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Mineral Precipitation by Epilithic Biofilms in the Speed River, Ontario, Canada

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Epilithic microbial communities, ubiquitously found in biofilms on submerged granite, limestone, and sandstone, as well as on the concrete support pillars of bridges, were examined in the Speed River, Ontario, Canada. Transmission electron microscopy showed that attached bacteria (on all substrata) were highly mineralized, ranging from Fe-rich capsular material to fine-grained (<1 μm) authigenic (primary) mineral precipitates. The authigenic grains exhibited a wide range of morphologies, from amorphous gel-like phases to crystalline structures. Energy-dispersive X-ray spectroscopy indicated that the most abundant mineral associated with epilithic bacteria was a complex (Fe, Al) silicate of variable composition. The gel-like phases were similar in composition to a chamositic clay, whereas the crystalline structures were more siliceous and had compositions between those of glauconite and kaolinite. The consistent formation of (Fe, Al) silicates by all bacterial populations, regardless of substratum lithology, implies that biomineralization was a surface process associated with the anionic nature of the cell wall. The adsorption of dissolved constituents from the aqueous environment contributed significantly to the mineral formation process. In this regard, it appears that epilithic microbial biofilms dominate the reactivity of the rock-water interface and may determine the type of minerals formed, which will ultimately become part of the riverbed sediment. Because rivers typically contain high concentrations of dissolved iron, silicon, and aluminum, these findings provide a unique insight into biogeochemical activities that are potentially widespread in natural waters.

The attachment and growth of microorganisms on submerged rocks are common in moderate- to fast-flowing rivers (17, 20). These epilithic microbial populations benefit from the high concentrations of organic and inorganic compounds that accumulate at the solid-liquid interface and are commonly more active in terms of their metabolic activity than their planktonic counterparts (15). To utilize the available nutrients for growth, microorganisms, such as bacteria, must have the ability to adsorb and concentrate ions from solution. The fixation of cations arises through electrostatic interaction with the anionic surfaces (carboxyl and phosphoryl groups) of the cell wall at circumneutral pH (6, 9), and these sites are known to be major repositories for metal deposition (2, 5, 21). Many cells also have an extracellular sheath or capsule composed of acidic polysaccharides, the molecular components of which are similarly reactive and consequently accumulate metals around the cell (10). Once bound to the bacteria, the metals can serve as nucleation sites for the formation and growth of authigenic mineral phases. The result is a mineralized cellular matrix that contains detectable concentrations of metal ions (as fine-grained minerals) that are not easily solubilized (3). Examples of bacterial mineral formation include phosphate mineralization in laboratory simulations of sediment diagenesis (4) and the precipitation of metal sulfides (11), calcite, gypsum (27), and complex (Fe, Al) silicates in natural environments (11, 19).

The colonization of a mineral substratum by microorganisms is directly influenced by the microtopology of the surface, with cells preferentially attaching themselves to highly abraded surfaces that afford protection from fluid turbulence and shear stress (24). Since susceptibility to abrasion is largely a function of the hardness of the rock-forming minerals, microbial population densities on softer rock types (e.g., limestone, which contains calcite and dolomite as major components) greatly exceed those on harder rock types (such as granite, which contains quartz and feldspar) in the same river or stream (12). While microbial population density is controlled by substratum type, in this study we were interested in determining what effects different rock substrata had on the precipitation of authigenic minerals by epilithic microorganisms.

MATERIALS AND METHODS

Epilithic microorganisms, growing in biofilms on submerged rocks (including granite, sandstone, and limestone) and the support pillars of a bridge (constructed of concrete), were collected from flowing areas (approximately 10 $cm \cdot s^{-1}$) of the Speed River near Guelph, Ontario, Canada. After the rocks were carefully removed from 1 m of water, sections (4.0 cm^2) of the biofilms were scraped off the hard substrata with sterile scalpels and immediately placed in 5-ml metal-free plastic tubes containing aqueous 2.0% (vol/vol) glutaraldehyde, a fixative for electron microscopy (7).

