Aggregation Phenomena in Cyanobacterial, Stromatolite Analogues

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Abstract

Earth's biological history is largely the story of microbial evolution. If one is to understand the evolution of such fundamental processes as photosynthesis, it is imperative to recognize and interpret microbial fossils. To these ends, we examine the forces that shape a modern cyanobacterial mat from the hot springs of Yellowstone that is thought to grow in a manner similar to an ancient structure called a conical stromatolite. Here we show that the initial stages in the growth of this mat is analogous to crystal-nucleation with long-range interactions. This result provides intuition for the forces that shape modern mats and, by analogy, conical stromatolites. Furthermore, the importance of long-range interactions suggests that many current models of stromatolite morphogenesis, which rely exclusively on local forcing, are not applicable to the early stages of growth.

To understand the evolution of life on early Earth, one must distinguish and interpret biotic and abiotic processes preserved in the rock record. Although there is often chemical evidence that signals the presence of life during the formation of a geological feature, many of the oldest presumed fossils are only identified by a distinctive morphology [8]. In such cases, how does one interpret a presumed fossil? One example that is abundant throughout Archean and Proterozoic formations is the conical stromatolite. These laminated, lithified sedimentary structures have long been thought to have formed when minerals precipitated within a microbial mat. The conical morphology of these deposits is thought to reflect the shape the microbial mat that grew it [1][4][5]. Assuming that these stromatolites are fossils, what does a conical morphology of a microbial mat imply about the types of microbes that grew it?

In order to understand why a microbial mat might develop a conical morphology, we study a population of modern, cone-forming cyanobacterial mats from the hot springs of Yellowstone National Park. These mats grow in springs that are supersaturated in silica or calcium carbonate; as a result cones are mineralized in-situ. As these mats grow, they produce laminated mineral-precipitates which passively preserve the mat's morphology. Since these mats grow in a manner similar to how some ancient stromatolites are believed to have formed, modern cone-forming mats offer a unique opportunity to test hypotheses about stromatolite morphogenesis [9]. In this paper, we examine the earliest stages of the stromatolite morphogenesis: the aggregation of bacteria.

It has been noted that the photosynthetic rate of bacteria within Yellowstone stromatolite analogues is substantially diminished by the presence of oxygen. However, the negative effects of oxygen are somewhat mitigated when the bacteria aggregate into clumps. It has been proposed that the clumps foster other microbial communities that consume the oxygen within the clump, thus maintaining a low-oxygen environment [10]. This observation suggests that the formation of clumps could be a response to oxygen. It has never been shown that oxygen sensitivity causes the formation of clumps.

To test the hypothesis that clumps form in response to oxygen, we observe the the behavior of small ($\sim 200 \mu$m) samples of bacteria in response to aerobic (5% CO_2, 21% O_2, 74% N_2) and anaerobic (5% CO_2, 5% H_2, 90% N_2) environments under a microscope. On the time scale of tens of minutes, samples in oxygenated water contracted slightly. Any filament that began to move away from the clump quickly retracted (Figure 1 (A) and (B)). However, samples in anoxic medium became more disperse within tens of minutes (Figure 1 (C) and (D)). We therefore conclude that aggregation is a behavioral response to oxygen.

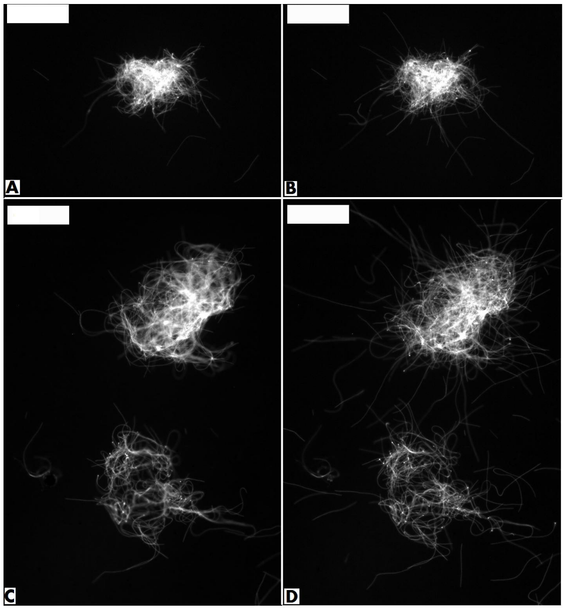

Figure 1: A clump of cyanobacteria under 50x magnification using chlorophyll epifluorescence. The scale bar is 0.02 cm. (A) A clump in oxygenated water at the beginning of an experiment. (B) The same clump one hour later. Any filament that leaves the clump is observed to quickly retract back into it. (C) A sample placed in anoxic medium at the beginning of an experiment. (D) The same sample one hour later. At the end of the experiment all of the filaments that left the clump continued to move away from regions of high density.

