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SPOTLIGHT

SLIME THROUGH TIME: THE FOSSIL RECORD OF PROKARYOTE EVOLUTION

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ABSTRACT

Prokaryota in natural environments form biofilms, which are benthic assemblages of a variety of microorganisms embedded within their extracellular mucilage. Biofilms are firmly attached to surfaces such as aquatic sediments. Quorum sensing by the many microbes in a biofilm is collective decision making and cooperation for responding to internal and external parameters affecting the community. This communication is based on chemical signaling affecting gene expression of the microorganisms. Microorganisms situated in a biofilm change behaviors and metabolic activities to comply with the requirements of the entire biofilm cooperative. Consequently, reconstruction of the evolution of prokaryotes



Paul Stoodley (right) received his Bachelor of Science degree in Environmental Sciences from Lancaster University. He then joined the Center for Biofilm Engineering (CBE) at Montana State University to work on industrial biofilms under Center founder, Bill Characklis. He received a Ph.D. in Biological Sciences at Exeter University under Hilary Lappin-Scott, before returning to the CBE for his self-funded postdoctoral training under mentor and friend, the late Bill Costerton, a pioneering figure in biofilm research. Dr. Stoodley then spent 5 years at Allegheny General Hospital developing techniques to detect and identify biofilms in clinical specimens. He currently holds a position of Reader (Associate Professor) in Microbial Tribology at the National Centre for Advanced Tribology in Engineering Sciences at the University of Southampton, UK. Dr. Stoodley has diverse research interests ranging from the prevention and monitoring of marine biofouling to the role of biofilms in chronic infections associated with medical devices, to the role of biofilms in oral health—all underpinned by a fascination with fundamentals of bacterial biofilm biology. Dr. Stoodley is Assistant Editor of Biofouling Journal and has over 130 publications in engineering, microbiology, and clinical journals. He has been involved in organizing the latest four international conferences of the American Society for Microbiology on Biofilms.

Nora Noffke (middle) received her Master of Science in geology-paleontology at the University of Tübingen, Germany, where she conducted research on trace fossils with her thesis advisor Dolf Seilacher. Following the advice of Robert Riding, Nora then joined the working group of Wolfgang E. Krumbein at the University of Oldenburg, Germany. Here she conducted her Ph.D. research on modern microbial mats in siliciclastic deposits, having the chance to work with one of the leading microbiologists in this research field, Dr. Gisela Gerdes, Director a.D. of the Marine Station of the Institute of Chemistry and Biology of Marine Environments. After a year as visiting professor at the University of Frankfurt, Germany, Nora migrated to the United States, and enjoyed a year in Boston as guest researcher of Andrew H. Knoll, Harvard University, and John P. Grotzinger, then at MIT. Nora finally accepted a faculty position at Old Dominion University. She has edited several volumes on modern and ancient biofilms and microbial mats, and has brought to life both the Gordon Research Conference on Geobiology and the SEPM Field Conference on Sandy Microbial Mats. Nora serves as coordinating editor of the volume Prokaryota of the *Treatise of Invertebrate Paleontology.*

Alan Decho (left), shown here working on carbonate microbiology in the Bahamas. Alan earned his Bachelors degree in Geology and Environmental Science at Eastern Connecticut State University, and a Masters degree in Zoology and Microbiology from Ohio University; he then went on to study for his Ph.D. at Louisiana State University. His initial work involved studying microbial interactions with small marine animals, called meiofauna. He then moved on to the study of biofilms as a Fulbright postdoctoral fellow under David Moriarty at the CSIRO Marine Laboratories, Queensland, Australia. Dr. Decho then moved to the U.S. Geological Survey at Menlo Park, California, where he worked with Samuel Luoma. His recent work is diverse and has focused on chemical communication (quorum sensing) within dense assemblages of bacteria, called microbial mats, including those of marine stromatolites and hypersaline ponds in tropical carbonate environments, the discovery of novel antibiotics from microbial mat systems, and inner workings of the biofilm environment. He has over 85 publications in microbiology, oceanography, and health-related research. This year, he will spend six months in Scotland and Europe, as part of a Fulbright Scholar Award and MAST Fellowship during his sabbatical. He is currently Professor, Graduate Director, and Associate Chair in the Department of ENHS at the University of South Carolina.