The biofilm samples were prepared for thin sectioning by washing in a solution of 0.05 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (Research Organics Inc., Cleveland, Ohio), pH 7.2, to remove excess glutaraldehyde. After being washed, the samples were dehydrated through a graded acetone series and embedded in epoxy resin (Epon 812; CanEM, Guelph, Ontario), as previously described

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by Graham and Beveridge (18). Thin sections, approximately 60 nm in thickness, were obtained with a Reichert-Jung Ultracut E ultramicrotome and mounted on Formvar-carbon-coated, 200-mesh copper grids. To increase the electron contrast of cytoplasmic material inside intact cells, some thin sections were stained with uranyl acetate and lead citrate before imaging in the electron microscope (7). Grids were viewed with a Philips EM400T transmission electron microscope (TEM) equipped with a model LZ-5 light element detector and an exL multichannel analyzer (both from Link Analytical). The TEM was operated at 100 keV, with a liquid nitrogen-cooled anticontamination device in place. Energy-dispersive X-ray spectroscopy (EDS) was conducted, using electron beam spot sizes of 400 nm with a beam current of 0.1 μA. Spectra were obtained by collecting counts for 100 s (live time). The d-spaces for crystalline mineral phases were examined by using selected area electron diffraction with a camera length of 800 mm. The elemental compositions of amorphous phases were characterized by EDS spot analyses run with the Link quantification program to determine stoichiometric ratios.

A water sample was collected 1 m from shore with a 500-ml Nalgene bottle; it was then acidified on site with 5 ml of analytical-grade HNO_3. Prior to multielement analysis, materials in suspension were removed in the laboratory by pressure filtration through 0.2-μm-pore-size Nuclepore membranes (Gelman Sciences Inc., Ann Arbor, Mich.). To determine the dissolved metal content, the water sample was analyzed by inductively coupled plasma-mass spectroscopy: an inductively coupled plasma-mass spectrometer, model PQ1, by Elemental Ltd. (Vancouver, British Columbia) was used. The Nuclepore membranes that contained the suspended sediments were allowed to air dry and subsequently were mounted on glass slides with double-sided tape. The mineralogy of the suspended sediment was determined by X-ray diffraction on a Rigaku rotating anode diffractometer (Co Kα). The scans were carried out at 160 kV and 45 mA, from 2 to 82° two-theta at a rate of 10°/min.

RESULTS

TEM analyses showed that complex microbial biofilm communities occurred on the submerged rocks. The epilithic bacteria (on all substrata) were highly mineralized, ranging from electron-dense Fe-rich capsules to fine-grained (<1 μm) authigenic mineral precipitates. Many of the cells possessed capsules, which varied greatly in their proportions; some encapsulated only an individual cell (Fig. 1A), but other microorganisms produced so much extracellular material that several cells became encapsulated to produce a microcolony (Fig. 1B to D). EDS analyses indicated that these capsular polymers sequestered significant amounts of iron from solution, along with detectable amounts of Ca, K, Si, and Mn (Fig. 2A). The accumulation of trace metals such as Mn, which was found in extremely low aqueous concentrations (65 μg/liter), indicates that epilithic biofilms are potent scavengers of dissolved metallic ions. This behavior correlates well with previous results which showed that transition metals were efficiently adsorbed by biofilms in acid mine drainage environments (13). Other major ions that were present in the Speed River water were Al (138 μg/liter), Fe (262 μg/liter), Mg (8,000 μg/liter), Ca (61,000 μg/liter), and K (1,800 μg/liter). Silicon was present at a concentration of 3,000 μg/liter.

The partial (Fig. 1B) and complete (Fig. 1C and D) encrustation of bacterial cells by different forms of mineralization was observed. These mineral grains, which were found associated with epilithic bacteria on all rock substrata, exhibited a wide range of morphologies, from amorphous gel-like phases to crystalline structures. EDS analyses indicated that the most abundant mineral phase precipitated in association with the epilithic microorganisms was a complex (Fe, Al) silicate of variable composition. With the exception of potassium, no other metals were detected in the mineralization around the bacterial cells. A ternary plot of Fe, Si, and Al (on an atomic percent basis), with the position of various ideal clay minerals labeled (Fig. 3) shows that the gel-like phases were initially similar in composition to a chamositic clay [$(Fe_5Al)(Si_3Al)O_{10}(OH)_8$], whereas the crystalline structures were more siliceous and had compositions between those of kaolinite [$Al_4(Si_4O_{10})(OH)_4$] and glauconite [$K(Al_{0.38}Fe_{1.28}Mg_{0.34})(Si_{3.7}Al_{0.3})O_{10}(OH)_2$]. Intermediate compositions, corresponding to the transition from a low-order state to a high-order state, were also recognized. Selected area electron diffraction patterns generated on the samples with good crystallinity indicated a hexagonal crystal habit (i.e., normal to *c* axis). The few diffractions obtained, however, do not correspond to any ideal clay mineralogy.