To further explore the role of oxygen in the formation of clumps, we followed the evolution of a mat as photosynthesis gradually raised the concentration of dissolved oxygen. Clumps were only observed in mats when the concentration of dissolved oxygen in the medium surpassed a certain value around 120 nM cm^{-3}. This observation suggest that there is a critical concentration of dissolved oxygen at which point bacteria cease to be repulsed by one-another and begin to form macroscopic clumps.

The observation that bacteria transition from a disordered state to an ordered state a critical oxygen concentration suggests the formation of bacterial aggregates is similar to a phase transition. Specifically, we explore an analogy between clumping and crystal nucleation. The formation of a clump requires an interface between the oxygenated water and the low-oxygen environment within the clump. Our observations are consistent with the hypothesis that when the surface-area-to-volume ratio of the clump is too high microbes leave the surface faster than they reproduce within it. Thus, small clumps dissolve while large clumps grow. Crystal nucleation is similar. This process occurs when the concentration of a solute approaches a critical value where fluctuations in the solute's local concentration become large. Some of these fluctuations concentrate enough material in a small enough volume such that there is a distinct interface between the solute crystal and solvent. Surface tension imparts an energy to the crystal-solvent interface. The resulting crystal is only stable if the change in the Gibbs Free Energy is sufficiently large to balance the surface energy. Since the surface energy scales with the surface area while the change in the Gibbs Free Energy scales with the volume, these energies are balanced at a critical radius: small crystals redissolve and large crystals grow [6]. We therefore suspect that the increased reproductive rate in a microaerophilic environment is the analogy of the change in the Gibbs Free Energy while the adverse effects of living on a clump's surface give rise to an effect similar to surface tension. Thus far, the analysis has only drawn a possible analogy between phase separation and clump formation. This analogy is only useful if the predictions for the growth of crystals during phase separation can give some intuition for the the evolution of a microbial mat.

At low concentrations of oxygen, bacteria glide away from one another and the system relaxes to a low-density state. If, however, oxygen levels are raised, there is some beneficial, short-range interaction that attracts the microbes into clumps with finite density. This process is analogous to crystal nucleation where bacterial density ρ is the order-parameter and the dimensionless oxygen concentration t is the control-parameter. Although there is no obvious thermodynamic potential that the microbial mat extremizes, the basic, short-range interactions that shape a mat are consistent with a Landau-Ginzberg equation.

$$-\beta\mathcal{H} = \int \mathbf{d}^2x \, \frac{t}{2}\rho^2 + u\rho^4 + \frac{K}{2}(\nabla\rho)^2. \quad (1)$$

This equation captures the basic phenomenology of the system: when t reaches a critical value near zero the equilibrium density transitions from zero to a finite value around $-\frac{t}{4u}$. The model becomes more interesting when one recognizes that there is also a long-range interaction due to the long distance that oxygen can diffuse between photosynthetic clumps of oxygen-sensitive bacteria. To model this behavior one must include a term of the form

$$-\beta\mathcal{H}_L = \int \mathbf{d}x\mathbf{d}y \, \rho(x)g(|x-y|)\rho(y) \quad (2)$$

where g is a positive function.

The process of phase-separation was first described theoretically by Lifshitz, Slyozov, and Wagner in the limit that nucleating crystals do not directly interact and the total amount of solute is constant. Since the change in the Gibbs Free Energy scales with the amount of super-saturated material which is constantly falling out of solution to form crystals, the the length-scale where the surface energy balances the Gibbs Free Energy increases in time. As the critical radius increases, the smallest crystals redissolve and recrystallize on larger crystals in a process known as Ostwald ripening. In the limit of infinite time there is only one, gigantic crystal. The theory predicts that the critical radius grows with the cube-root of time [6]. If there is also a long-range repulsive force, the picture changes slightly. The long-range interaction prevents the formation of crystals larger than some size that depends on the nature of the interaction. In this case, rather than forming a single crystal, the system develops a multitude of small crystals with a characteristic size. Interactions between neighboring crystals also impose a characteristic spacing between the crystals. In two dimensions, the crystals are typically arranged in a regular hexagonal lattice; which maximizes the density of crystals subject to the constraint that there is a fixed lattice spacing. This result has been shown for systems with a variety of long-range interactions including the the Coulomb force [3] and more complected forces such as those that describe Langmuir monolayers [7]. If the formation of clumps in a microbial mat is indeed analogous to Ostwald Ripening with a long-range interaction, we expect clumps to form a hexagonal lattice with a lattice spacing set by the diffusion of oxygen.

