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The Rise of Complexity: Pavilion Lake Microbialites Suggest a Pathway toward Macroorganismic Communities

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Abstract

We propose that freshwater calcite microbialites may represent an alternative trajectory toward a community ecology aggregate that achieves some of the biological functions perfected by macroorganismic communities. A distinctive assemblage of microbialites studied at Pavilion Lake, British Columbia, Canada, could be a prototypical example of this transitional stage of biological organization. The community composition, and, significantly, the identification of quorum sensing molecules in these microbialites, is consistent with the view that they represent an independent and incomplete trajectory toward colonial functionality. This, in turn, supports the general view that biological complexity has evolved independently, under a variety of conditions and on a number of occasions in the history of life. Because these microbialites have formed at temperatures between 0 and 10°C, they provide an example of incipient macroorganismic communities emerging in cold, freshwater environments, analogous to most of the other planetary bodies of our Solar System.

Keywords: complexity, quorum sensing, genetic program, ecosystem, microbialite, multicellular, colonial organism

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Introduction

The emergence of eukaryotes and multicellularity are two landmarks in the evolution of complex macroorganisms on Earth. Neither the conditions nor the critical selective variables for these two events are clearly understood, however, and transitional forms in both cases are poorly represented in the fossil record. Knowledge of putative transitional forms could thus shed light, not only on the way the transformations occurred on Earth, but how the transitions might occur on other worlds.

Colonial communities, such as those that gave rise to the Proterozoic stromatolites, represent early bacterial aggregates that functioned independently but aggregated into a macroorganismic superstructure. Sponges appeared in the same geological era [1] and even today are still characterized by an obligatory association with *cyanobacteria*. [2]. The Paleozoic appearance of coral reefs represent a geologically modern superstructure of much greater complexity, consisting of a mixture of colonial and independent multicellular organisms cohabiting in an intricate ecological community.

Hypothesis

Calcite microbialites are complex assemblages of organisms occupying a mineral superstructure that they build, analogous to corals and stromatolites. We suggest that microbialites are a colonial intermediate between the exclusively prokaryotic colonial precursors of the stromatolites and the multicellular organismic aggregates that gave rise to coral reefs.

A distinctive assemblage of freshwater calcite microbialites discovered at Pavilion Lake, British Columbia, Canada [3] may show this intermediate level of complexity. In support of this possibility, we here present our observations on the composition and biodiversity of the microbialites at Pavilion Lake, along with our hypothesis about a new and alternative trajectory for the evolution of complex community structures. We also make note of the astrobiological implications of this hypothesis.

Pavilion Lake Setting

Pavilion Lake (50°51'57"N, 121°44'20"W), located in Marble Canyon in the interior of British Columbia, Canada, is 5.7 km long and has a width of up to 0.8 km. A freshwater lake with a maximum-recorded depth of 65 m, it is slightly alkaline (mean pH = 8.3). Deeper than 20 m water temperature ranges from 3 to 10°C [4], and shallower than 10 m water temperature ranges from 0 to 20°C. The lake hosts unusual, large (meter scale) freshwater carbonate structures at depth deeper than 10m, referred to as microbialites [3] due to the hypothesized biological role in their formation (Fig. 1).

Observations

Morphology

Collections of microbialites forming reefs oriented perpendicularly to the shoreline were reported from depths of 10 m – 35 m in selected regions of Pavilion Lake and categorized into three depth groupings: (1) mounds found at shallow to intermediate depths of 10-20 m, with a height of several centimeters to a few decimeters (Fig. 1a); (2) cone-shaped seepage structures with hollow internal conduits that open at the top of the cones [3], decimeters to meters in diameter, and up to 3 m in height at intermediate depths of approximately 20-35 m, (Fig. 1b, d); and artichoke-type mounds, centimeters to meters in diameter (Fig. 1c).

