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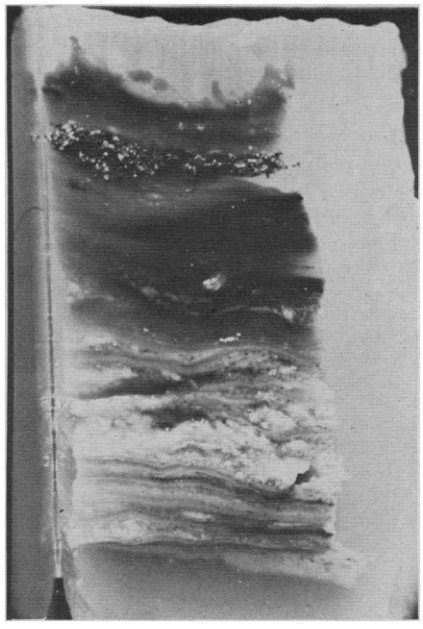
Bacterial Stromatolites: Origin of Laminations

Abstract. *Laminated mats composed of motile filamentous photosynthetic bacteria and nonmotile unicellular blue-green algae occur in a large number of Yellowstone hot springs at temperatures between 55° and 70°C. Field studies indicate that the bacteria are the predominant mat-forming component. Under low light intensities, mats composed exclusively of bacteria can be formed. The bacteria undergo a diurnal migration, moving on top of the algae during the night and becoming mixed again with the algae during the day as a result of algal growth. Thus, the laminations probably arise as a result of differential migration of the bacteria in daily response to reduced light intensities. This response to light is exactly opposite to that previously reported for filamentous stromatolite-forming, blue-green algae, but the net result is the same—formation of a laminated mat.*

If the present is the key to the past, a study of modern growing stromatolites may help in interpreting the origin and significance of Precambrian stromatolites and the environmental conditions under which they were formed. It has been conventionally assumed that Precambrian stromatolites are formed by filamentous blue-green algae (*1*), yet laminated mats are also being formed today by filamentous bacteria in the effluent channels of hot springs. In earlier work (*2*) it was shown that the main structural component of these mats was bacterial, but at that time it was not known that the bacteria were photosynthetic. Since then it has been shown (*3, 4*) that these filamentous bacteria contain bacteriochlorophylls and are capable of light-stimulated fixation of CO_2. These bacteria, now classified in the genus *Chloroflexus* (*5*), are widespread in hot springs of neutral to alkaline *p*H throughout the world. They are capable of both anaerobic and aerobic growth, and can grow photosynthetically as well as heterotrophically. Under conditions of high sulfide, mats are formed composed exclusively of these bacteria (*6*), but in most cases they are found in nature in association with unicellular, nonmotile blue-green algae of the genus *Synechococcus*. It is our purpose here to provide data showing that it is the bacterial component which controls mat structure, and that the laminae arise as a result of differential response of the bacteria to changing light conditions over the diurnal cycle.

The distinction between the *Chloroflexus* mats under study in this report and the Conophyton-like stromatolitic structures described earlier from hot springs by Walter *et al.* (*7*) should be emphasized.The Conophyton-like structures are formed by an association of a filamentous blue-green alga *Phormidium tenue* and *Chloroflexus*, but in most cases the alga is dominant and is capable of forming the structures in the complete absence of the bacteria (*8*). The mats under study in this report are generally flat rather than conical, although, as noted below, nodular structures are sometimes formed (*9*). Detrital silica derived from the siliceous sinter of the geyser basins is frequently incorporated into the *Chloroflexus* mats, being carried onto the mats with runoff from occasional thunderstorms. Between storms, the mats are relatively undisturbed, and *Chloroflexus* migrates rapidly around and on top of the detrital material which falls on a mat, as can be readily observed by sprinkling carborundum powder onto the mat. *Chloroflexus* is thus a sediment-trapping organism, as are the stromatolite-forming blue-green algae.