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in Earth history must consider the biofilm way of microbial life. Biogenic sedimentary structures might not represent certain microbial groups, but in fact may be relics of modified cooperative microbial activities. Future research should focus on detectable biosignatures caused by biofilm consortia as a whole instead of on the appearance or extinction of individual microbial groups. Such sedimentary structures as stromatolites and microbially induced sedimentary structures (MISS) are intrinsically controlled by biofilms, but also affected by extrinsic (environmental) conditions.

INTRODUCTION

In order to understand the evolution of Earth's earliest life in the rock record, scientists commonly search for fossils or biochemical signatures that are indicative of the existence or initial appearance of specific prokaryotic groups such as photoautotrophic cyanobacteria, sulphate-reducing microorganisms, or methanogenic bacteria. Most early ancestors of prokaryotes, like their modern representatives, may have existed not as individual cells, but in organized assemblages of cooperating microbes called biofilms. In consequence, it might be necessary to advocate for the search for phenotypes of biofilms in the paleontological exploration of Earth's Precambrian history.

Biofilms are assemblages of microorganisms and their adhesive extracellular polymeric substances (EPS) that attach firmly to an interface (Costerton et al., 1978; Hall-Stoodley et al., 2004). In a biofilm, individual prokaryotic (and eukaryotic) cells arrange themselves into positions that facilitate interactions with their neighboring cells (Stoodley et al., 2002). The microbial interactions include chemical communication with each other, leading to joint decision making on strategies important for the community: for example, coordination of metabolic action, gene exchange, EPS secretion, or defense against intruding hostile microbes (Costerton et al., 1999; Camilli and Bassler, 2006). Through such coordination, the biofilm contains and employs the collective knowledge and capabilities of most or all members of the community. This affords a biofilm an arsenal of capabilities to rapidly respond and adapt to adverse conditions—for example, stressful environmental events—more quickly and effectively than any of the individual cells, or than any homogeneous populations of planktonically growing cells (Boles et al., 2004; Stewart and Franklin, 2008).

Biofilms are well-known objects of study in medical sciences, however, only more recently has the relevance and importance of biofilms in the geological record of life been recognized (Noffke, 2010). This is surprising, since the Earth's surface constitutes the largest interface in nature, and as long as water and bacteria are present, a biofilm can form; consequently, most natural surfaces are overgrown by biofilms. The central question in understanding the history of prokaryotic life is whether biofilm activities cause structures, textures, or chemical signatures in sediments and whether these structures and signatures have become preserved in the consolidated rock record.

Much controversy has centered on differences and similarities in the microbial communities of present day versus the early Earth. Reports on the Earth's historical appearance or existence of an individual group of prokaryotes must be regarded with caution, however. One reason is centered on the concept of a biofilm, where a prokaryotic (as well as eukaryotic) group may express a different phenotype than in solitary existence—the latter of which is possible in an artificial laboratory setting but has not yet been shown to occur in nature. A second major reason is that in the fossil record, the morphologies of most microbially induced sedimentary structures (MISS) and many stromatolites have not changed significantly over 3.5 billion years. One conclusion must be that the ancient structure-forming biofilms and microbial mats were functioning in the sediment as cooperatives with some level of similarity to today (Noffke, 2010).

WHAT A BIOFILM LOOKS LIKE

In the present-day world, the majority of prokaryotes exist as biofilms (Costerton et al., 1978). A biofilm is a group of individual prokaryotes (and microeukaryotes) that can be found in any natural environment where water is present. A biofilm also includes copious amounts of EPS, which give rise to the viscous-elastic properties of the biofilm. Indeed, first reports describing biofilms used such names as mucous blob or slime. The architectural framework of biofilms is the EPS—adhesive mucilages that maintain cells in a suitable position relative to each other within the consortium (Kirisits et al., 2007; Flemming and Wingender, 2010). The initial production of long-chained EPS requires high energy that is most often provided by autotrophic primary producers. Subsequent partial degradation and resecretion of this primary EPS by other (heterotrophic) members of the biofilms remodels the mucilaginous, structural framework to adapt its function to enhance metabolic and protective processes. Biofilms and their macroscopic accumulations into microbial mats are transient, that is, after a time of cooperative existence the opportunistic microbes disperse, seeking new attachment elsewhere (Stoodley et al., 2002).