Although the surfaces of the biofilm exposed to the water column were expected to trap detrital grains in the encompassing exopolymer, the crystalline phases described in this study appear to be strictly of authigenic origin, because they differ mineralogically from the suspended sediment of the Speed River, which is composed of quartz, feldspars, calcite, dolomite, siderite, micas, smectite, chlorite, and lithic fragments of variable provenance. Also, while metallic ions and other dissolved species are capable of diffusing into the biofilms and reacting to precipitate minerals around the cell, it seems unlikely that penetration of detrital grains would be equally as efficient.

DISCUSSION

The role of bacteria in the formation of authigenic minerals involves a complex interaction between metals in solution and the reactive components of the cell. For metallic ions, the anionically charged cell wall and extracellular polymers (capsule and sheath) provide special microenvironments for the deposition of iron and other soluble cationic species. Ferric iron, which exhibits unstable aqueous chemical phases, was bound in significant amounts; previous studies indicate that this may be sufficient to induce hydrolytic transformations to the more stable insoluble hydroxide form [e.g., ferrihydrite ($Fe_2O_3 \cdot 9H_2O$)] (14).

From our present study, we believe that, through progressive mineralization, the bound iron subsequently served as nucleation sites for the precipitation and growth of more complex authigenic mineral phases. In this context, the (Fe, Al) silicates may have been directly precipitated when dissolved silicon and aluminum, as well as trace amounts of potassium, reacted with cellularly bound iron, as might be expected given the large surface area and high chemical reactivity of ferrihydrite (8). Moreover, hydroxyl groups or hydronium ions bonded to only one Fe, Si, or Al atom are abundant and exhibit a high adsorptive affinity for additional metals (28). Eventually, continued aggregation of these hydrous precursors resulted in the formation of low-order, gel-like phases, with a chemical composition similar to that of a chamositic clay. These amorphous phases have previously been observed in metal-contaminated lake sediment (11) and in the surface waters of Amazonian rivers (19), implying that this form of mineralization may be widespread.