The diffusion length of oxygen is the scale where oxygen diffuses away from a clump at the same rate that is produced by photosynthesis. If the distance between neighboring clumps is λ and each one produces oxygen at a rate $\mathcal{R}$, the time τ before there is a significant change in the concentration of oxygen C is

$$\tau \sim \frac{C\lambda^3}{\mathcal{R}}. \tag{3}$$

If the rate at which oxygen is produced is faster than the rate at which it diffuses away oxygen levels will increase around the clumps and, eventually, become toxic. Thus, oxygen must diffuse away from the clumps at the same rate that it is produced. The time-scale for gas to diffuse away from the clump is,

$$\tau \sim \frac{\lambda^2}{D} \tag{4}$$

where D is the diffusion constant of oxygen in water. Therefore, the scale at which diffusion balances production is

$$\lambda \sim \frac{\mathcal{R}}{CD}. \tag{5}$$

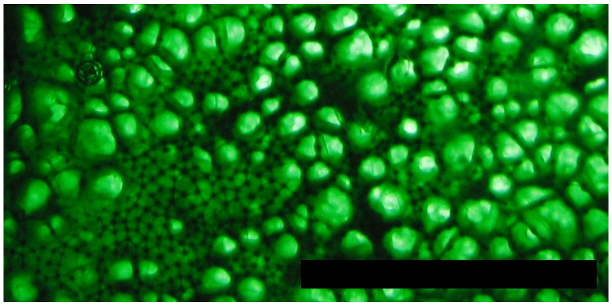

Figure 2: Clumps that grow on a thin biofilm typically form a roughly hexagonal lattice. The scale bar is one centimeter.

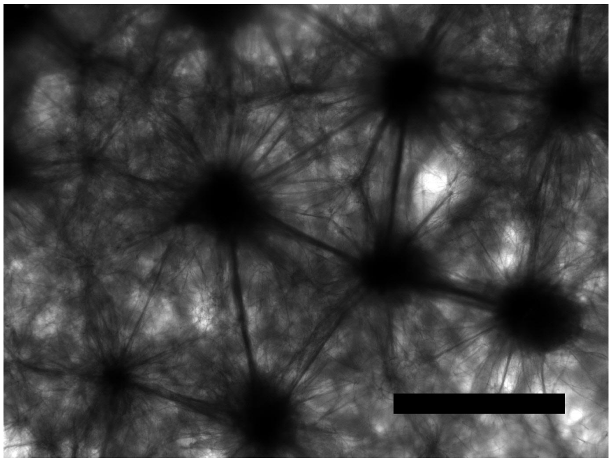

Figure 3: Small clumps grown on smooth agar form a roughly hexagonal lattice. This image is magnified 50 times. The scale bar is 0.05 cm.

To test the hypothesis that oxygen-sensitive cyanobacterial mats can be described as two-dimensional crystallization with long-range interactions, we observed the evolution of a thin biofilm growing on a bed of silica sand in an atmosphere originally composed of 5% CO_2, 5% H_2, 90% N_2. As the mat grew and photosynthesized a certain proportion of CO_2 was converted to O_2.

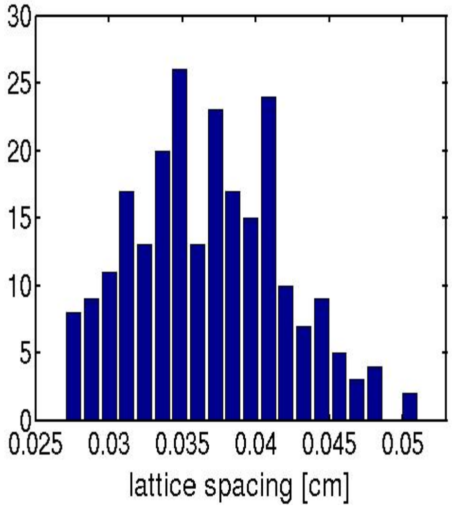

Figure 4: Histogram of the lattice spacing for clumps grown on a thin biofilm. The lattice spacing is consistent with the diffusion length of oxygen.

Within two weeks, the microbial mat developed roughly 200 clumps (Figure 2). Each clump generally had between five and seven neighbors (Figure 3). 95% of of neighboring clumps were between 0.027 cm and 0.046 cm from one another (Figure 4). Oxygen microelectrodes were used to determine the characteristic photosynthetic rate (8 10^{-15} mole sec^{-1}) and oxygen concentration (3 10^{-7} mole cm^{-3}). The diffusion constant of oxygen in water is of order 10^{-5} $\mathrm{cm}^2\mathrm{sec}^{-1}$ These values correspond to a diffusion length of order 0.01 cm. Hexagonal lattices with similar spacings were reproduced by mats growing on smooth agar, textured filter paper, and smooth plastic.

The formation of a hexagonal lattice of microbial clumps with a spacing consistent with the diffusion of oxygen suggests that the aggregation of oxygen-sensitive, oxygen-producing bacteria can be usefully modeled as two-dimensional phase-separation with a long-range interaction. Although it has not been demonstrated that the interactions between bacteria can be modeled as forces in the strict sense of the term, this observation provides intuition for the processes which shape modern stromatolite analogues. This work suggests one of the most important processes in the early evolution of these cyanobacterial mats is the diffusion of oxygen over long distances. This result contradicts the current model for the formation of conical stromatolites, which suggests that the mat evolves due to local forcing by light gradients generated by differences in the mat's height [2][9]. The importance of the diffusion of oxygen also informs the interpretation of the Precambrian fossil record. Given that the aggregation of bacteria in modern stromatolite analogues is driven by the diffusion of oxygen, we suspect that similar processes may have been important in the formation of some of Earth's earliest fossils.

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