there other environments in our own solar system where we could settle the question of habitability?

6. Conclusions

The experience we have gained with microbial mats in interaction with ice the subglacial lakes of Antarctica has been shown to be relevant for the future exploration of the solar system.

6.1. FROM THE ANTARCTIC SUBGLACIAL LAKES TO EUROPA

The satellite system of Jupiter has three of its four Galilean satellites locked in the so-called Laplace resonance. Consequently, the energy and angular momentum they exchange among themselves and with Jupiter contribute to various degrees to the internal heating sources of the satellites. Europa is one of the best candidates for the search for life in our solar system, for the "equation of life" is fulfilled in

this world: not only there is expected to be a supply of biogenic elements, also an energy source we have just described, but in addition there is compelling evidence gathered by the Galileo mission for an internal ocean of salty liquid water, possibly ten times deeper that the deepest point in the Pacific Ocean.

The Jupiter System plays a prominent role in the Cosmic Vision Plan proposed by the European Agency to build its ambitious scientific program over the period 2015–2025 (Blanc and the LAPLACE consortium, 2008). Of the three key questions that have been raised, its habitability is the most urgent. However, if a lander is to be used for a most direct test of biogenicity of the sulfur patches that were discovered during the Galileo mission, new cutting-edge technology has to be envisaged. One such technology has been reviewed in a recent paper (Smith et al., 2008).

6.2. TECHNOLOGY REQUIRED FOR THE EXPLORATION OF EUROPA

Penetrators allow key scientific investigations of airless solar system bodies, such as Europa, or the Earth's satellite via affordable precursor missions. In fact, it is difficult to envisage any other method that allows globally spaced surface exploration of airless planetary bodies that is not prohibitively expensive. Penetrators are small probes that impact planetary bodies at high speed and bury themselves into the planetary surface. Before reaching Europa with LAPLACE, our own satellite, the Moon, can be used as an intermediate stage. We have proposed deployment on the Moon of ~13 kg penetrators that are designed to survive impact at high speed (~300 m s^{-1}) and penetrate ~ 2–5 m. In earlier work (Chela-Flores and Kumar, 2008), and in the present paper we have presented arguments that militate in favor of introducing mass spectrometry as appropriate instrumentation for the penetrators. The results of this paper shown in Fig. 1 (and its corresponding explanation in Section A3) suggest that with this new technology just a modest penetration of a few millimeters would be sufficient for deciding on biogenicity. Indeed, with the optimum stopping depth we have calculated, SRB would leave a measurable trace of their activity that is not affected by the harsh external environment. Penetrators that are in the process of being developed might succeed in making reliable measurements of the $\delta^{34}S$ parameter that are not affected by the presence of harsh radiation of the Jovian magnetosphere.

6.3. ON DEEPER IMPLICATIONS OF MICROBIAL MATS

In addition, in this work we have also pointed out the significance of microbial mutualism (a syntrophic association between various genera of bacteria within the microbial mats). This means that we have related sulfur transport in microbial mats that are known to take place in the Antarctica subglacial lakes with a possible mechanism that might rationalize what is taking place on the icy surface of Europa

on its sulfur patches. Lake Vostok gave us some further hints from microorganisms in the accreted ice that lies just above the still-unexplored liquid water.

Depending upon the conditions established by the opposing gradients of light intensities from above and sulfide concentrations below, different kinds of bacteria may develop. It is conceivable that once phototropic sulfur bacteria have evolved under favorable conditions of pH, salinity, temperature, availability of organic carbon in anoxygenic condition, they could survive with energy conserving processes in the dark. This argues in favor of a biogenic interpretation of the sulfur patches on the Europan icy surface. Thus, it is suggestive that under such similar environment, the Jovian satellite Europa can harbor life, or may be in process for its emergence. The icy surface of Europa is in the extremely low temperature range 50–110 K. However, below the ice we may have warm water that could harvest SRB.

6.4. SULFUR, A POSSIBLE BIOMARKER FOR THE ICY SURFACE OF EUROPA

On Europa there is another source of sulfur and that is energetic sulfur coming from the nearby Jovian atmosphere, namely from Jupiter. Now if sulfur from biological origin does exist in the underlying ocean and is carried to the surface by convective currents then this sulfur will mix with the implanted sulfur coming from Jupiter. Now if a probe with appropriate instruments tries to measure the $\delta^{34}S$ parameter, then this value would be some average value for that between the biogenic sulfur and implanted sulfur. Then any interpretation based on this value would be incorrect. Therefore, it is important that we know up to what depth does the sulfur from Jupiter penetrates the surface of Europa. Based on the theory of ion implantation in material science (discussed further in Section A3), we have plotted in Fig. 1, the density distribution of implanted sulfur as a function of the (x/R_p). Here "x" is the depth in centimeters and R_p (4.8×10^{-5} cm) is the maximum range of implanted sulfur. We have taken on the "X" axis x/R_p, which is dimensionless just for convenience. It just reduces the scale. As seen from figure, the maximum density is at $x/R_p = 1$, which means that $x = R_p$. In other words, the maximum density is at R_p. After distance R_p the density of implanted sulfur is zero. So this means that any probe that wants to measure sulfur of biogenic origin, it has to go beyond 4.8×10^{-5} cm because beyond this depth only sulfur of biogenic origin will exist.

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Appendix A

A1. SIGNIFICANCE OF THE $\Delta^{34}S$ PARAMETER

Sulfur is one of the key elements of life. Sulfur exists in four stable isotropic forms: ^{32}S (95.02%), ^{33}S (0.75%), ^{34}S (4.21%), and ^{36}S (0.02%). Sulfur isotope ratios are typically reported in the delta notation, as deviations with respect to the standard that is the troilite of the Cañon Diablo meteorite (CDT):

$$\delta^x S = \left[\left({}^x S / {}^{32} S \right)_{sample} \Big/ \left({}^x S / {}^{32} S \right)_{std} - 1 \right] \times 10^3 \ [‰, \text{CDM}] \tag{1}$$

where $x = 33$, 34 or 36. As pointed out (Kaplan, 1975; Chela-Flores, 2006), metabolic pathways of sulfur bacteria have enzymes that preferentially select the isotope ^{32}S over ^{34}S. This implies that where there is an abundance of sulfur bacteria, the value of the $\delta^{34}S$ parameter would be negative. Bacterial (dissimilatory) sulfate reduction (BSR) is a naturally occurring process. Under anaerobic conditions, sulfate is used by bacteria as an electron acceptor for oxidation of organic carbon (from pyruvate, lactate, formate, ethanol, methanol, amongst others), according to the following generalized reaction,

$$2 < CH_2O > + SO_4^{2-}(aq) + 2H^+(aq) + 2CO_2(g) + H_2S(g) + 2H_2O \tag{2}$$

The above reaction is also called "sulfate respiration." The product H_2S in the above reaction is highly enriched in ^{32}S. According to a model (Farquhar and Wing, 2003, Johnston et al., 2007) the parameter\ $\delta^{34}S_{SO_4^{2-}} - \delta^{34}S_{H_2S}$,

$$\delta^{34} S_{SO_4^{2-}} - \delta^{34} S_{H_2S} = \left[\frac{\left({}^{34} S / {}^{32} S \right)_{SO_4^{2-}} - \left({}^{34} S / {}^{32} S \right)_{H_2S}}{\left({}^{34} S / {}^{32} S \right)_{std}} \right] \times 10^3 (‰) \tag{3}$$

(The model has been referred to as the "the Rees–Farquhar model".) The above parameter is initially negative, becomes less negative in the process of sulfate respiration. Indeed, by the metabolic activity of SRB, the quantity $\left({}^{34}S / {}^{32}S \right)_{SO_4^{2-}}$ becomes less negative, while $\left({}^{34}S / {}^{32}S \right)_{H_2S}$ becomes more negative. This basically means that during the process of sulfate respiration the isotope ^{32}S increases in the product H_2S and is reduced in SO_4^{2-}. Accelerated sulfate reduction by bacterial communities is known to occur in the presence of organic carbon (Fauville et al., 2004).

