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Microbial biomass and community structure of a stromatolite from an acid mine drainage system as determined by lipid analysis

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Abstract

Lipids were extracted to determine the microbial biomass and community structure of a Fe-rich stromatolite from acid mine drainage (AMD) at the Green Valley coal mine site (GVS) in western Indiana. The distribution of biomarkers correlated well with layers in the laminated stromatolite. Our results show that the biomass of the top layer of the stromatolite was dominated by phototrophic organisms which constituted 83% of the total biomass. Biomass of the lower layers was dominated by prokaryotic microorganisms. The presence of terminal methyl-branched fatty acids and mid methyl-branched fatty acids suggests the presence of Gram-positive and sulfate-reducing bacteria, respectively. Fungi appear to also be an important part of the AMD microbial communities as suggested by sterol profiles and the presence of polyunsaturated fatty acids. Hydroxy fatty acids and C_{19} cyclopropane fatty acids were also detected and likely originated from acid-producing, acidophilic bacteria. The presence of Archaea is indicated by abundant phospholipid ether-linked isoprenoid hydrocarbons (phytane and phytadienes). The AMD Fe-rich stromatolites at GVS thus appear to be formed by interactions of microbial communities composed of all three domains of life; Archaea, Bacteria, and Eukarya. The identification of microeukaryote-dominated stromatolites implicates the prominent role of these organisms in the formation and preservation of these structures. In addition, the production of oxygen through photosynthesis by these organisms in AMD systems may be important for retrodicting the interaction of microbial communities in Precambrian environments in the production of microbially-mediated sedimentary structures and oxygenation of Earth's early atmosphere.

Keywords: Acid mine drainage; Archaea; Bacteria; Lipids; Phototrophs; Stromatolites

1. Introduction

Stromatolites, the mineralized counterparts of microbial mats (Jahnke et al., 2004), are thought to be one of the most tangible morphological, biological and chemical evidences for life on early Earth (Hofmann

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et al., 1999). These laminated structures were widely distributed in shallow marine seas in the first 3 billion years (Ga) of Earth history (Walter, 1994). Much paleoenvironmental and paleobiological information is preserved in the microstructures of stromatolites (e.g., LaBerge, 1973). It is believed that these layered structures were built mainly by mat-building prokaryotes — cyanobacteria, which were presumably the first to develop the oxygenic atmosphere (Golubic, 1994). These structures may be representative of some of Earth's earliest living communities and thus may also provide information on the evolution of the biosphere, atmosphere and geosphere on Earth as well as possibly extraterrestrial bodies.

Modern stromatolites are living analogs for studying the origin, evolution and distribution of life and biogeochemical processes that may leave preserved biosignatures in fossil stromatolites on Earth. Researchers have drawn the close resemblance of modern stromatolites in various extreme environments (hot springs, shallow marine intertidal environments, etc.) to those formed on early Earth and preserved in the geologic record (Jahnke et al., 2004; Burns et al., 2004). However, previous research has focused primarily on interpreting the types of organisms that are represented by currently accepted microbial fossils of extant prokaryotes considered to be the earliest and most primitive life forms (Knoll, 1999) as prokaryotes dominated the early history of the Earth. Eukaryotes are often overlooked in the study of early Earth since their body fossils are rarely preserved in the geologic record. Recent work on Fe-rich stromatolites formed in acid mine drainage (AMD) systems provides new perspective on the role of eukaryotes in the formation of stromatolites and oxygenation of the atmosphere on early Earth. Brake et al. (2002, 2004) suggests that living stromatolites in the AMD environment may be modern analogs of Late Archean–Early Proterozoic banded iron formation (Brake et al., 2002, 2004). The biogenic origin of AMD stromatolites has been demonstrated by the identification of biogenically-derived macrotextures and microtextures, and the presence and arrangement of microbes and their precipitates (Brake et al., 2002, 2004). The unique aspects of these AMD stromatolites are that they exhibit distinct laminated textures that were formed primarily by photosynthetic eukaryotes (*Euglena mutabilis*) (Brake et al., 2002). Thus, the AMD system offers the opportunity to study relatively unique aspects of biodiversity and biogeochemistry of modern living stromatolites (Brake et al., 2001a,b). Biomarkers found in organisms adapted to the AMD environment may be reliable molecular fossils for interpreting the biogeochemical record of eukaryotes and their contribution to the formation of stromatolitic deposits and the rise of oxygen in the atmosphere, both of which have been attributed to the action of prokaryotes and abiotic processes.

Lipids are important biological components of prokaryotic and eukaryotic cells. Lipid analyses of potential ancient stromatolite analogues offer some direct evidence of their biogenic origin and provide invaluable insights into the microbial diversity and biological activities associated with stromatolitic structures (e.g., Jahnke et al., 2004). Phospholipid fatty acids (PLFA) are also important structural components of microbial cells that have been used extensively as proxies for determining microbial biomass and community composition in modern microbial ecosystems (White et al., 1997; Fang et al., 2006). A great number of structurally and functionally diverse lipids exist in microorganisms allowing the extraction of taxonomic information and inference of microbial community composition. PLFA are degraded rapidly upon cell death, and therefore provide a real time analysis of the biodiversity of prokaryotic and eukaryotic communities. Lipid biomarkers are also used to establish possible links between modern microbial communities and their ancient counterparts (Brocks et al., 1999, 2003; Jahnke et al., 2004), and indicate the evolutionary history of organisms, particularly in the Precambrian from which cellular or body fossils are very rare (e.g., Brocks et al., 2003). For instance, acyclic hydrocarbons, hopanes, and steranes are well-preserved biogenic molecules in the geological record that offer insights into the presence of specific types of microorganisms in ancient environments. The presence of 2α-hopanes in the 2.5-Ga-old Mt. McRae shale in the Hamersley Basin, Australia, provides direct evidence that cyanobacteria lived in Archean environments (Summons et al., 1999). Steranes in 2.7-Ga-old shale from the Pilbara Craton, Australia, provided biogeochemical evidence for the early rise of eukaryotes and put the increase in atmospheric oxygen levels 0.5 to 1 Ga years prior to their microfossil record (Brocks et al., 1999).

In this study, we use lipid biomarkers to determine microbial biomass and characterize the microbial communities once present in and that might be responsible for the construction of Fe-rich stromatolites in acid mine drainage at the Green Valley coal mine site (GVS) in Indiana. As far as we know, this is the first study undertaken to identify lipid biomarkers as proxies for the microbial communities that may have constructed these stromatolites, although microbiological and neoichnological research has been on going with respect to the morphology and microbial communities of the Fe-rich