Those mounds found in the shallowest strata are noted to be covered by photosynthetic microbial communities and their calcified remains. At the lower intermediate to deeper depths of deeper than 20m, the mounds can be described as a combination of “cone-shaped and leaf-like” morphologies [3], sometimes capped by ‘chimney like’ formations as shown in Fig. 1b. A single internal conduit is seen in all sampled cases, running the length of these ‘chimneys’ (Fig. 1d).

Raw ages ranging from 12,300 ± 1,400 to 3,650 ± 860 years were derived for two whole mounds collected from depths of 27 and 32 m, using thermal ionization mass spectrometry, plus one bulk analysis by alpha spectrometry of the center of a third mound at a depth of 32 m [3]. These values are interpreted to represent the maximum possible age of the carbonates. As such, these structures appear to have begun formation nearly 11,000 years ago, after the glacial retreat of the Cordilleran Ice Sheet. They extended at rates of up to 0.05 mm per year [5].

Community Structure

Assays were carried out on a total of 14 water samples and two microbialite samples at or near Pavilion Lake by phospholipid fatty acid (PLFA) analysis to measure viable microbial biomass, community composition and nutritional status [5-8]. Using this technique, several biomarkers were identified which are typical of both prokaryotic and eukaryotic microbes. A biomarker indicative for diatoms was found as well.

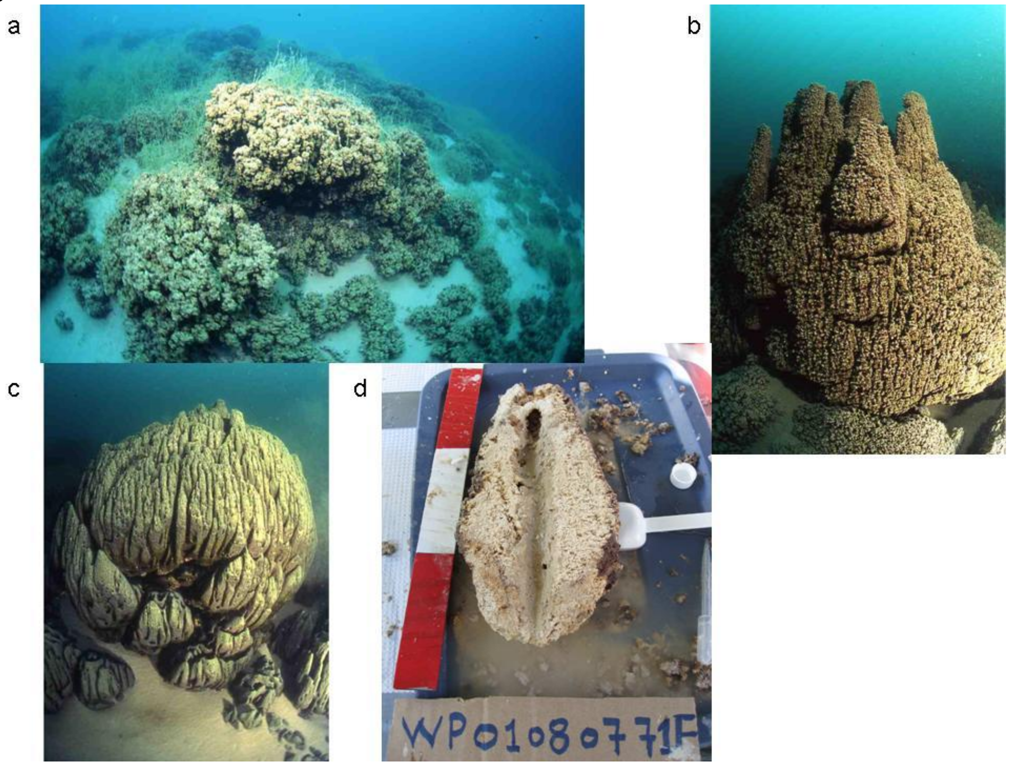
A molecular approach was used to assess the microbial diversity of the microbialites. We used real-time quantitative polymerase chain reaction (Q-PCR) to estimate absolute and relative abundance of archaea, bacteria and eukarya, using rDNA as a marker. In order to establish the taxonomic composition of microbialites we used terminal restriction fragment length polymorphism analysis (T-RFLP) to quantitatively characterize relative abundance of rDNA phylotypes. Phylogenetic identity was assigned to phylotypes based upon sequence analysis of rDNA clone libraries.