The relevance of the $\delta^{34}S$ parameter for biogenic sulfur on the icy surface of Europa has been reviewed (Chela-Flores, 2006; Chela-Flores and Kumar, 2008). Life requires an input of energy and must be able to control the flow of energy

through redox chemistry, which is a universal concept. As life is based on organic chemistry, such chemistry must be allowed to operate. An extremophile must either live within the extreme environmental parameters, or guard against the outside world in order to maintain these conditions. For example, certain cold-tolerant cyanobacteria (Vincent, 2007) have a variety of strategies to minimize stresses of freeze–up. Like sea-ice microbiota, the mat-forming species in the McMurdo Ice shelf form copious quantities of exopolymeric substances already referred to as EPS in Section 2. This material shows the flow of liquid water during freeze-up and thaw, and may also force crystal formation to occur well away from the cells. Experiments indicate that EPS is critical to surviving desiccation, as well as freeze-up (Tamaru et al., 2005). Also EPS are the source of organic carbon. We now examine the influence on temperature and radiation on biosignatures suitable for Europa and suggest a comparison with their counterparts on Earth.

A2. TEMPERATURE

The magnitude of isotope fractionation by microbial sulfate reduction also depends upon temperature (Kaplan and Rittenberg, 1964; Canfield et al., 2006). The isotope fractionation factor (a) is (Ono, 2008):

$$ {}^{x}\alpha_{SO_4^{2-}-H_2S} = \frac{[{}^{x}SO_4^{2-}]/[{}^{32}SO_4^{2-}]}{[{}^{x}H_2S]/[{}^{32}H_2S]} \tag{4} $$

where x = 33, 34, and 36. The isotope fractionation factor at equilibrium can be derived from the ratios of the partition function (Q),

$$ {}^{34}\alpha_{SO_4^{2-}-H_2S} = \frac{{}^{34}Q_{SO_4^{2-}}}{{}^{32}Q_{SO_4^{2-}}} \frac{{}^{32}Q_{H_2S}}{{}^{34}Q_{H_2S}} \tag{5} $$

The partition function is

$$ \mathrm{Q} = \prod_i u_i \exp\left[-u_i /_{2]}\right][1-\exp(-u_i)] \tag{6} $$

where $u_i = h\upsilon_i / kT$ (h is the Planck constant, k is the Boltzmann constant, T is the temperature in kelvin and υ_i is the ith vibrational frequency of the molecule). A plot (cf., Fig. 2) of 1,000 × ln $({}^{34}\alpha_{SO_4^{2-}-H_2S})$ as a function of temperature shows that as temperature decreases, the value of 1,000 × ln $({}^{34}\alpha_{SO_4^{2-}-H_2S})$ increases, which is a signature of biological process.

Below the ice, the evidence gathered by the Galileo mission suggests that there is an ocean of liquid water, which could in principle harvest sulfur bacteria.

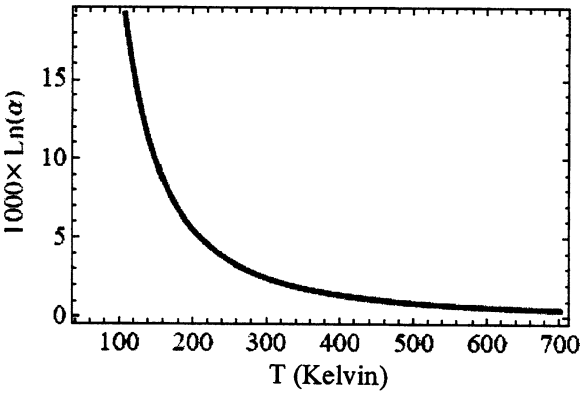

Figure 2. A plot of 1,000 × ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$ as a function of temperature shows that as temperature decreases the value of 1,000 × ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$ increases, which is a signature of biological process. The temperature on the surface of Europa is about 110 K. This corresponds to 1,000 × ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$ ~15 while the temperature in the sea water below in contact with the ice is 270 K which corresponds to 1,000 × Xln $((^{34}\alpha_{SO_4^{2-}-H_2S}))$ ~4.5.

The temperature of such an underlying sea just in contact with the ice is estimated to be near 270 K. This temperature is appropriate for BSR as it is evident from Fig. 2. A surface temperature on the surface of Europa of about 110 K corresponds to 1,000 × ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$ ~ 15, while the temperature in the sea water below in contact with the ice of temperature of the order of 270 K, corresponds to 1,000 × ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$ ~ 4.5. In comparison, the average temperature of the dry valley Antarctic lakes is in the range 273–280 K, which means that 1,000 × ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$ ~ 3. The algal mats in these lakes are known to exist about 4 m below the surface of the frozen lakes and are also capable of lifting off, floating, and freezing in ice. These mats selectively remove a huge quantity (40–104 kg) of sulfur annually (discussed in Section 3.1). It may be speculated that similar algal mats, if they exist beneath the icy surface of Europa, may eventually be transported to the surface by surficial ablation from above (produced by the micrometeoroids and refreezing form below the ice layer, thus contributing to the surficial sulfur patches. It is interesting that Europa offers a wide temperature range suitable for a wide variety of microorganisms to exist. Moreover, low temperature favors enhanced biological activity of sulfate respiration. This would be reflected in the extremely high values of 1,000×ln $(^{34}\alpha_{SO_4^{2-}-H_2S})$, or by highly negative values of $\delta^{34}S_{SO_4^{2-}} - \delta^{34}S_{H_2S}$. These effects are subject to measurement by miniature mass spectrometer in future missions (Blanc and the LAPLACE consortium, 2008). The possibility of highly negative $\delta^{34}S$ value due to hydrothermal process is ruled out since extremely high temperatures (>550 K) is required for such a process. The possibility of bacterial life well below the sea underneath with temperatures exceeding 350 K cannot be ruled out since we know examples of sulfur-dependent

extremophiles such as *Sulfolobus acidocaldarius* an archaea that flourishes at pH 3 and > 350 K in Yellowstone National Park (USA) (Rothschild and Mancinelli, 2001).

There are thermophiles among the bacteria (*Bacillus*, *Clostridium*, *Thiobacillus*, *Desulfotomaculum*), the archaea (*Pyrococcus*, *Thermococcus*, *Thermoplasma*, *Sulfolobus*), and the methanogens that exist in the temperature range 330–390 K. In contrast, the upper limit for eukaryotes is 330 K, a temperature suitable for some protozoa, algae, and fungi.

A3. SULFUR ION IMPLANTATION ON THE SURFACE OF EUROPA

On Europa, however, there is another source of sulfur, namely, the energetic sulfur ions coming from the nearby Jovian atmosphere. These energetic ions after striking the surface of Europa will penetrate a certain depth into the icy surface. If we assume that there are sulfur bacteria on the surface and below the icy crust of Europa and the initial value of the $\delta^{34}S$ parameter is negative then this mechanism of ion implantation will change the value of the $\delta^{34}S$ parameter and make it less negative or even positive. A probe on the surface of Europa that tries to measure the $\delta^{34}S$ parameter would then wrongly detect the absence of sulfur bacteria. Consequently, it is important that the probe goes well beyond the maximum penetration depth of the ions to measure the $\delta^{34}S$ parameter. For this it is essential to know the density distribution of the implanted ions as a function of depth as well as time of implantation. Based on the LSS (Lindhard, Scharff and Schiøt) theory of ion implantation, the implant profile in an amorphous material can be described by the equation (Sze, 1988):

$$n(x) = n_o \exp\left(-\frac{(x - Rp)^2}{2\Delta R_P}\right) \tag{7}$$

where, $n_o = \frac{\phi}{t}\sqrt{2\pi}\Delta R_P$, ϕ is the implanted dose, t is the time of implantation, R_p is the projection range and is equal to the average distance an ion travels before it stops and ΔR_p is the standard deviation of R_p which is roughly $1/5R_p$ from the known data for different ions and impact surface. The value of R_p for sulfur ion for the Europan surface is 4.8×10^{-5}cm and $\phi = 9.0\times 106$ $(cm^2\ s)^{-1}$ (Cooper et al., 2001) (cf., Fig. 1). It is therefore clear from Fig. 1 that the implanted sulfur is heavily distributed around the maximum depth R_p. This implies that a penetrator has to go beyond R_p to measure biogenic sulfur without any contamination from implanted sulfur.

8. References

Blanc, M. and the LAPLACE consortium (2008) LAPLACE: a mission to Europa and the Jupiter System, Astrophysical Instruments and Methods, in press. A digital version can be consulted in http://www.ictp.it/~chelaf/ss186.html (Team members full list is available at http://www.ictp.it/~chelaf/ss164.html).

Baumstark-Khan, C. and Facius, R. (2002) Life under conditions of ionizing radiations, In: G. Horneck and C. Baumstark-Khan (eds.) *Astrobiology: The Quest for the Conditions of Life.* Springer, Dordrecht, The Netherlands, pp. 261–296.