Details of methods will be published elsewhere (*10*). Growth rates of the mats were followed by marking with carborundum powder. *Chloroflexus* contains both bacteriochlorophylls a and c. Bacteriochlorophyll a was assayed by extraction into methanol and measurement of absorption with a Beckman DB-G spectrophotometer at 770 nm, the absorption peak of bacteriochlorophyll a in this solvent. Bacteriochlorophyll c (which absorbs in solvents at virtually the same wavelength as algal chlorophyll a) was measured in vivo by homogenizing mat material in a solution of sucrose (1.4 g/ml) to reduce scatter and reading the in vivo absorption spectrum of bacteriochlorophyll c at 740 nm. Algal chlorophyll a was determined similarly, by using the absorption peak at 665 nm in methanol or the in vivo peak in sucrose suspension at 680 nm. Photosynthesis was measured by the ^{14}C method as described by Brock and Brock (*11*), and the effect of light intensity on photosynthesis was determined by using calibrated nylon mesh as described by Brock and Brock (*12*). To distinguish between algal and bacterial photosynthesis, the inhibitor of algal photosynthesis 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) was used, at a final concentration of about $5 \times 10^{-5} M$. In preliminary experiments it was shown that at this concentration light-stimulated uptake of CO_2 by the alga is completely inhibited, whereas bacterial CO_2 fixation is unaffected. In some photosynthesis experiments, an infrared filter (Tiffen 87 C) was used which eliminated all wavelengths of light to which the alga responds, but passed infrared radiation used by the bacterium. These two methods of measuring bacterial photosynthesis gave similar results. Mats were sampled with cork borers of various sizes. In some experiments, layers of the mat were cut in the field by using a dissecting microscope. To preserve delicate structures for later microscopy, cores were allowed to fall gently into vials containing melted 2 percent water agar. After the agar had hardened, it was removed from the vial and the core was cut longitudinally. The study areas in Yellowstone Park—Pool A, Toadstool Geyser, and Twin Butte Vista—have been described by Bauld and Brock (*4*). These areas may be considered representative of hundreds of springs in Yellowstone Park where *Chloroflexus* is present. In the study areas, the laminated mats develop in shallow, gently flowing water, the flow characteristics being essentially



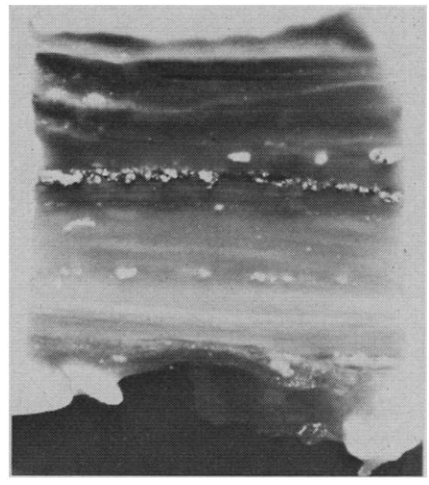


Fig. 1 (left). Vertical section through a mat from Pool A, Yellowstone Park (58°C). The total length of the section is about 1.5 cm. A carborundum layer is seen at about 2.7 mm below the surface; the carborundum had been sprinkled on the surface of the mat 1 month before sampling. The light-colored layers in the lower portion are composed primarily of detrital silica. Fig. 2 (right). Vertical section through a mat from Toadstool Geyser (61°C). The total length of the section is about 0.75 cm. A carborundum layer is seen at about 3.0 mm below the surface; the carborundum had been sprinkled on the surface of the mat 1 month before sampling.

laminar. Mats also develop in areas of turbulent flow, but the morphology is less regular.

Sections through typical laminated bacterial mats are shown in Figs. 1 and 2. The microstructure of the mat is complex and is not easily seen in these sections. At the top of the mat the laminae are probably very thin and appear in photographs such as Figs. 1 and 2 as a combined layer. This top layer, approximately 1 mm thick, is usually dark green due to the presence of the blue-green alga, but this region also contains the photosynthetic bacterium, which provides the structural matrix. On top of the dark green layer, pink or pinkish green nodes or colonies are occasionally seen in which the photosynthetic bacterium predominates. Under the dark green surface layer there is usually an orange or pink layer 1 to 2 mm thick containing almost exclusively the photosynthetic bacterium, and the bacteria in this region are still photosynthetically active. Below this region, orange, tan, or whitish layers are seen, composed primarily of unicellular bacteria and dead hulks of photosynthetic bacteria. In this region laminae appear thicker, probably because they represent a number of individual laminae which have become compacted and have coalesced within the depths of the mat. These layers are often separated by layers of detrital silica. It is of considerable interest that in this region the blue-green alga is virtually absent, and not even ghosts or empty hulks are seen under the microscope. The blue-green alga may be destroyed by other bacteria present, since it does not appear in axenic culture to be unusually prone to lysis. *Chloroflexus* must be considerably more resistant to decomposition than the blue-green alga, as empty hulks of this organism constitute the bulk of the mat matrix throughout the whole of the lower region where organic matter is present. Under the organic-rich region, all that is left after decomposition are the detrital silica layers, which often retain the grossly laminated morphology quite clearly (Fig. 1). The accretion rate of these mats, as shown by carborundum marking, is about 0.1 mm per day, although decomposition and compression in the lower portions of the mat must occur at the same rate since there is no net upward growth of the mats.