The Q-PCR data indicated that bacteria dominate the community in all locations, typically comprising over 90% of recoverable rDNA signatures. The dominant taxa in all communities were proteobacteria (up to 61%), mainly of the common freshwater bacterium *Delftia lacustris*. However, rDNA signatures of eukaryotic and archaea were also present. The cyanobacterial genera *Pseudoanabaena*, *Oscillatoria*, and *Calothrix*, which were reported from morphological studies [3] were confirmed by the presence of their rDNA signatures. Furthermore, *Leptolyngbya*, a common cyanobacterial genus identified in marine stromatolites in Highborne Cay, Bahamas and Shark Bay, Western Australia, and also recorded as an abundant morphotype in Pavilion Lake microbialites [9-10], were also present.

Quorum Sensing Molecules

Quorum sensing molecules are chemical signals that bacteria use to communicate and coordinate their activities via either diffusion to the extracellular environment or release into the intracellular environment. A wide range of diffusible signals that act extracellularly or are attached to the outside membrane of the cell is currently recognized. These include homocysteine, which can be reprocessed from S-adenosyl-homocysteine (SAH) by a one-step

Fig. 1 Examples of varying microbialite morphologies in Pavilion Lake: (a) Shallow to Upper Intermediate (10-15m); (b) Intermediate to Lower Intermediate (about 20m); (c) Deep (25-35m); (d) Internal structure of microbialite (b) from a sample obtained in August 2007. Image credit for Fig. 1a,b, and c goes to Donnie Reid.



process using SAH-hydrolase, or a two-step process, involving enzymatic conversion by the Pfs and LuxS enzymes.

The freshwater microbialites at Pavilion Lake resemble ancient calcareous structures found in the Early Cambrian reef belts. The Pavilion Lake calcite microbialites support diverse bacterial communities including species such as *Fischerella sp.*, *Pseudoanabaena sp.* and *Synechoccus sp*, all bacteria which have been shown to engage in cell-to-cell signaling via quorum sensing molecules [11]. If the Pavilion Lake microbialites represent an alternative trajectory toward macroorganismic communities, we would expect a communication system to exist between the different microbes, contributing to the precipitation and subsequent colonization of the microbialites.

Using degenerative primers and touchdown PCR for the quorum sensing molecules, we found LuxS and SahH genes in the intermediate depth microbialite samples from Pavilion Lake. We therefore predict that quorum sensing molecules will actually be found to be expressed within these community aggregates.

Discussion

The microbialites of Pavilion Lake bear analogy to ancient stromatolites – the mineralogical remnants of paleomicrobialites prominent in the fossil record. Pavilion Lake microbialites differ from ancient stromatolites in their modern (essentially contemporary) origin and in a substantial eukaryotic contribution to their biomass. The complexity of the resulting community structure, in fact, suggests the possibility of a functional organismic aggregate capable of more complex biological processes than those likely to have been carried out by the precursors of ancient stromatolites. Another though more remote analogy to the Pavilion Lake microbialites is their resemblance to that of a coral reef, which is built from modular organisms that deposit a mineral superstructure that serves as a habitat for an entire community ecosystem. The microbialite communities found at Pavilion Lake differ from coral reefs in being composed primarily of microorganisms, and predominantly of bacteria and archaea. However, based on microscopy [3] and direct observations by human divers, the shallow and intermediate depth microbialites in Pavilion Lake also support snails, isopods, and diatoms, amongst other eukaryotic organisms. These organisms are found along the surface of the microbialites (in the case of diatoms and snails), and moving between the larger pore spaces of the microbialites (in the case of isopods).