Canfield, D.E., Olesen, C.A. and Cox, R.P. (2006) Temperature and its control of isotope fractionation by a sulfate-reducing bacterium. Geochim. Cosmochim. Acta **70**: 548–561.

Canfield, D.E. and Des Marais, D.J. (1991) Aerobic sulfate reduction in microbial mats. Science **251**: 1471–1473.

Chela-Flores, J. (2006) The sulfur dilemma: are there biosignatures on Europa's icy and patchy surface? Int. J. Astrobiol. **5**: 17–22.

Chela-Flores, J. and Kumar, N. (2008) Returning to Europa: can traces of surficial life be detected? Int. J. Astrobiol. 7: 263–269.

Christner, B.C., Roysto-Bishop, G., Foreman, C.M., Arnold, B.R., Tranter, M., Welh, K.A., Lyons, W.B., Tspain, A.I., Studinger, M. and Priscu, J.C. (2006) Liminological conditions in subglacial Lake Vostok, Antarctica. Limnol. Occeanogr. **51**: 2485–2501.

Chyba, C. (2000) Energy for microbial life on Europa. Nature **403**: 381–383.

Cohen, Y. (1984) Oxygenic photosynthesis, anoxygenic photosynthesis and sulfate reduction in cyanobacterial mats, In: M.J. Klug and C.A. Reddy (eds.) *Current Perspectives in Microbial Ecology.* ASM Press, Washington, DC, pp. 435–441.

Cohen, Y. and Rosenberg, E. (1989) *Microbial Mats. Physiological Ecology of Benthic Microbial Communities*. ASM Press, Washington, DC, 494 pp.

Cooper, J.F., Johnson, R.E., Mauk, B.H., Garrett, H.B. and Gehrels, N. (2001) Energetic ion and electron radiation of the icy Galelian satellites. Icarus **149**: 133–159.

Davey, M.E. and O'Toole, G.A. (2000) Microbial biofilms from ecology and molecular genetics. Microbiol. Mol. Biol. Rev. **64**: 847–867.

Doran, P.T., Wharton, R.A. Jr. and Berry Lyons, W. (1994) Paleolimnology of the McMurdo Dry Valleys, Antarctica. J. Paleolimnol. **10**: 85–114.

Doran, P.T. Stone, W., Priscu, J., McKay, C., Johnson, A. and Chen, B. (2007) Environmentally Non-Disturbing Under-ice Robotic ANtarctiC Explorer (ENDURANCE). American Geophysical Union, Fall Meeting, abstract #P52A-05.

Farquhar, J. and Wing, B.A. (2003) Multiple sulfur isotopes and the evolution of the atmosphere. Earth Planet. Sci. Lett. **213**: 1–13.

Fauville, A., Mayer, B., Frömmichen, F., Friese, K. and Veizer, J. (2004) Chemical and isotopic evidence for accelerated bacterial sulphate reduction in acid mining lakes after addition of organic carbon: laboratory batch experiments. Chem. Geol. **204:** 325–344.

Greenberg, R. (2005) *Europa – The Ocean Moon*. Springer and Praxia Publishing, Chichester, 328 pp.

Johnston, D.T., Farquhar, J. and Canfield, D.E. (2007) Sulfur isotope insights into microbial sulfate reduction: when microbes meet models. Geochim. Cosmochim. Acta **71**: 3929–3947.

Jørgensen, B.B. (1982a) Mineralisation of organic matter in the sea bed–the role of sulphate reduction. Nature **296**: 643–645.

Jørgensen, B.B. (1982b) Ecology of bacteria of sulfur cycle with special reference to anoxic–oxic interface environments. Philos. Trans. R. Soc. Lond. B **298**: 543–562.

Kaplan, I.R. and Rittenberg, S.C. (1964) Microbiological fractionation of sulfur isotopes. J. Gen. Microbiol. **34**: 195–212.

Kaplan, I.R. (1975) Stable isotopes as a guide to biogeochemical processes. Proc. R. Soc. Lond. B **189**: 183–211.

Konhauser, K. (2007) *Introduction to Geomicrobiology.* Blackwell, Oxford, pp. 235–259.

Lisle, J.T. and Priscu, J.C. (2004) The occurrence of lysogenic bacteria and microbial aggregates in the lakes of McMurdo dry valleys, Antarctica. Microb. Ecol. **47**: 427–439.

Lovley, D.R., Phillips E.J.P., Lonergan D.J. and Widman P.K. (1995) Fe(III) and S(0) reduction by *Pelobacter carbinolicus*. Appl. Environ. Microbiol. **61**: 2132–2138.

Mikell, A.T., Parker B.C. and Simmons, G.M. Jr. (1984) Response of an Antarctic lake heterotrophic community to high dissolved oxygen. Appl. Environ. Microbiol. **47**: 1062–1066.

Minz, D., Fisbain, S., Green, S.J., Muyzer, G., Cohen, Y., Rittmann B.E. and Stahl, D.A. (1999) Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemoline in contrast to eukaryotic preference for anoxia. Appl. Environ. Microbiol. **65**: 4659–4665.

Ono, S. (2008) Multiple-sulfur isotope biosignatures. Space Sci. Rev. Springer, Dordrecht, **135**: 203–220.
Oren, A. (2008) Life at low water activity. Halophilic microorganisms and their adaptations. Biochem. **30**(4): 10–13.
Parker, B.C. (1982) Comparative ecology of plankton communities in seven antarctic oasis lakes. J. Plankton Res. **4**: 271–286.
Parker, B.C., Simmons, G.M. Jr., Seaburg, K.G. and Wharton, R.A. Jr. (1980) Ecological comparisons of oasis lakes and soils. Antarct. J. U.S. **15**: 167–170.
Parker, B.C., Simmons, Jr., G.M., Gordon Love, F., Wharton, Jr., R.A. and Seaburg, K.G. (1981) Modern stromatolites in Antarctic dry valley lakes. BioScience **31**: 656–661.
Parker, B.C. and Wharton, R.A. (1985) Physiological ecology of blue green algal mats (modern stromatolites) in Antarctic oasis lakes. Arch. Hyrobiol. Suppl. **71**: 31–348.
Pernthaler, A., Dekas, A.E., Brown. T., Goffredi, S.K., Embaye, T. and Orphan, V.J. (2008) Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. Proc. Natl. Acad. Sci. USA **105**: 7052–7057.
Pfennig, N. and Widdel, F. (1982) The bacteria of the sulphur cycle. Philos. Trans. R. Soc. Lond. B **298**: 433–441.
Postgate, J.R. (1984) *The Sulphate-Reducing Bacteria,* 2nd ed. Cambridge University Press, Cambridge, pp. 1–208.
Priscu, J.C., Adams, E.E., Lyons, W.B., Voytek, M.A., Mogk, D.W., Brown, R.L., McKay, C.P., Takacs, C.D., Welch, K.A., Wolf, C.F., Krishtein, J.D. and Avci, R. (1999) Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. Science **286**: 2141–2144**.**
Priscu, J.C., Bell, R.E., Bulat, S.A., Ellis-Evans, C.J., Kennicutt, M.C., Lukin, V.V., Petit, J.-R., Powell, R.D., Siegert, M.J. and Tabacco, I. (2003) An international plan for Antarctica subglacial lake exploration. Polar Geogr. **27**: 69–83.
Rabus, R., Hansen, T. and Widdel, F. (2006) Dissimilatory sulfate- and sulfur reducing prokaryotes, In: M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt (eds.) *The Prokarotes. Vol. 2: Ecophysiology and Biochemistry.* Springer, New York, pp. 659–768.
Rothschild, L.J. and Mancinelli, R.L. (2001) Life in extreme environments. Nature **409**: 1092–1101.
Schink, B. (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol. Mol. Biol Rev. **61**: 262–280.
Schink, B. (2002) Synergistic interactions in the microbial world. Antonie Van Leeuwenhoek **81**: 257–261.
Schink, B. and Stams, A.J.M. (2002) Syntrophism among prokaryotes, In: M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt (eds.) *The Prokarotes. Vol. 2: Ecophysiology and Biochemistry.* Springer, New York, pp. 309–335.
Schulze-Makuch, D. and Irwin, L.N. (2002) Energy cycling and hypothetical organisms in Europa's Ocean. Astrobiology **2**: 105–121.
Seckbach, J. and Chela-Flores, J. (2007) Extremophiles and chemotrophs as contributors to astrobiological signatures on Europa: a review of biomarkers of sulfate-reducers and other microorganisms, In: R.B. Hoover, G.V. Levin, A.Y. Rozanov and P.C.W. Davies (eds.) *Instruments, Methods, and Missions for Astrobiology X.* Proc. SPIE **6694**: 66940W.
Seckbach, J. and Oren, A. (2007) Oxygenic photosynthetic microorganisms in extreme environments, In: J. Seckbach (ed.) *Algae and Cyanobacteria in Extreme Environments.* Springer, Dordrecht, The Netherlands, pp. 4–25.
Shen, Y. and Buick, R. (2004) The antiquity of sulfate reduction. Earth-Sci. Rev. **64**: 243–272.
Siegert, M.J., Carter, S., Tabacco, I., Popov, S. and Blankenship, D.D. (2005) A revised inventory of Antarctic subglacial lakes. Antarctic Sci. **17**: 453–460.
Siegert, M.J., Tranter, M., Ellis-Evans, J.C., Priscu, J.C. and Lyons, W.B. (2003) The hydrochemistry of Lake Vostok and the potential for life in Antarctic subglacial lakes. Hydrol. Process. **17**: 795–814.
Siegert, M.J., Ellis-Evans, J.C., Tranter, M., Mayer, C., Petit, J.R., Salamatin, A. and Priscu, J.C. (2001) Physical, chemical and biological processes in Lake Vostok and other Antarctic subglacial lakes. Nature **414**: 603–609.