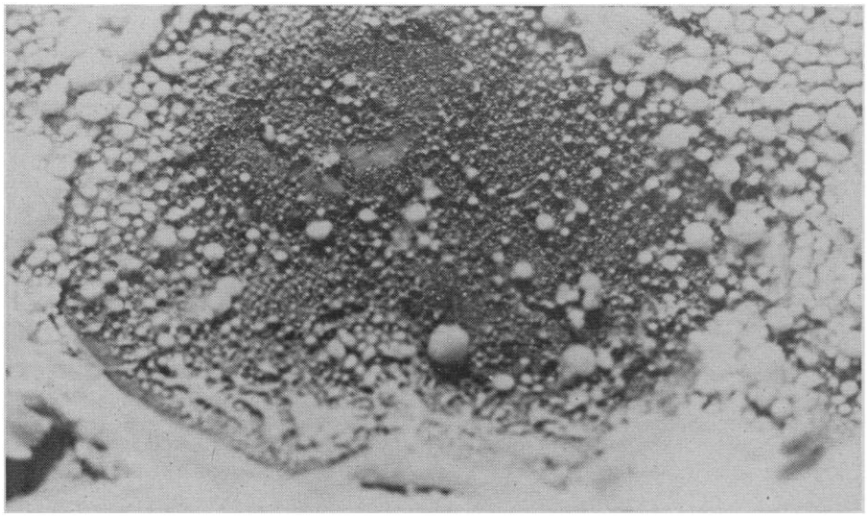


Fig. 3. *Chloroflexus* nodes which had developed in 1 week on top of a carborundum layer under reduced light intensity (maximum, 1000 lux). The diameter of the carborundum circle is about 85 mm.

As shown by Bauld and Brock (*4*) and confirmed by the work described here, most photosynthetic activity is confined to the top 2 to 3 mm of the mat. Additionally, light penetration studies on sections of cores showed that light intensity below about 2 to 3 mm was reduced to less than 1 percent of full sunlight, and since full sunlight intensity is about 70,000 lux, the light intensity 2 mm below the surface of the mat was about 700 lux. Studies with infrared filters showed that both visible and infrared radiation are attenuated to the same extent; most of the light attenuation must be due to scattering rather than absorption in these compact mats. As shown by Brock and Brock (*12*), the optimum light intensity for algal photosynthesis is at full sunlight, whereas Bauld and Brock (*4*) showed that the optimum light intensity for photosynthesis by bacteria taken from the orange layer beneath the green layer was quite low, around 1000 lux. We have now con-

firmed these findings. Thus, both alga and bacterium are photosynthetically active in the top dark green layer, but the bacterium is able to photosynthesize at lower light intensities than the alga.

As noted above, pink or pinkish-green nodes or colonies occasionally develop on top of the flat mat. Radioisotope studies were done with these structures, and the extent to which photosynthesis was due to bacteria was determined by using the inhibitor DCMU. Bacterial photosynthesis was found to be a significant or dominant fraction of the photosynthetic activity of these structures. This shows that even at the top of the mat, the photosynthetic bacterium was able to carry out light-stimulated CO_2 fixation.