Because they lack a regular crystal structure, these hydrous

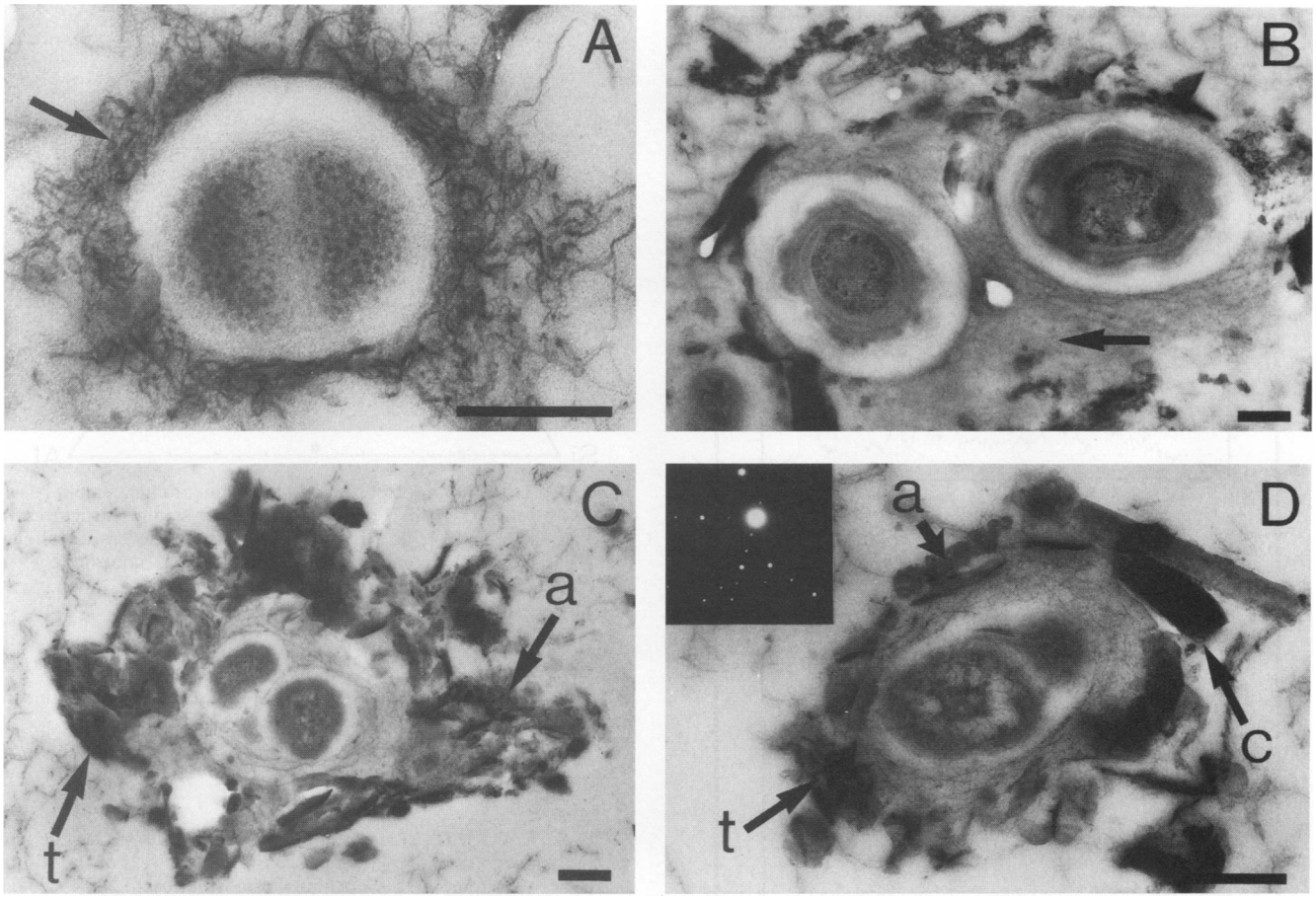

FIG. 1. TEM of epilithic bacteria (stained with uranyl acetate and lead citrate) from the Speed River. (A) Bacterial cell (from limestone) with iron-rich capsule (arrow). (B) Partially encrusted bacterial cells (from sandstone) with an iron-rich capsule (arrow). (C) Completely encrusted cells (from granite) with a poorly ordered gel-like phase (a) and transition phase (t). The latter is distinguished from the gel-like phase by having denser aggregates with definite shape present within an encompassing amorphous (highly hydrated) gel. These aggregates correspond to precipitates with a greater degree of crystallization due to loss of water. (D) Completely encrusted cell (from concrete) with a poorly ordered gel-like phase (a), transition phase (t), and crystalline phase (c) with selected area electron diffraction pattern. Scale bars = 300 nm.

compounds are probably unstable and will, over time, dehydrate, converting to more stable crystalline forms. In our study area, the hydrous chamosite-like clay appeared especially reactive to silicic acid (H_4SiO_4). Continued adsorption of silicon, through hydrogen bonding of the hydroxyl groups in the bound cations with the hydroxyl groups in the soluble silica (26), seems to have accompanied the solid-state transformation from the low-order chamosite-like phase to the crystalline phase (22). Eventually this process led to the complete encrustation of the same bacterial cells within a Si-rich mineralized matrix. In our opinion, these compositions are those of single mineral phases, rather than intimate mixtures; however, further investigation is required to verify this point of view.

As the final stages of mineral formation appear to be inorganically driven, the preferential accumulation of silicon may reflect the higher concentration of dissolved silicon to dissolved iron (3,000 μg of Si per liter compared to 262 μg of Fe per liter), as well as the different behavior of these two elements in the aqueous system. For example, dissolved iron readily adsorbs to anionically charged organic material (1), including the microorganisms, whereas dissolved silicon (in the form of silicic acid) has a large negative surface charge (23), which makes the silica highly reactive to metal hydroxides (30), similar to those bound on the cells. Under appropriate conditions when metal hydroxide sites are available, clay authigenesis may preferentially reduce dissolved silicon to extremely low levels (30). In addition, water analysis of the Speed River indicates that the concentration of dissolved silica is below quartz saturation (6 ppm at 25°C [16]) and, therefore, at the low end for clay formation in terms of silica concentration (25). This suggests that other factors, such as redox and pH conditions proximal to the bacteria, could be influencing the authigenic mineral reactions.