Simmons, G.M. Jr, Parker, B.C., Allnut, F.T.C., Brown, D., Cathey, D. and Seaburg, K.G. (1979) Ecological comparison of oasis lakes and soils. Antarctic J. U.S. **14**: 181–183.

Smith, A., Crawford, I.A., Gowen, R.A., Ball, A.J., Barber, S.J., Church, P., Coates, A.J., Gao, Y., Griffiths, A.D., Hagermann, A., Phipps, A., Pike, W.T., Scott, R., Sheridan, S., Sweeting, M., Talboys, D., Tong, V., Wells, N., Biele, J., Chela-Flores, J., Dabrowski, B., Flannagan, J, Grande, M., Grygorczuk, J., Kargl, G., Khavroshkin, O.B., Klingelhoefer, G., Knapmeyer, M., Marczewski, W., McKenna-Lawlor, S., Richter, L., Rothery, D.A., Seweryn, K., Ulamec, S., Wawrzaszek, R., Wieczorek, M. and Wright, I.P. (2008) LunarEX – a proposal to cosmic vision. Exp. Astron. **10.**1007/s10686-008-9109-6 (August 21, 2008).

Stal, L.J. (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytolog. **131**: 1–32.

Stone, R. (1999) Paleobiology: permafrost comes alive for Siberian researchers. Science **286**: 36.

Stoylar, S., Dien, S.V., Hillesland, K.L., Pinel, N., Lie, T.J., Leigh, J.A. and Stahl, D.A. (2007) Metabolic modeling of a mutualistic microbial community. Mol. Systems Biol. **3**(92): 1–13.

Sze, S.M. (1988) *VLSI Technology*. McGraw-Hill, New York, p. 333.

Tamaru, Y., Takani, Y., Yoshida, T. and Sakamoto, T. (2005) Crucial role of extracellular polysaccharides in dessication and freezing tolerance in the terrestrial cyanobacterium Nostoc commune. Appl. Environ. Microbiol. **71**: 7327–7333.

Vincent, W.F. (1988) *Microbial Ecosystems of Antarctica*. Cambridge University Press, Cambridge, 304 pp.

Vincent, W.F. (2007) Cold tolerance in cyanobacteria, In: J. Seckbach (ed.) *Algae and Cyanobacteria in Extreme Environments.* Springer, Dordrecht, pp. 289–301.

Wharton, R.A. Jr., Parker, B.C. and Simmons, G.M. Jr. (1983) Distribution, species composition and morphology of algal mats in Antarctic dry valley lakes. Phycologia **22**: 355–365.

Widdel, F. (1988) Microbiology and ecology of sulfate and sulfur reducing bacteria, In: A.J.B. Zehnder (ed.) *Biology of Anaerobic Organisms.* Wiley, New York, pp. 469–585.

Zolotov, M.Y. and Shock, E.L. (2003) On energy for biologic sulfate reduction in a hydrothermally formed ocean on Europa. J. Geophys. Res. **108** (E4): 3.1–3.9.

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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.

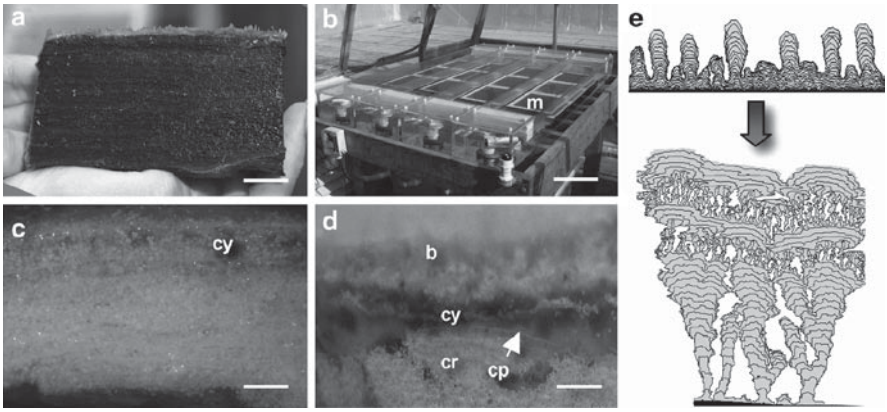

Figure 1. Microbial mat models for astrobiological research. (**a**) Hypersaline microbial mat sampled from Guerrero Negro, Baja California that have been incubated under artificial growth conditions in situ. Bar, 2 cm. (**b**) Recirculating water flume located at the NASA Ames greenhouse facilitates 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

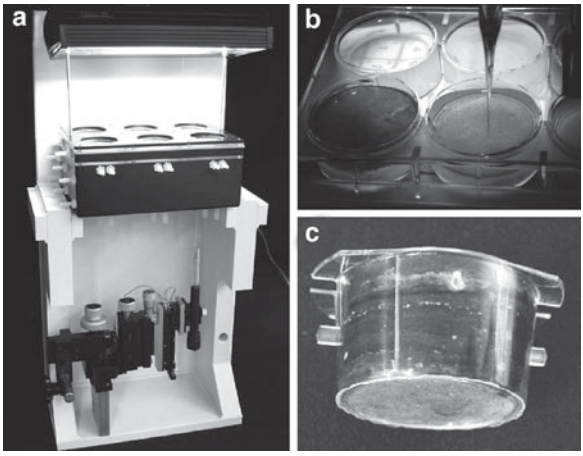

Figure 2. Microbial Assay Technology System (MATS). (**a**) MATS station containing a six chamber module and gas sampling ports. (**b**) Individual chambers can be sampled for chemical profiles (e.g., oxygen microsensor shown here). (**c**) Within each chamber artificial mats are contained within an 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

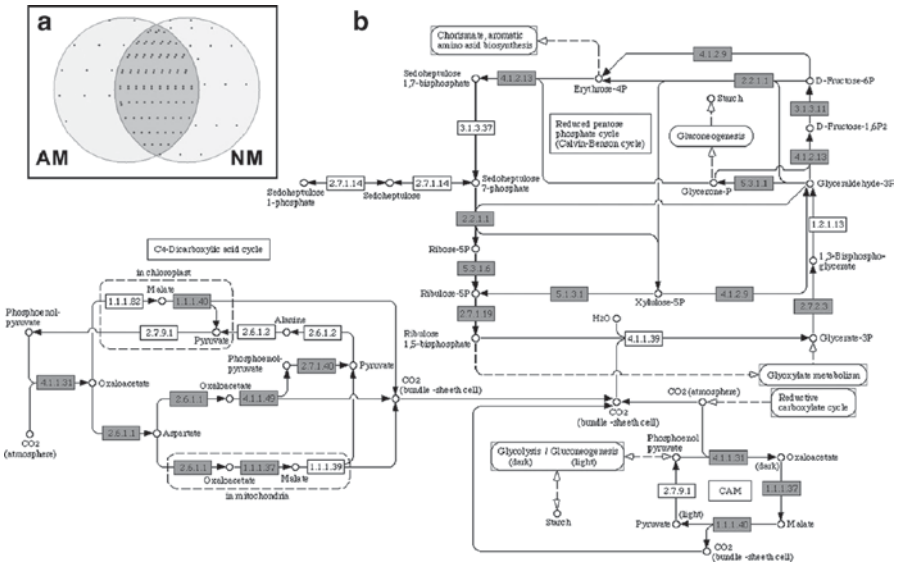

Figure 3. Metagenomics in the modern marine microbialites of Highborne Cay, Bahamas. (**a**) Venn diagram depicting the extent of overlap in the recovered metagenomic sequences in the artificial (AM) and natural (NM) microbialites. (**b**) Carbon fixation pathways in photosynthetic organisms. Rectangles 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.

