Iron phosphate precipitation by epilithic microbial biofilms in Arctic Canada

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On Ellesmere Island, in the Canadian Arctic, dark-colored biofilms proliferate on moist surfaces, including exposed grano-diorite outcrops. Transmission electron microscopy of these biofilms indicates that complex epilithic microbial communities developed, consisting of cyanobacteria and fungi symbiotically associated in a lichen, along with a consortium of free-living algae and gram-negative bacteria. The epilithic cyanobacteria and bacteria were shown to extensively precipitate phosphatic minerals, ranging from relatively large polyphosphate granules (approximately 250 nm in diameter) within their cytoplasmic membranes to smaller iron phosphate grains (generally less than 50 nm in diameter) associated with the periplasmic space and encompassing capsule. Complete encrustation of some bacterial cells by the iron phosphates was observed. Energy-dispersive X-ray spectroscopy suggested that these grains are compositionally similar to the mineral strengite ($FePO_4 \cdot 2H_2O$). This study clearly indicates that the Arctic supports a thriving microbial community that influences the biogeochemical cycling of PO_4 in an environment of low nutrient availability. Nutritional requirements by the microorganisms were actively maintained through a relatively closed recycling mechanism, which restricted the immediate loss of phosphorus from the biofilm.

Sur l'île d'Ellesmere, dans l'Arctique canadien, des biofilms de couleur foncée prolifèrent sur les surfaces humides, inclu-ant les affleurements de granodiorite. L'étude de ces biofilms au microscope électronique à transmission révèle que des com-munautés microbiennes épilithiques complexes se sont développées, constituées de cyanobactéries et de champignons vivant en symbiose dans les lichens, avec un consortium d'algues autonomes et de bactéries non colorées par le réactif de Gram. L'étude montre que les cyanobactéries et les bactéries épilithiques ont pour rôle de précipiter en quantité abondante des miné-raux phosphatés, allant de granules relativement gros (diamètre approximatif de 250 nm) de polyphosphates, localisés dans leurs membranes cytoplasmiques, à des grains plus petits (généralement de diamètre inférieur à 50 nm) de phosphate de fer associés à l'espace périplasmique et à la capsule enveloppante. Certaines cellules bactériennes sont complètement incrustées par des phosphates de fer. La spectroscopie des rayons-X à énergie dispersive révèle que la composition de ces grains est analogue à celle du minéral strengite ($FePO_4 \cdot 2H_2O$). Cette étude indique clairement qu'il existe dans l'Arctique une com-munauté microbienne vigoureuse, et qui influence le cycle de PO, dans un milieu pauvre en éléments nutritifs. Les besoins en éléments nutritifs de ces microorganismes sont satisfaits par un mécanisme de recyclage, relativement fermé, limitant les pertes du phophore des biofdms.

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Introduction

Over the past two decades, a great deal of information has been published on the activity of microorganisms in extreme polar surface environments (see Vincent 1988 for a review). Their uniqueness arises from their ability to grow in hostile environments that are often devoid of higher forms of life. In many parts of the Antarctic and Arctic, epilithic and endolithic microbial life-forms predominate, respectively, on or within rock outcrops. These sessile rnicrobial populations benefit from the high concentration of organics and inorganics that accumulate at such interfaces, as well as the large pool of nutrients available to the cells from the rock substrate. Although the microbial communities are often severely constrained by their environment, they are able to exert profound influences on their surroundings: the physical and chemical erosion of the exposed rock surfaces, the release of metallic ions through mineral dissolution, and the formation of neogenic weathering products (Jones et al. 1980; Jones et al. 1981; Friedmann 1982).

The chemical weathering of rock surfaces has been largely attributed to those strains of fungi that produce citric and (or) oxalic acid (Henderson and Duff 1963; Jones et al. 1981). In circumstances where there is contact between acid-producing microorganisms and their rock substratum, soluble metal com-plexes may form. Both common silicate (e.g., plagioclase) and ferromagnesian (e.g., olivine, augite) minerals are susceptible to dissolution by organic acids, and in some instances only siliceous relics remain after the depletion of aluminum, iron, and magnesium from the primary rock-forming minerals (Jones et al. 1981). Apart from weathering, lichens have also been shown to form poorly ordered authigenic mineral phases, such as ferrihydrite (Jones et al. 1980), and insoluble organic salts, most notably oxalates (Verrecchia 1990). On a larger scale, biologically mobilized iron in some Antarctic sandstones was reported to form iron-rich surface crusts on the exposed rocks (Friedmann 1982).