We were surprised to discover that all epilithic bacterial populations examined in the Speed River biomineralized in the same manner. Although the rock substrata differed extensively from granites (composed of crystalline quartz and feldspar), sandstone (cemented quartz and feldspar grains), limestone (calcite and/or dolomite), and concrete (sand, gravel, and crushed rock as the aggregate, with limestone and shale as the cement), the epilithic bacteria consistently formed (Fe, Al) silicates. Although acid-producing microorganisms may partly solubilize their rock substratum, it seems improbable that a carbonate-bearing rock such as limestone could serve as a source of Fe, Al, and Si for the bacterial populations. Clearly,

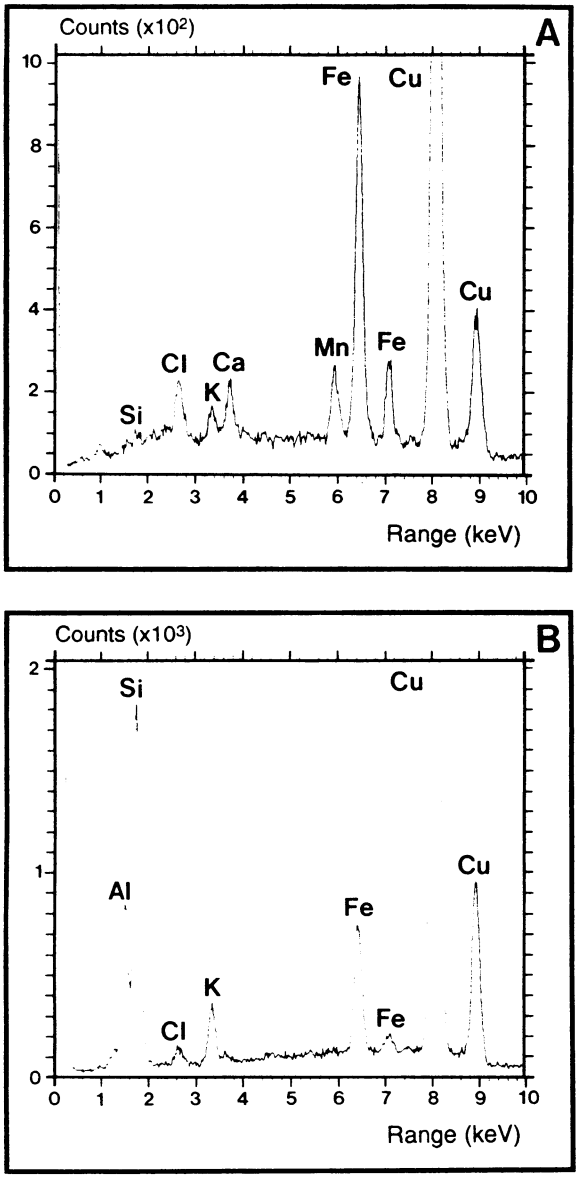

FIG. 2. EDS spectra of (A) mineralized capsule and (B) crystalline (Al, Fe) silicates precipitated by epilithic bacteria. Cu peaks are from the supporting grid; the Cl peak is due to contamination from epoxy resin.

rock lithology has limited influence on authigenic mineral formation, implying that biomineralization was more of a surface process, associated with the anionic nature of bacterial cell walls. It seems more likely that the adsorption of dissolved constituents (from the aqueous environment) contributed significantly to the mineral formation process. In this regard, epilithic microbial biofilms dominate the reactivity of the rock-water interface and influence the chemical composition of the minerals which ultimately form the riverbed sediment. Because rivers typically contain high concentrations of iron, silicon, and aluminum, these findings provide a unique insight into biogeochemical activities that are potentially widespread in natural waters.

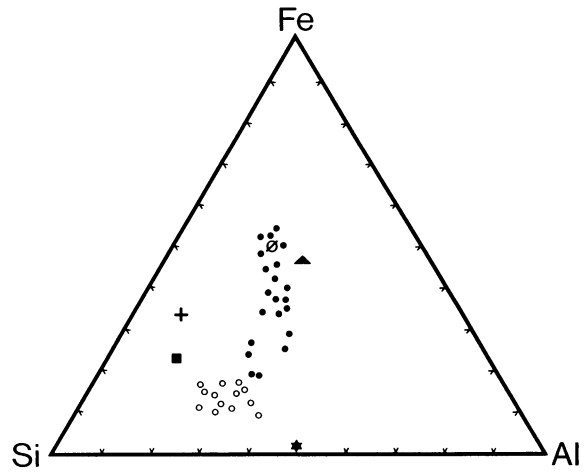

FIG. 3. Distribution of Fe, Al, and Si (on an atomic percent basis) in poorly ordered gel-like phases (●) and crystalline structures in epilithic bacteria (○). Fe, Al, and Si contents for several ideal clay minerals, including chamosite (ø), kaolinite (★), nontronite (+), glauconite (■), and berthierine (▲), are labeled.

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