5. References

Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. **59**: 143–169.

Awramik, S.A. (1971) Precambrian columnar stromatolite diversity: reflections of metazoan appearance. Science **174**: 825–827.

Awramik, S.A. (1984) Ancient stromatolites and microbial mats, In: Y. Cohen, R.W. Castenholz and H.O. Halvorson (eds.) *Microbial Mats: Stromatolites.* Alan R. Liss, New York, pp. 1–22.

Batchelor, M.T., Burne, R.V., Henry, B.I. and Watt, S.D. (2000) Deterministic KPZ model for stromatolite laminae. Physica **A 282**: 123–126.

Batchelor, M.T., Burne, R.V., Henry, B.I. and Jackson, M.J. (2004) A case for biotic morphogenesis of coniform stromatolites. Physica **A337**: 319–326.

Baumgartner, L.K., Reid, R.P., Dupraz, C., Decho, A.W., Buckley, D.H., Spear, J.R., Przekop, K.M. and Visscher, P.T. (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment. Geol. **185**: 131–145.

Bebout, B.M., Carpenter, S.P., Des Marais, D.J., Discipulo, M., Embaye, T., Garcia-Pichel, F., Hoehler, T.M., Hogan, M., Jahnke, L.L., Keller, R.M., Miller, S.R., Prufert-Bebout, L.E., Raleigh, C., Rothrock, M. and Turk, K. (2002) Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating Earth's present and past field environments. Astrobiology **2**: 383–402.

Bebout, B.M., Hoehler, T.M., Thamdrup, B., Albert, D., Carpenter, S.P., Hogan, M., Turk, K.A. and Des Marais, D.J. (2004) Methane production by microbial mats under low sulphate concentrations. Geobiology **2**: 87–96.

Beech, I., Hanjagsit, L., Kalaji, M., Neal, A.L. and Zinkevich, V. (1999) Chemical and structural characterization of exopolymers produced by *Pseudomonas* sp. NCIMB 2021 in continuous culture. Microbiology **145**: 1491–1497.

Beukes, N.J. and Lowe, D.R. (1989) Environmental control on diverse stromatolite morphologies in the 3000 Myr Pongola Supergroup, South Africa. Sedimentology **36**: 383–397.

Bhaya, D., Grossman, A.R., Steunou, A.S., Khuri, N., Cohan, F.M., Hamamura, N., Melendrez, M.C., Bateson, M.M., Ward, D.M. and Heidelberg, J.F. (2007) Population level functional diversity in a microbial community revealed by comparative genomic and metagenomic analyses. ISME J. **1**: 703–713.

Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M. and Visscher, P.T. (2007) Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. Geobiology **5**: 401–411.

Brassell, S.C., Wardroper, A.M.K., Thompson, I.D., Maxwell, J.R. and Eglington, G. (1981) Specific acylic isoprenoids as biological markers of methanogenic bacteria in marine sediments. Nature **290**: 693–696.

Breitbart, M., Hoare, A., Nitti, A., Siefert, J., Haynes, M., Dinsdale, E., Edwards, R., Souza, V., Rohwer, F. and Hollander, D. (2009) Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Cienegas, Mexico. Environ. Microbiol. **11**: 16–34.

Briones, A. and Raskin, L. (2003) Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. Curr. Opin. Biotechnol. **14**: 270–276.

Brocks, J.J. and Pearson, A. (2005) Building the biomarker tree of life. Rev. Mineral. Geochem. **59**: 233–258.

Buffan-Dubau, E., Pringault, O. and de Wit, R. (2001) Artificial cold-adapted microbial mats cultured from Antarctic lake samples. 1. Formation and structure. Aquat. Microb. Ecol. **26**: 115–125.

Cady, S.L., Farmer, J.D., Grotzinger, J.P., Schopf, J.W. and Steele, A. (2003) Morphological biosignatures and the search for life on Mars. Astrobiology **3**: 351–368.

Canfield, D.E. and Des Marais, D.J. (1991) Aerobic sulfate reduction in microbial mats. Science **251**: 1471–1473.

Cohen, Y., Jørgensen, B.B., Revsbech, N.P. and Poplawski, R. (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl. Environ. Microbiol. **51**: 398–407.

Conrad, R., Frenzel, P. and Cohen, Y. (1995) Methane emission from hypersaline microbial mats: lack of methane oxidation activity. FEMS Microbiol. Ecol. **31**: 311–341.

Decho, A.W. (2000) Microbial biofilms in intertidal systems: an overview. Continental Shelf Res. **20**: 1257–1273.

Delano, J.W. (2001) Redox history of the Earth's interior since 3900 Ma: implications for prebiotic molecules. Orig. Life Evol. Bio. **31**: 311–341.

Des Marais, D.J. (1995) The biogeochemistry of hypersaline mats. Adv. Microb. Ecol. **14**: 251–274.

Des Marais, D.J. (1997) Long-term evolution of the biogeochemical carbon cycle, In: J.E. Banfield and K.H. Nealson (eds.) *Geomicrobiology: Interaction Between Microbes and Minerals.* Mineralogical Society of America, Washington DC, pp. 427–448.

Des Marais, D.J., D'Amelio, E.D., Farmer, J.D., Jørgensen, B.B., Palmisano, A.C. and Pierson, B.K. (1992) Case study of a modern microbial mat-building community: the submerged cyanobacterial mats of Guerrero Negro, Baja California Sur, Mexico, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere: A Multidisciplinary Study.* Cambridge University Press, Cambridge, pp. 325–333.

Des Marais, D.J., Nuth, J.A., 3rd, Allamandola, L.J., Boss, A.P., Farmer, J.D., Hoehler, T.M., Jakosky, B.M., Meadows, V.S., Pohorille, A., Runnegar, B. and Spormann, A.M. (2008) The NASA astrobiology roadmap. Astrobiology **8**: 715–730.

Desnues, C.G., Rodriguez-Brito, B., Rayhawk, S., Kelley, S., Tran, T., Haynes, M., Lui, H., Hall, D., Angly, F.E., Edwards, R.A., Thurber, R.V., Reid, R.P., Siefert, J., Souza, V., Valentine, D., Swan, B., Breitbart, M. and Rohwer, F. (2008) Biodiversity and biogeography of phages in modern stromatolites and thrombolites. Nature **452**: 340–345.

Donachie, S.P., Foster, J.S. and Brown, M.V. (2007) Culture clash: challenging the dogma of microbial diversity. ISME J **1**: 97–102.

Dupraz, C. and Visscher, P.T. (2005) Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol. **13**: 429–438.

Dupraz, C., Visscher, P.T., Baumgartner, L.K. and Reid, R.P. (2004) Microbe–mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthra Island, Bahamas). Sedimentology **51**: 1–21.

Dupraz, C., Pattisina, R. and Verrecchia, E.P. (2006) Translation of energy into morphology: simulation of stromatolite morphospace using a stochastic model. Sediment. Geol. **185**: 185–203.

Farmer, J.D. (1992) Grazing and bioturbation in modern microbial mats, In: J.W. Schopf and C. Klein (eds.) *The Proterozoic Biosphere: A Multidisciplinary Study.* Cambridge University Press, Cambridge, pp. 295–298.

Fenchel, T. (1998a) Artificial cyanobacterial mats: cycling of C, O, and S. Aquat. Microb. Ecol. **14**: 253–259.

Fenchel, T. (1998b) Artificial cyanobacterial mats: structure and composition of the biota. Aquat. Microb. Ecol. **14**: 241–251.

Fenchel, T. (1998c) Formation of laminated cyanobacterial mats in the absence of benthic fauna. Aquat. Microb. Ecol. **14**: 235–240.