In principle, therefore, Pavilion Lake microbialites could be viewed as intermediate in complexity between the purely bacterial colonies that gave rise to the Paleozoic stromatolites, and the complex community ecosystems represented by the Cambrian and subsequent coral reef formations. However, in view of their modern and fresh water origin, they clearly represent an independent lineage in the evolution toward a more complex community structure.

An indication that the microorganismic components of the microbialites are more than passive aggregates is the presence of genes for LuxS and SahH quorum sensing molecules. We hypothesize that future research will confirm the expression of these quorum sensing pathways for communicating between the different members of the microbialites. We further predict that quorum sensing molecules will not be found to be produced by the multicellular inhabitants of the microbialite microenvironment such as snails and isopods, which use the microbialites for food and/or protective habitation, but are not an integral part of their superstructure.

Certainly, the complex interaction of these microbial cells is not equivalent to the collaboration of cells within an individual multicellular organism, where each cell has the same genetic information, but differential gene expression provides well-defined cellular specializations. However, their long-term intimate co-habitation is well suited for intercellular chemical signalling and functional coordination, and increases the opportunity for some degree of genetic exchange over evolutionary time that could encode their mutual interactions into a “community” genetic program. It is not inconceivable that a community genetic structure could give rise to a multicellular aggregate which functions in a coordinated manner somewhat like a multicellular organism, much as the semi-autonomous cells of a sponge function as a quasi-multicellular organism.

As is the case with many other microbial associations (such as the formation of biofilms and stromatolites), microbialites tend to form when living conditions are adverse [12-14]. But whereas stromatolites usually form in favorable conditions for growth (moderate temperatures), these microbialites form under unfavorable conditions (very cold temperatures). Therefore, the evolutionary pressure for the development of efficient means of collaboration will tend to be stronger, speeding the emergence of cell-to-cell communication, even among different domains of life. Although these microbialites may not represent a *de facto* transitional form from unicellular to multicellular beings, they could represent an alternative path to macroorganismic complexity that fuses different cell types into functionally multicellular forms.

A more likely scenario, however, is that the Pavilion Lake microbialites represent a heterogeneous microbial community ecosystem that gives rise to macroorganismic superstructures, similar to ancient stromatolites but more complex and diverse in function. They could thus be viewed as an independent and intermediate level of biological complexity between the colonial organizations represented by the Proterozoic stromatolites and the Paleozoic coral reef communities. While consistent with the generally accepted view that multicellularity was polyphyletic in origin [15], the microbialites of Pavilion Lake add an important new, explicit example of what an early trajectory toward multicellularity in a novel (cold, freshwater) environment could have looked like.

The microbialites may also have astrobiological implications. On worlds where evolutionary trajectories could have proceeded to a stage comparable to eukaryotic unicellular life on Earth, structures such as the Pavilion Lake microbialites could in theory be constructed. Mars is one such planet on which early life may have been arrested by cooling and desiccation at an advanced microbial state [16]. Jupiter’s moon Europa, Saturn’s moon Enceladus, and Neptune’s moon Triton are other bodies in the Solar System known to be dynamic enough to suggest endogenous energy sources, yet likely to have existed in their current state of subsurface liquids encased in frozen outer shells for billions of years [17]. The fact that the Pavilion Lake microbialites have been generated at cool to cold temperatures is compatible with formation of analogous microbialites on other worlds under frigid conditions, which characterizes most of the other planetary bodies of our Solar System and probably beyond [18].

Further studies of these unusual microbialite communities may provide additional clues to their functional interactions, and suggest models for the evolution of complex life on this and other worlds.

Conclusions

Freshwater calcite microbialites at Lake Pavilion consist of a complex community of microorganisms that collectively form large, ordered structures suggestive of aggregate functions similar to those of more complex colonial community ecosystems, or even conceivably of multicellular macroorganisms. They may represent a prototypical stage in the evolution of complexity wherever life has emerged.