Most studies of microbial mineral weathering and biominer-alization in polar regions have been limited to the Antarctic. Therefore, our present work focused on determining whether microbial cornrnunities in the Arctic are involved in similar biogeochernical activities. We collected samples of epilithic biofilms (ubiquitously found in dense mats on moist surfaces, including several granodioritic outcrops) near the village of
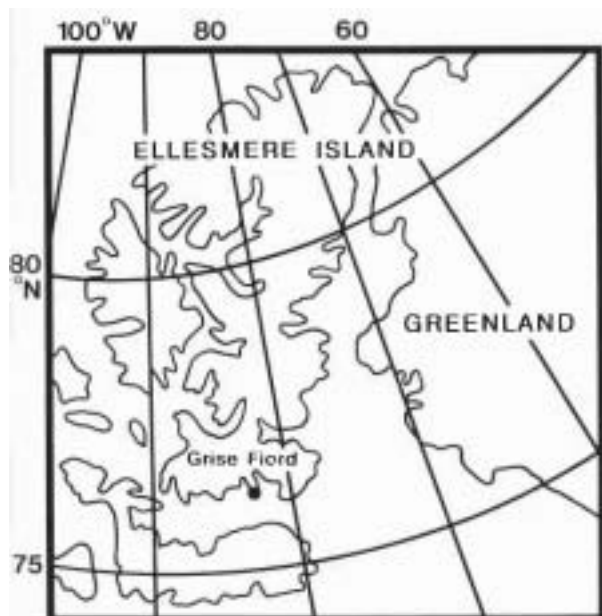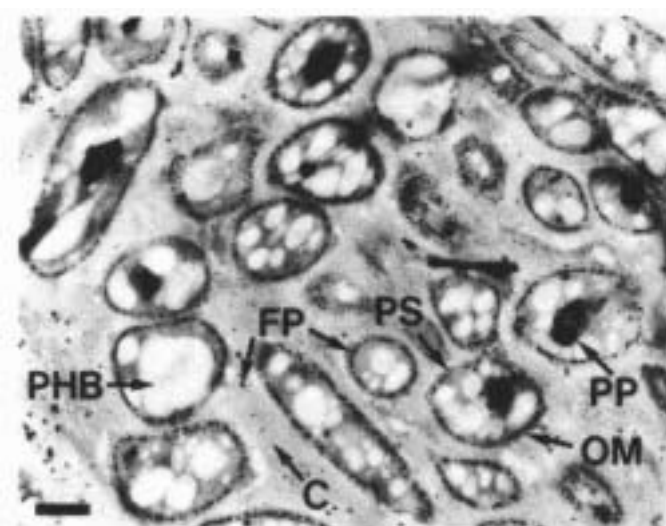
FIG. 1. Location of study area.

FIG. 2. Transmission electron micrograph of the epilithic bacterial community (stained with uranyl acetate and lead citrate) from the surface of a granodiorite outcrop. Arrows indicate the intracellular polyphosphate granules (PP) and the numerous iron phosphate grains (FP) that are associated with the periplasmic space (PS) and the encompassing capsule (C). On some cells the outer membrane (OM) is clearly visible, and the large electron-translucent granules within the bacterial cytoplasm are, presumably, polyhydroxy butyrate bodies (PHB). Scale bar = 500 nm.

Grise Fiord on Ellesmere Island, Canada (Fig. 1). These biofilms appear dark in hand sample, a characteristic that may be attributed to both the dark-colored nature of the rocky substratum, encrusted by the microorganisms (Weber 1977), and microbial pigmentation (Fletcher et al. 1985). The latter may be an environmental adaptation, since the black pigment (presumably scytonemin) may function as a photoprotectant against uv radiation (Vincent and Roy 1993), and is commonly found in high concentrations in some microbial (e.g., cyanobacteria) cornmunities that live in habitats exposed to direct sunlight (Turian 1985).

Methods

Sections of the biofilms (approximately 4.0 cm^2) were scraped off the hard substratum with a sterile scalpel and immediately placed in 5 mL metal-free plastic tubes containing aqueous 2.0% (vol./vol.) glutaraldehyde, a fixative for electron microscopy (Beveridge et al. 1993). The microorganisms were prepared for thin sectioning by washing in a solution of 0.05 M N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) buffer (Research Organics Inc., Cleveland), adjusted to pH 7.2 to remove excess glutaraldehyde. After washing, the sarnples were dehydrated through a graded acetone series and embedded in epoxy resin (Epon 812, CanEM, Guelph). Thin sections, approximately 60 nm, were obtained using a Reichert-Jung Ultracut E ultramicrotome, and subsequently mounted on Formvar and 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 (Beveridge et al. 1993). Grids were viewed with a Philips EM400T transmission electron microscope − scanning transmission electron microscope (TEM−STEM) equipped with a model LZ-5 light element detector and an exL multichannel analyzer (both from Link Analytical) operating at 100 keV, with a cold trap in place. Energy-dispersive X-ray spectroscopy (EDS) was conducted using an electron beam current of 0.1 μA and a spot size of 400 nm. Spectra were obtained by collecting counts for

100 s (live time). Crystalline mineral phases were determined using selected area electron diffraction (SAED) with a camera length of 800 mm. Amorphous phases were identified by EDS spot analyses run with the Link quantification software program to determine stoichiometric ratios.