Fenchel, T. and Kühl, M. (2000) Artificial cyanobacterial mats: growth, structure, and vertical zonation patterns. Microb. Ecol. **40**: 85–93.

Fernandez, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C. and Tiedje, J. (1999) How stable is stable? Function versus community composition. Appl. Environ. Microbiol. **65**: 3697–3704.

Foster, J.S., Green, S.J., Ahrendt, S.R., Hetherington, K.L., Golubic, S., Reid, R.P. and Bebout, L. (2009) Molecular and morphological characterization of cyanobacterial diversity in the marine stromatolites of Highborne Cay, Bahamas. ISME J. **3**: 572–587.

Frias-Lopez, J., Shi, Y., Tyson, G.W., Coleman, M.L., Schuster, S.C., Chisholm, S.W. and DeLong, E.F. (2008) Microbial community gene expression in ocean surface waters. Proc. Natl. Acad. Sci. USA **105**: 3805–3810.

Garrett, P. (1970) Phanerozoic stromatolites: noncompetitive ecologic restriction by grazing and burrowing animals. Science **169**: 171–173.

Green, S.J., Blackford, C., Bucki, P. and Jahnke, L.L. (2008) A salinity and sulfate manipulation of hypersaline microbial mats reveals stasis in the cyanobacterial community structure. ISME J. **2**: 457–470.

Grotzinger, J.P. and Knoll, A.H. (1999) Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? Annu. Rev. Earth Planet. Sci. **27**: 313–358.

Grotzinger, J.P. and Rothman, D.H. (1986) An abiotic model for stromatolite morphogenesis. Nature **383**: 423–425.

Grötzschel, S., Abed, R.M.M. and De Beer, D. (2002) Metabolic shifts in hypersaline microbial mats upon addition of organic substances. Environ. Microbiol. **4**: 683–695.

Habicht, K.S., Gade, M., Thamdrup, B., Berg, P. and Canfield, D.E. (2002) Calibration of sulfate levels in the Archean ocean. Science **298**: 2372–2374.

Hammes, F. and Verstraete, W. (2002) Key roles of pH and calcium metabolism in microbial carbonate precipitation. Rev. Environ. Sci. Biotechnol. **1**: 3–7.

Handelsman, J. (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. **68**: 669–685.

Havemann, S.A. and Foster, J.S. (2008) Comparative characterization of the microbial diversities of an artificial microbialite model and a natural stromatolite. Appl. Environ. Microbiol. **74**: 7410–7421.

Hurtgen, M.T., Arthur, M.A., Suits, N.S. and Kaufman, A.J. (2002) The sulfur isotopic composition of Neoproterozoic seawater sulfate: implications for a snowball Earth? Earth Planet. Sci. Lett. **203**: 413–429.

Jahnke, L.L., Orphan, V.J., Embaye, T., Turk, K.A., Kubo, M.D., Summons, R.E. and Des Marais, D.J. (2008) Lipid biomarker and phylogenetic analyses to reveal archaeal biodiversity and distribution in hypersaline microbial mat and underlying sediment. Geobiology **6**: 394–410.

Kan, J., Hanson, T., Ginter, J.M., Wang, K. and Chen, F. (2005) Metaproteomic analysis of Chesapeake Bay microbial communities. Saline Syst. **1**: 7.

Kardar, M., Parisi, G. and Zhang, Y.C. (1986) Dynamic scaling of growing interface. Phys. Rev. Lett. **56**: 889–892.

Kasting, J.F. (2001) Earth history. The rise of atmospheric oxygen. Science **293**: 819–820.

Kawaguchi, T. and Decho, A.W. (2000) Biochemical characterization of cyanobacterial extracellular polymers (EPS) from modern marine stromatolites (Bahamas). Prep. Biochem. Biotechnol. **30**: 321–330.

Kawaguchi, T. and Decho, A.W. (2002) Isolation and biochemical characterization of extracellular polymeric secretions (EPS) from modern soft marine stromatolites (Bahamas) and its inhibitory effect on $CaCO_3$ precipitation. Prep. Biochem. Biotechnol. **32**: 51–63.

Kolenbrander, P.E., Andersen, R.N., Blehert, D.S., Egland, P.G., Foster, J.S. and Palmer Jr., R.J. (2003) Communication among oral bacteria. Microbiol. Mol. Biol. Rev. **66**: 486–505.

Kunin, V., Raes, J., Harris, J.K., Spear, J.R., Walker, J.J., Ivanova, N., von Mering, C., Bebout, B.M., Pace, N.R., Bork, P. and Hugenholtz, P. (2008) Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol. Syst. Biol. **4**: 198.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Sogin, M.L. and Pace, N.R. (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. **72**: 3685–3695.

May, R.M. (1973) Stability and complexity in model ecosystems. Monogr. Popul. Biol. **6**: 1–235.

Nübel, U., Garcia-Pichel, F., Clavero, E. and Muyzer, G. (2000) Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ. Microbiol. **2**: 217–226.

Oremland, R.S. and King, G.M. (1989) Methanogenesis in hypersaline environments, In: Y. Cohen and E. Rosenberg (eds.) *Microbial Mats: Physiological Ecology of Benthic Microbial Communities.* American Society for Microbiology, Washington, DC, pp. 180–190.

Omoregie, E.O., Crumbliss, L.L., Bebout, B.M. and Zehr, J.P. (2004) Determination of nitrogen-fixing phylotypes in *Lyngbya* sp. and *Microcoleus chthonoplastes* cyanobacterial mats from Guerrero Negro, Baja California, Mexico. Appl. Environ. Microbiol. **70**: 2119–2128.

Orphan, V.J., Jahnke, L.L., Embaye, T., Turk, K.A., Pernthaler, A., Summons, R.E. and Des Marais, D.J. (2008) Characterization and spatial distribution of methanogens and methanogenic biosignatures in hypersaline microbial mats of Baja California. Geobiology **6**: 376–393.

Owen, T., Cess, R.D. and Ramanathan, V. (1979) Enhanced CO_2 greenhouse to compensate for reduced solar luminosity on early Earth. Nature **277**: 640–642.

Paerl, H.W., Steppe, T.F., Buchan, K.C. and Potts, M. (2003) Hypersaline cyanobacterial mats as indicators of elevated tropical hurricane activity and associated climate change. Ambio **32**: 87–90.

Parro, V., Moreno-Paz, M. and Gonzales-Toril, E. (2007) Analysis of environmental transcriptomes by DNA microarrays. Environ. Microbiol. **9**: 453–464.

Pavlov, A.A., Kasting, J.F., Brown, L.L., Rages, K.A. and Freedman, R. (2000) Greenhouse warming by CH_4 in the atmosphere of early Earth. J. Geophys. Res. **105**: 11981–11911.

Poretsky, R.S., Bano, N., Buchan, A., LeCleir, G., Kleikemper, J., Pickering, M., Pate, W.M., Moran, M.A. and Hollibaugh, J.T. (2005) Analysis of microbial gene transcripts in environmental samples. Appl. Environ. Microbiol. **71**: 4121–4126.

Post, W.M., Izaurralde, R.C., Jastrow, J.D., McCarl, B.A., Amonette, J.E., Bailey, V.L., Jardine, P.M., West, T.O. and Zhou, J.Z. (2004) Enhancement of carbon sequestration in US soils. Bioscience **54**: 895–908.

Prufert-Bebout, L., Heyenga, G. and Bebout, B.M. (2005) Microbial assay technology system (MATS): hardware for coordinated microbial systems biology research. *NASA Ames Instrumentation Workshop*, Moffett Field, CA.

Ram, R.J., VerBerkmoes, N.C., Thelen, M.P., Tyson, G.W., Baker, B.J., Blake, R.C., 2nd, Shah, M., Hettich, R.L. and Banfield, J.F. (2005) Community proteomics of a natural microbial biofilm. Science **308**: 1915–1920.

Raynaud, D., Jouzel, J., Barnola, J.M., Charrellaz, J., Delams, R.J. and Lorius, C. (1993) The ice record of greenhouse gases. Science **259**: 926–934.

Reeburgh, W.S. (1980) Anaerobic methane oxidation: rate depth distributions in Skan Bay sediments. Earth Planet. Sci. Lett. **47**: 345–352.

Reeburgh, W.S. (1996) 'Soft Spots' in the Global Methane Budget, In: M.E. Lidstrom and F.R. Tabita (eds.) *Microbial Growth on C1 Compounds.* Kluwer, Dordrecht, The Netherlands, pp. 334–342.