HOW A BIOFILM COOPERATES AND INTERACTS WITH ITS ENVIRONMENT

This consortium of microbes functions as a cooperative, its many microbial members collectively interacting to form a complex society where they can strategically manipulate their immediate environment. Through chemical communication between individual cells and even between different taxonomic groups (Camilli and Bassler, 2006), and in response to such environmental stimuli (extrinsic parameter) as shear stress and nutrient levels, the cells optimize the buildup of the biomechanical structure of the biofilm and its attachment to a surface to achieve efficiency in metabolic activities. Present-day biofilms are known to employ chemical cues to coordinate gene expression, which allows bacteria to change the physical structure of the biofilm (Davies et al., 1998), and to produce light (i.e., bioluminescence) in symbiotic associations with marine animals. Chemical signals are now recognized to have additional functions to cells. For example, they are used to probe the diffusion properties (diffusion sensing) of their proximate environment (Redfield, 2002; Hense et al., 2007).

In natural environments, biofilms are subjected to sudden changes in photochemical and geochemical gradients, aggressive erosive shear, and rapid nutrient fluctuations (Stoodley et al., 2002). Coordinated interaction and the collective search for solutions allow a biofilm to respond much more efficiently to these stresses than as individual cells (Fuqua and Greenberg, 2002; Camilli and Bassler, 2006). Of immediate importance is that modern, lithifying stromatolites are known to produce many kinds of chemical signals (Decho et al., 2009). These stromatolites occur in open marine waters where strong wave action occurs, and require the microbial community to remain firmly attached to the surfaces of the stromatolite structures. The specific functions that chemical signaling afford cells in natural environments such as those of stromatolites, however, is currently unknown but is likely to have many different roles in these diverse microbial communities (Decho et al., 2011).

PROKARYOTES AND BIOFILMS IN EARTH HISTORY

Structure Formation and Preservation

Such structures as MISS and stromatolites have occurred since early Archean time and indicate that the ability of prokaryotes to assemble as biofilms appears to have persisted throughout the geological record of life (Costerton and Stoodley, 2003; Noffke, 2010). In order to explore this matter the fundamental questions must be answered: Which biofilm functions induce a mark in the sedimentary deposits, and which of these features remain detectable even after the diagenetic transformation of sediment into rock? A biofilm has both physical and chemical effects on its sedimentary media. For example, syndepositional microbial