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References

[1] Nichols S, Wörheide G. Sponges: new views of old animals. *Integrative and Comparative Biology,* (2005) 45, 333-334.
[2] Trautman, D.A., Hinder, R., Borowitzka, M.A. Population dynamics of an association between a coral reef sponge and a red macroalga. *Journal of Experimental Marine Biology and Ecology*, (2000) 244, 87-105.
[3] Laval, B., Cady, S.L., Pollack, J.C., McKay, C.P., Bird, J.S., Grotzinger, J.P., Ford, D.C., and Bohm, H.R. Modern freshwater microbialite analogues for ancient dendritic reef structures, (2000) *Nature* 407, 626-629.
[4] Lim, D.S.S., B.E. Laval, G. Salter, D. Antoniades, A.L. Forrest, W. Pike, R. Pieters, M. Saffari, D. Reid, D. Schulze-Makuch, D. Andersen, and C.P. McKay. Limnology of Pavilion Lake, B.C., Canada – Characterization of a microbialite forming environment. *Fundamental and Applied Limnology*, (2009) 173, 329-351.
[5] Brady, A.L., Slater, G., Laval, B., Lim, D.S. Constraining carbon sources and growth rates of freshwater microbialites in Pavilion Lake using ^{14}C analysis. Geobiology (2009) 7, 544-555.
[6] Edlund, A., Nichols, P.D., Roffey, R., White, D.C. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from Desulfovibrio species. *Journal of Lipid Research*, (1985) 26, 982-988.
[7] Dowling, N.J.E., Widdel, F., White, D.C. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulfate reducers and other sulfide-forming bacteria. *J. Gen. Microbiol.*, (1986) 132, 1815-1825.
[8] Bhat, R.U., Carlson, R.W. A new method for the analysis of amide-linked hydroxy fatty acids in lipid-A from gram-negative bacteria. *Glycobiology*, (1992) 2, 535-539.
[9] Foster, J.S., Green, S.J., Ahrendt, S.R., Golubic, S., Reid, R.P., Hetherington, K.L., Bebout, L. Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas . *The ISME Journal*, (2009) 3, 573-587
[10] Goh, F., Allen, M.A., Leuko, S., Kawaguchi, T., Decho,A.W., Burns, B.P., Neilan, P.A. Determining the specific microbial populations and their spatial distribution within the stromatolite ecosystem of Shark Bay. *The ISME Journal* , (2009) 3, 383–396
[11] Fuqua, W. C., Winans, S. C., Greenberg, E. P. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol*, (1994)176, 269-275.
[12] Grotzinger, J.P., Knoll, A.H. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annu. Rev. Earth Planet Sci.*, (1999) 27, 313-58.
[13] Sheehan, P.M., Harris, M.T. Microbialite resurgence after the late Ordovician extinction. *Nature* 430, (2004), 75-78.
[14] Xie, S., Pancost, R.D., Yin, H., Wang, H., Evershed, R.P. Two episodes of microbial change coupled with Permo/Triassic faunal mass extinction. *Nature* 434, (2005), 494-497.
[15] Abedin, M. & N. King. 2010. Diverse evolutionary paths to cell adhesion. *Trends Cell Biol.* 20: 734-42.
[16] Schulze-Makuch, D., Irwin, L.N., Lipps, J.H., LeMone, D., Dohm, J.M., Fairén, A.G Scenarios for the evolution of life on Mars. *J Geophys Res - Planets*, (2005) 110: E12S23, doi:10.1029/2005JE002430.
[17] Irwin, L. N. & Schulze-Makuch, D. 2010. *Cosmic Biology: How Life Could Evolve on Other Worlds*. Praxis. New York
[18] Schulze-Makuch, D. & Irwin, L.N. 2008. *Life in the Universe: Expectations and Constraints*, 2nd ed. Springer-Verlag. Berlin.