Rothschild, L.J. and Mancinelli, R.L. (1990) Model of carbon fixation in microbial mats from 3,500 Myr ago to the present. Nature **345**: 710–712.

Simoneit, B.R. (2002) Molecular indicators (biomarkers) of past life. Anat. Rec. **268**: 186–195.

Simoneit, B.R. (2004) Biomarkers (molecular fossils) as geochemical indicators of life. Adv. Space Res. **33**: 1255–1261.

Smith, J.M., Green, S.J., Kelley, C.A., Prufert-Bebout, L. and Bebout, B.M. (2008) Shifts in methanogen community structure and function associated with long-term manipulation of sulfate and salinity in a hypersaline microbial mat. Environ. Microbiol. **10**: 386–394.

Spear, J.R., Ley, R.E., Berger, A.B. and Pace, N.R. (2003) Complexity in natural microbial ecosystems: the Guerrero Negro experience. Biol. Bull. **204**: 168–173.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R. and Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl. Environ. Microbiol. **69**: 5157–5169.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., Macintyre, I.G. and Thompson, J.A. (1998) Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am. Mineral. **83**: 1482–1493.

Visscher, P.T., Gritzer, R.F. and Leadbetter, E.R. (1999) Low molecular weight sulfonates, a major substrate for sulfate reducers in marine microbial mats. Appl. Environ. Microbiol. **65**: 3272–3278.

Walker, J.C.G., Hays, P.B. and Kasting, J.F. (1981) A negative feedback mechanism for the long-term stabilization of Earth's surface temperature. J. Geophys. Res. **86**: 9776–9782.

Walter, M.R. (1976) *Developments in Sedimentology: Stromatolites*. Elsevier, Amsterdam.

Wieland, A., Kühl, M., Mcgowan, L., Fourcan, A., Duran, R., Caumette, P., De Oteyza, T.G., Grimalt, J.O., Sole, A., Diestra, E., Esteve, I. and Herbert, R.A. (2003) Microbial mats on the Orkney Islands revisited: microenvironment and microbial community composition. Microb. Ecol. **46**: 371–390.

Witten, T.A. and Sander, L.M. (1981) Diffusion-limited aggregation, a kinetic critical phenomenon. Phys. Rev. Lett. **47**: 1400–1403.

Witten, T.A. and Sander, L.M. (1983) Diffusion limited aggregation. Phys. Rev. **27**: 5686–5697.

Wolfram, S. (1984) Universality and complexity in cellular automata. Physica D **10**: 1–35.

Yannarell, A.C., Steppe, T.F. and Paerl, H.W. (2007) Disturbance and recovery of microbial community structure and function following Hurricane Frances. Environ. Microbiol. **9**: 576–583.

PART 6:
OUTLOOK AND SUMMARY

Seckbach
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SUMMARY AND CONCLUSIONS

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1. General Background

This volume encompasses many aspects of microbial mats. Many of the chapters dealt with their description, their geographical distribution, and their environmental properties. They presented the characteristics of the mats and of the microorganisms of which they are composed. The chapters included descriptions of microbial mats in fresh water, soils, seepages, marine settings, and in hypersaline areas. Among the microorganisms encountered are green algae, diatoms, cyanobacteria, lichens, and many others. Geographical locations included marine settings such as the Baltic Sea, the hypersaline mats of Guerrero Negro, the acidic environment of the Rio Tinto, and the cold Antarctic Dry Valleys. Some chapters discussed the molecular aspects of the mats, their osmotic adaptation, biosignatures, and other properties of the microbial communities. Other chapters dealt with the ancient mats (the paleoenvironment of early Earth), comparing their properties with those of modern mats. Their occurrence in extreme environments could serve as a model for similar structures which might possibly exist in extraterrestrial bodies such as on Europa (satellite of Jupiter), Mars, and other celestial bodies.

2. Fossil Mats

The study of microbial mats is important not only for understanding modern environments, but also for interpreting the geological record. Microbial cells are rarely preserved in the fossil record and, as has been demonstrated by various

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investigators, simple microbe-like morphologies such as spheres and filaments may be formed abiologically. Other biosignatures, including fossil microbial mats, have therefore become important in investigating the abundance and the environments of life on the early Earth. Fossil mats provide a microenvironmental context for individual microbial fossils and a record of microbial activity in the absence of such structures.

Very fine microbial mat structures preserved by early silicification are present in the rocks of the Kaapvaal Craton in southern Africa and the Pilbara Block in northwestern Australia. The diversity of mat types represented and their depositional association with varying, and often extreme, environmental settings suggest that early life had occupied a variety of niches on the Earth as early as 3.5 billion years ago. Examining these and other fossil mats with a combination of traditional petrographic and chemical techniques and new high-resolution analyses will result in a more confident and detailed understanding of the record of life on Earth.

3. Biofilms Form Everywhere on and Within Clastic Sediments

An important concept to understand is the continuum from thin and vulnerable (in terms of preservation) biofilms through tough leathery (fibrous, filamental) microbial mats to the range of subtle features formed and preserved within clastic sedimentary environments by the interaction of mat growth, mat metabolism, mat decay, mat destruction, and sedimentation processes. The concomitant time scale related to this continuum varies from several hours for biofilms, to several weeks for fully formed leathery mats to reach fruition (e.g., Gerdes and Klenke, 2007), to periods probably measurable in months to even years for the secondary structures alluded to above (e.g., Eriksson et al., this volume). The continuum is interpreted from the clastic sedimentary record based on a relatively large (ca. 50) set of essentially subtle or proxy features, which result from the highly diverse interaction of evolving mats and unconsolidated sediment, varying mostly from sand grades down to mud-sized particles. Mostly, it is features indicative of sediment behavior incompatible with the normal physical and chemical controls on clastic sedimentation processes that suggest a role for microbial mats within the observed sedimentation system.

The inherent complexity resulting from the sum of the above basic observations rivals and possibly exceeds that inherent in the relationship between the physically (and to a lesser extent, chemically) formed sedimentary structures and their genetic processes and host (paleo-) environments. However, this picture has been subject to some bias and confusion due to the number of studies carried out on modern and ancient equivalent examples from shallow marine tidal to supratidal settings as well as hypersaline lagoons (e.g., Gerdes et al., 1985a, b, c; Gerdes, 2007). Consequently, some diagnostic prejudice toward such specific settings has emerged in the literature (e.g., Noffke et al., 2006; Noffke, 2007). This viewpoint contrasts with a more widely supported one where a non-facies-specific relationship

appears to be paramount for the mat–sediment interactions (e.g., Schieber et al., 2007a, c; three case studies in Eriksson et al., this volume). What is remarkable and possibly even characteristic of microbial mats within clastic sedimentary settings, is their enormous environmental range, known from at least shallow marine environments through a full range of continental aqueous settings, even to deserts (Schieber, 1998; Eriksson et al., 2000; Schieber et al., 2007a, c; Gerdes, 2007). However, when abundance and variety of mat-related structures are considered, there is a measure of truth to a littoral sandy setting bias (Schieber et al., 2007c). Microtopographic variability within any specific environmental setting also plays a role in determining preservation and even type of mat-related structures, particularly within the littoral settings (e.g., Eriksson et al., this volume).

It is commonly asserted that metazoan grazing in settings younger than the Neoproterozoic–Phanerozoic boundary significantly reduced the abundance of microbial mats, effectively restricting their occurrence to stressed environmental settings (see also in this issue, papers by: Green; Oren) such as desiccating tidal flats or hypersaline lagoons; however, Schieber et al. (2007b) question this simple assertion, also pointing out that the broad adaptability of mats is the same on either side of this boundary. This question is discussed in some detail by Krumbein in this volume.

Microbial mats and the binding properties they provide for clastic sediment particles can potentially result in differences in clastic sediment architecture and even in sequence stratigraphic stacking patterns (e.g., Sarkar et al., 2005; Banerjee and Jeevankumar, 2005; Catuneanu and Eriksson, 2007), but much more work is still needed to understand this. Analogously, a large body of work is required to tie the different mat-related structural features to specific environments and subenvironments, to make these sedimentary features equally useful in palaeoenvironmental interpretative studies as their much better known physically and chemically formed sedimentary structure equivalents.