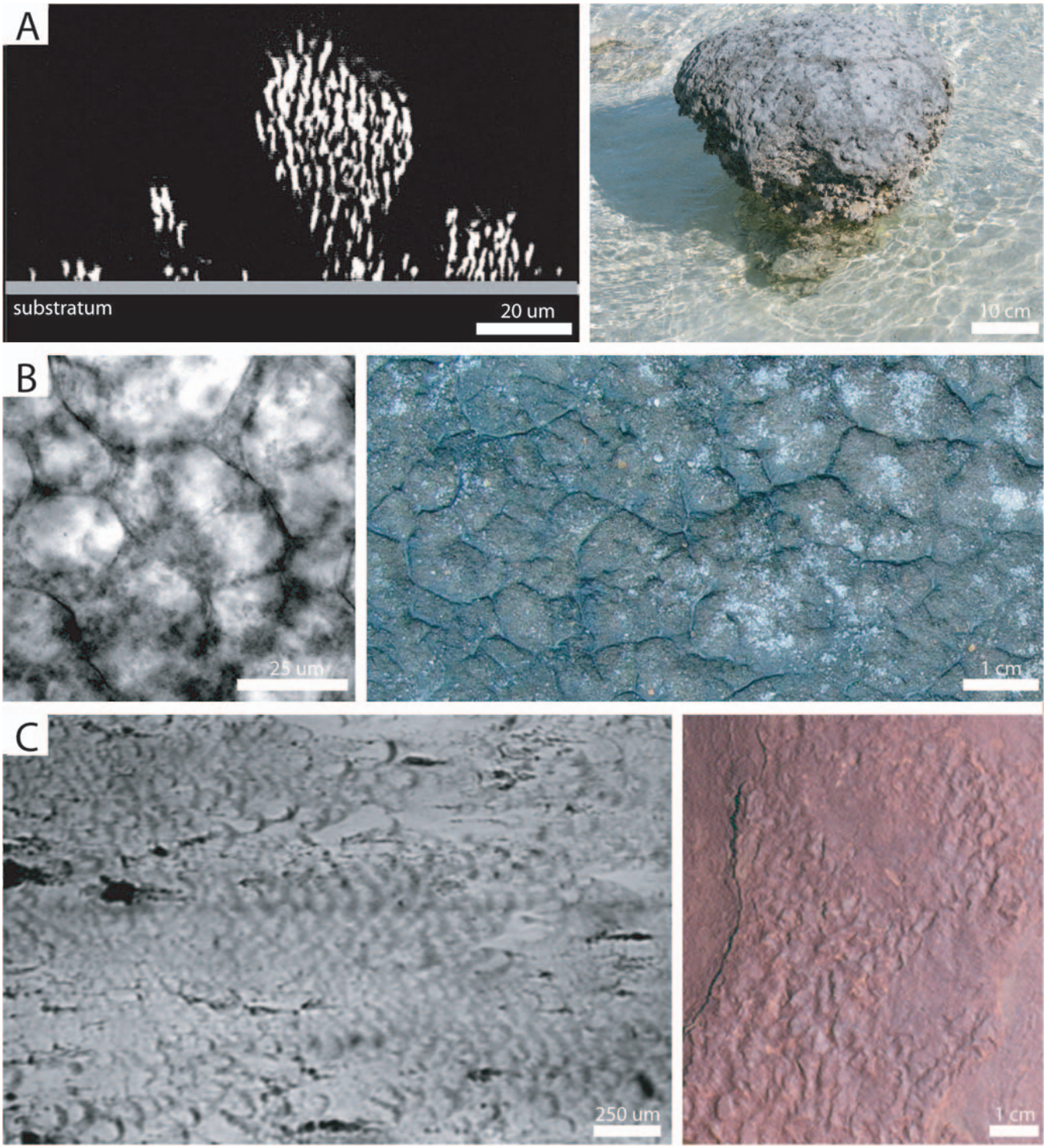

FIGURE 1—Biofilm structures; laboratory cultures (left) and lithified (right): A) In quiescent hydraulic conditions, growing biofilms commonly develop a mushroom shape; scale: 20 µm; (Stoodley et al., 1999; copyright Taylor & Francis). This mushroom shape is characteristic for many stromatolites as this example, right, from Gabla Point, Western Australia, documents; scale 10 cm. B) A honeycomb pattern establishes on surfaces of biofilms of various prokaryotic groups. The laboratory biofilm on the left is formed by *Staphylococcus epidermitis* under completely quiescent conditions (Schaudinn et al., 2007); scale: 25 µm. The example shown on the right is a modern *Microcoleus chthonoplastes*-dominated microbial mat typical for modern tidal flats; scale: 2 cm. However, honeycomb patterns are recognizable in ancient biofilms of up to Archaean ages. C) Biofilms grown in the laboratory under turbulent flow formed ripples and streamers (Stoodley et al., 1999, copyright Taylor & Francis); scale: 2 mm. In the fossil record, sandstone surfaces display similar ripple marks; scale: 3 cm.

sediment fixation (biostabilization; Paterson et al., 2010) or baffling and trapping of mineral particles form characteristic MISS. Biostabilization requires copious amounts of EPS to fix sedimentary particles. If MISS caused by biostabilization are preserved in the fossil rock such structures point to the former presence of ancient biofilms that included autotrophic members producing abundant EPS. Syndepositional baffling and trapping of sedimentary grains causes yet another type of MISS that, if fossilized, documents the existence of mobile filamentous prokaryotes in the ancient biofilm. In the presence of rapid, biologically induced precipitation of minerals, stromatolites are built

up. In present-day stromatolites, cautiously considered analog models of Precambrian forms, the structured precipitation of laminae ($CaCO_3$ layers) building up the stromatolite is delicately balanced by spatially organized bacterial cells and their EPS (Reid et al., 2000; Dupraz et al., 2009). In the fully lithified rock, pyrite, hematite, or siderite minerals constitute fossil cells (filaments, trichomes, cocci). The minerals derive from the activities of heterotrophic biofilm members (sulfur reducers, Fe-oxidizers; methanogenic bacteria) decomposing the organic matter of the primary producers, be they photoautotrophic or chemoautotrophic (Wacey et al., 2011).

Is Morphology the Result of Extrinsic or Intrinsic Factors?

A long-standing question is whether the morphology of stromatolites and MISS is a consequence of intrinsic or extrinsic parameters. This issue would be important for a biostratigraphical use of stromatolites (K. Grey and S. Awramik, personal communications, 2011).