Knowing the light intensity preferences of the photosynthetic bacterium and alga, we studied the effect of experimentally reducing the light intensity over portions of the mat in the field. To do this, we prepared a neutral density filter about 0.7 by 0.7 m by fastening black, closely woven, nylon material (sold for manufacturing rainwear) to a wooden frame. This filter passed about 2 percent of full sunlight (around 1000 lux). Within several days after filters were placed over areas of mat, the mat had become pink, and within a week most of the algae had disappeared and the mat was composed of virtually only bacteria. The algae presumably disintegrated, as they do naturally when buried in the dark depths of the mat. Despite the absence of algae, the mat retained its structure, and nodes and other macrostructures were formed. That this exclusively bacterial mat grew after light reduction was shown by placing carborundum on the mat at the time the filter was installed. Figure 3 shows the type of nodulated structures which developed on top of the carborundum at low light intensity. When photosynthesis in these structures was measured at low light intensity, it was virtually exclusively bacterial. At high light intensity, total photosynthesis was higher and more algal photosynthesis was found, but bacterial photosynthesis still predominated. In one experiment, 100 percent of the photosynthesis in these structures was due to bacteria.

In other locations, covers which excluded all light were placed over the mats. The covers were placed at 0930 hours; within 5 hours (1415) the mats were noticeably pinker than the mats in full light, indicating that the *Chloroflexus* had responded to darkness by moving up through the algal layer. This experiment was repeated several times with similar results. Because of the rapid response, it seemed likely that similar migration of *Chloroflexus* would occur every night. To confirm this, observations were made throughout the night by using a flashlight and taking color photographs with electronic flash. Analysis of the photographs showed that the mat did become pink at night, and became green again during the next morning. Confirmatory evidence was obtained by placing thin sheets of lens paper on mats either in the evening or early in the morning. *Chloroflexus* migrates up through the mesh of the paper and microscopy of the paper can be used to determine if differential migration occurred. Lens papers removed in the morning after overnight placement generally had predominately *Chloroflexus*, whereas in papers placed in the morning and removed at the end of the day, *Synechococcus* was predominant.

It seems possible that the upward migration of *Chloroflexus* in response to low light or darkness, and the lack of migration during daylight, could account for the origin of the laminated mats. It has been shown (*3*) that *Chloroflexus* is capable of moving at rates of 30 to 150 μm/hour, and hence an accretion rate of 0.1 mm/day (100 μm/day), as measured by carborundum marking, is possible. It is of interest that work with blue-green algal stromatolites (*13*) has shown that upward migration of algal filaments occurs in the day, followed by horizontal growth at night. Work on blue-green algal stromatolites in Yellowstone hot springs (*8*) has shown a similar migration pattern. Thus, the migration of the bacteria is exactly opposite to that of the blue-green algae, yet the result is the same, the formation of a laminated mat.

Although these results say nothing directly about Precambrian stromatolites, they do show that stromatolites might have been formed solely by filamentous photosynthetic bacteria. *Chloroflexus* is capable of phototrophic growth under anaerobic conditions, as are other photosynthetic bacteria, and oxygen is presumably not evolved since DCMU has no inhibitory effect. *Chloroflexus* resembles a blue-green alga morphologically, and was for many years classified as one (*14*). Organisms morphologically similar to *Chloroflexus* are seen in thin sections of Precambrian rocks (*15*). If Precambrian stromatolites were formed by similar photosynthetic bacteria, the time at which stromatolites first appear in the fossil record could not be used to date the time of origin of blue-green algae, or the time at which biologically produced oxygen first appeared in the atmosphere. Since photosynthetic bacteria (including *Chloroflexus*) are capable of completely autotrophic growth under anaerobic conditions when sulfide is present, they could have been responsible for the production of some of the organic matter found in Precambrian rocks.

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5. The earlier spelling of this genus, *Chloroflexis* [R. W. Castenholz, in *The Biology of Blue-green Algae*, N. G. Carr and B. A. Whitton, Eds. (Blackwell, London, 1973), chap. 19] is being changed to the correct form, *Chloroflexus*, in the taxonomic publication on this organism (B. K. Pierson and R. W. Castenholz, in preparation).
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9. A key difference between the two types of mats is the temperature at which they form. Conophyton-like mats form only at temperatures below about 55° to 59°C, and are most common at temperatures around 35° to 45°C. The *Chloroflexus* mats, on the other hand, form best in the temperature range of 55° to 65°C, although they occur over a temperature range from about 50° to 70°C. It should be emphasized that at temperatures above 60° to 62°C filamentous blue-green algae are never found anywhere in the world [T. D. Brock, *Science* **158**, 1012 (1967); R. W. Castenholz, *Bacteriol. Rev.* **33**, 476 (1969)] although unicellular blue-green algae, thought probably not to be able to form mats by themselves, are present at temperatures up to 70° to 73°C.
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