4. Mats in Extreme Environments on Earth and Elsewhere

Microorganisms form the major part of all life on Earth, and they are also organized in microbial mats and into biofilms. For this reason, this volume's main focus is the description of different aspects of microbial mats that are fundamental for our deeper understanding of a major cross-section of microbiology in general, and for astrobiology. We have discussed multiple implications of the study of microbial mats. Prominent among them is the understanding of the early Earth, before multicellularity evolved. Microbial mats may help us to understand the possibility of life elsewhere in our own solar system, such as on Europa, Mars, Enceladus (one of the innermost moons of Saturn), and Titan (satellite of Saturn).

Indeed, microbial mats are ubiquitous in extreme environments: at high and low temperatures; in hypersaline water bodies; in hot springs, where they not only survive, but thrive as exemplified by the startling colored microbial mats that live

in Yellowstone National Park. Microbial mats are also present in volcanic vents on the ocean floor, called black smokers. Other environments suitable for microbial mats are deserts and, specifically the Dry Valleys of Antarctica in the MacMurdo region that is traversed by striking glaciers. Some of the most interesting lakes in this region are permanently covered by ice. These extraordinary environments present us with an ideal window to glance at significant events that are relevant for ancient life, and even for paleolimnology that is suggestive of the possible perseverance of life on Mars in an earlier Eden-like epoch.

The extremophiles that are trapped in microbial mats may also be living under the Taylor Glacier in the Taylor Valley, a region that is bounded by the Ferrar Glacier and the Asgard Range. These microbes probably lived in the ocean at one time, but when the floor of the Dry Valleys rose more than a million years ago, the glacier covered seawater when it advanced and trapped the microorganisms in pockets of water. An intriguing feature, named Blood Falls, suggests the presence of microbial mats underneath the Taylor Glacier. The name is due to its resemblance to a blood-red color waterfall at the glacier's extreme end. This coloring is analogous to the colored microbial mats that live in the hot springs of the Yellowstone National Park. Isotopic measurements of sulfate, water, carbonate, and ferrous iron as well as gene analyses imply that a microbial consortium facilitates a catalytic sulfur cycle that could be analogous to the metabolic events that may sustain life elsewhere in the Solar System (Mikucki et al., 2009). This is especially relevant to the icy satellites of the outer Solar System, including Europa, where sulfur patches were discovered by the Galileo Mission (1995–2003). These stains on the icy surface of the Jovian satellite are suggestive of chemosynthetic products of metabolism.

From the point of view of geology and microbiology, some of the best studied frozen lakes are in the Taylor Valley, namely Lake Fryxell and Lake Hoare. Further north, in the Wright Valley, Lake Vanda is also remarkable for its biota. Among the microbial mats that are permanently thriving in the frozen lakes there are examples of both prokaryotes and eukaryotes. Besides, some of the most interesting geologic paleoindicators for reconstructing the history of these lakes are stromatolites. In the Dry Valleys these structures consist of various species of cyanobacteria, such as *Phormidium frigidum* Fritsch, a prokaryote that forms the matrix of most mat types (Wharton et al., 1983).

Modern organisms analogous to ancient life are to be found in the Dry Valley lakes. What is most significant is that single-celled eukaryotes are amply represented in this Antarctic biota. Among the related paleoindicators that have been found are diatom frustules, while cyst-like structures, most likely of crysophycean origin have also been identified. These intriguing lakes contain various taxa of planktonic and benthic microorganisms. These environments are dominated by lower life forms inviting us to search for biomarkers of an earlier biota since grazing, for instance, is totally absent (Doran et al., 1994). Microbial mats in Lakes Bonney, Chad, Fryxell, Hoare, and Vanda have been thoroughly documented, especially since the 1980s. For instance, in these environments microbial

mats are known to include not only the above-mentioned cyanobacteria, but also heteotrophic bacteria, eukaryotic algae (mainly diatoms), and fungi (Baublis et al., 1991). There are some dinoflagellates *Gymnodinium* and *Glenodinium* in Lake Fryxell, where, in addition, protozoan taxa were associated with the algal mats (Cathey et al., 1981).

5. References

Banerjee, S. and Jeevankumar, S. (2005) Microbially originated wrinkle structures on sandstone and their stratigraphic context: palaeoproterozoic Koldaha Shale, central India. Sediment. Geol. **176**: 211–224.

Baublis, J.A., Wharton, R.A. Jr. and Volz, P.A. (1991) Diversity of micro-fungi in an Antarctic dry valley. J Basic Microbiol. **31**: 3–12.

Cathey, D.D., Parker, B.C., Simmons, G.M., Jr., Yongue, W.H., Jr. and Van Brunt, M.R. (1981) The microfauna of algal mats and artificial substrates in Southern Victoria Land lakes of Antarctica. Hydrobiologia **85**: 3–15.

Catuneanu, O. and Eriksson, P.G. (2007) Sequence stratigraphy of the Precambrian. Gondwana Res. **12/4**: 560–565.

Doran P.T., Wharton, R.A., Jr. and Lyons, W.B. (1994) Paleolimnology of the McMurdo Dry Valleys, Antarctica. J. Paleolimnol. **10**: 85–114.

Eriksson, P.G., Simpson, E.L., Eriksson, K.A., Bumby, A.J., Steyn, G.L. and Sarkar, S. (2000) Muddy roll-up structures in siliciclastic interdune beds of the ca. 1.8 Ga Waterberg Group, South Africa. Palaios **15**: 177–183.

Gerdes, G. (2007) Structures left by modern microbial mats in their host sediments, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 5–38.

Gerdes, G. and Klenke, T. (2007) States of biogenic bedding as records of the interplay of ecologic time and environment (a case study of modern siliciclastic sediments, Mellum Island, southern North Sea). Senckenb. Marit. **37**: 129–144.

Gerdes, G., Krumbein, W.E. and Holtkamp, E.M. (1985a) Salinity and water activity related zonation of microbial communities and potential stromatolites of the Gavish Sabkha, In: G.M. Friedman and W.E. Krumbein (eds.) *Hypersaline Ecosystems: the Gavish Sabkha.* Springer, Berlin, pp. 238–266.

Gerdes, G., Krumbein, W.E. and Reineck, H.E. (1985b) The depositional record of sandy, versicoloured tidal flats (Mellum Island, southern North Sea). J. Sediment. Petrol. **55**: 265–278.

Gerdes, G., Krumbein, W.E., and Reineck, H.E. (1985c) Verbreitung und aktuogeologische Bedeutung mariner mikrobieller Matten im Gezeitenbereich der Nordsee. Facies **12**: 75–96.

Mikucki, J.A., Pearson, A., Johnston, D.T., Turchyn, A.V., Farquhar, J., Schrag, D. P., Anbar, A.D., Priscu, J.C. and Lee, P.A. (2009) A contemporary microbially maintained subglacial ferrous "ocean". Science **324**: 397–400.

Noffke, N., Eriksson, K.A., Hazen, R.M. and Simpson, E.L. (2006) A new window into Early Archean life: microbial mats in Earth's oldest siliciclastic deposits (3.2 Ga Moodies Group, South Africa). Geology **34**: 253–256.

Noffke, N. (2007) Microbially-Induced Sedimentary Structures (MISS) of Early and Middle Archaean ages – Moodies Group, Pongola Supergroup, Witwatersrand Supergroup (South Africa), In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann, and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 153–157.

Sarkar, S., Banerjee, S., Eriksson, P.G. and Catuneanu, O. (2005) Microbial mat control on siliciclastic Precambrian sequence stratigraphic architecture: examples from India. Sediment. Geol. **176**: 195–209.

Schieber, J. (1998) Possible indicators of microbial mat deposits in shales and sandstones: examples from the Mid-Proterozoic Belt Supergroup, Montana, USA. Sediment. Geol. **120**: 105–124.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (2007a) Prologue: an introduction to microbial mats, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 1–3.

Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sarkar, S., Altermann, W. and Catuneanu, O. (eds.) (2007b) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2.* Elsevier, Amsterdam, 311 pp.

Schieber, J., Bose, P.K., Eriksson, P.G. and Sarkar, S. (2007c) Palaeogeography of microbial mats in terrigenous clastics – environmental distribution of associated sedimentary features and the role of geologic time, In: J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann, and O. Catuneanu (eds.) *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record. Atlases in Geoscience 2*. Elsevier, Amsterdam, pp. 267–275.

Wharton, R.A., Jr., Parker, B.C. and Simmons G.M., Jr (1983) Distribution, species composition and morphology of algal mats in Antarctic Dry Valley lakes. *Phycologia* **22:** 355–365.

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