In specifically designed studies, present-day biofilms and microbial mats show evidence of being shaped simultaneously by both intrinsic and extrinsic factors. Intrinsic factors include all biologically controlled parameters—for example cell replication, production of EPS, active movement of cells and filaments. Extrinsic factors are all environmental conditions affecting the biofilm, such as shear stress by water currents and wave action, intensity of solar radiation, and presence of nutrients. Laboratory cultures document that biofilms grow into clusters and mushroom-like stalked blobs in quiescent hydraulic conditions (Stoodley et al., 1999) (Fig. 1A, left). Interestingly, the basic structure of such a mushroom-shaped biofilm corresponds to that of a stromatolite (Fig. 1A, right). Mass transfer limitation is thought to control the shape of the biofilm. The biofilm grows upward into the bulk fluid and forms the wide cap where the concentration of nutrients is high. In contrast, the nutrient shortage at the base of the biofilm limits growth. Cell death and decay support the formation of the stalk. Computer models also predict this biofilm growth towards a mushroom shape. Sometimes, better-adapted bacteria in the population dominate in the stalk whereas others move upward to form the caps. The arrangements of cells within a biofilm influences the efficiency of cell-signaling.

Other biofilms, undisturbed by any water motion, may form a honeycomb pattern on their surfaces (Fig. 1B, left) (Shepard and Sumner 2010; Dubey and Ben-Yehuda, 2011). This phenomenon of unknown function is observed in biofilms constructed by very different prokaryotic groups including the photosynthetic cyanobacteria *Microcoleus chthonoplastes* and *Oscillatoria limosa* (Fig. 1B, right). The organization of such a honeycomb pattern therefore must correspond to very antique genetic information. Indeed, fossil microbial mats of Archean ages already show this surface pattern (Shepard and Sumner, 2010).

With the onset of hydraulic dynamics the shape of biofilms is altered, however. Biofilms are viscoelastic fluids and can be shaped by water shear and drag. For example, cell signaling ceases in *Pseudomonas aeruginosa* (bacterial) biofilms exposed to high water flow so that extrinsic effects may surmount the intrinsic control parameter (Stoodley et al., 1999). Ripple formation in biofilms was observed in this example. During ripple migration, pieces of EPS are ripped off the biofilms, dragged along by turbulent eddies and deposited at the lee sides of mini ripples (Fig. 1C, left). Fossil examples are most abundant, dominating the spectrum of MISS in marine paleoenvironments (Fig. 1C, right). Even if extrinsic parameters have their say in biofilm appearance, the continual growth in biofilms also increasingly affects its immediate environment. In natural marine settings, microbial mats actively bioengineer their own habitat by stabilizing their sedimentary medium and creating a window of hydraulic quiescence (Noffke, 2010). This bioengineering capability is a matter of survival for the biofilm and its microorganisms because of the constant danger that marine microbial mats may be buried by sediment or eroded by currents.

CONCLUSIONS

Much controversy has centered on differences and similarities in the microbial communities of the present day versus the early Earth. Reports on the Earth's historical appearance or existence of an individual group of prokaryotes, however, must be regarded with caution. One reason is that in a biofilm, a prokaryotic group may express a different phenotype than in solitary existence, which might be possible in an artificial laboratory setting, but has not yet been shown to be realized in nature. Another reason is that in the fossil record, the morphologies of most MISS and many stromatolites have not changed significantly over 3.5 billion years. One conclusion must be that the ancient structure-forming biofilms and microbial mats were functioning in the sediment as cooperatives with some level of similarity to today. In consequence, as the search for evidence of microbial life in the Precambrian (and even elsewhere beyond Earth) increases, several parameters, which are uniquely inherent to biofilms, should be emphasized in the analyses of biofilm fossils. These include the macroscopic buildups, the microspatial arrangements of cells, or remnants of sharp geochemical gradients (typical of a biofilm), the potential vestiges of quorum-sensing signals, and the lithified EPS matrix itself. Sedimentary rocks such as carbonates or chert might serve well to understand fine-scaled textures and ancient gradients, because of the quick precipitation of minerals in such sediments (Berelson et al., 2011) entombing biochemical signals within minutes. With improvements in Raman confocal microscopy, NanoSIMS, and quorum-sensing molecular detection using high-resolution mass spectrometry, the continuing development of analytical technologies should provide us with enormous possibilities. As scientists continue to search for the early vestiges and chemical signatures of their existence, studying biofilms becomes necessary to understand the state in which these early cells may have collectively existed.

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