Biofilms were prepared for epifluorescence microscopy, in the laboratory, by preparing wet mounts of small (−5 mm^2) sections of sample. These sections were viewed on a Zeiss Axioskop epifluorescence microscope equipped with a No. 20 filter block (excitation at 546 nm) to determine the presence of phycoerythrin-containing microorganisms, such as cyanobacteria.

The percentages of major elements (given as SiO_2, Al_2O_3, total Fe as Fe_2O_3, K_2O, CaO, MgO, P_2O_5, and Na_2O) in the rock samples were determined by X-ray fluorescence spectrometry (XRF) on a Philips PW-1450 automatic sequential spectrometer. Mineralogies were determined by X-ray diffraction (XRD) on a Rigaku rotating anode diffractometer (Co K_α). The scans were carried out at 160 kV and 45 mA, from 2−82" 2θ at a rate of 10°/min.

Results

TEM analyses indicated that a diverse microbial community developed on the exposed surfaces of the rock. The principal microorganisms included cyanobacteria and fungi symbiotically associated in a lichen (taxonomically undetermined), together with a consortium of free-living algae and gram-negative bacteria, encapsulated within an extensive matrix of extruded exopolymer.

The epilithic cyanobacteria and bacteria in the biofilms were highly mineralized, with precipitates ranging from relatively large polyphosphate granules (approximately 250 nm in diameter) within their cytoplasmic membranes to smaller iron phosphate grains (generally less than 50 nm in diameter) associated
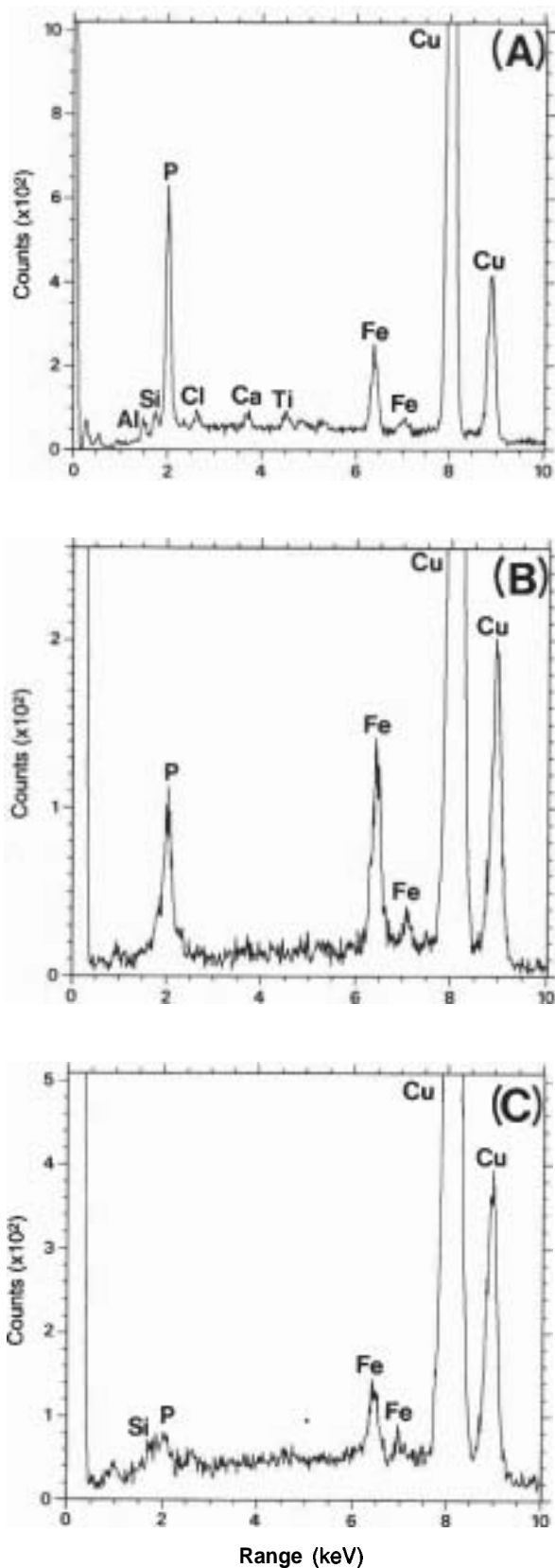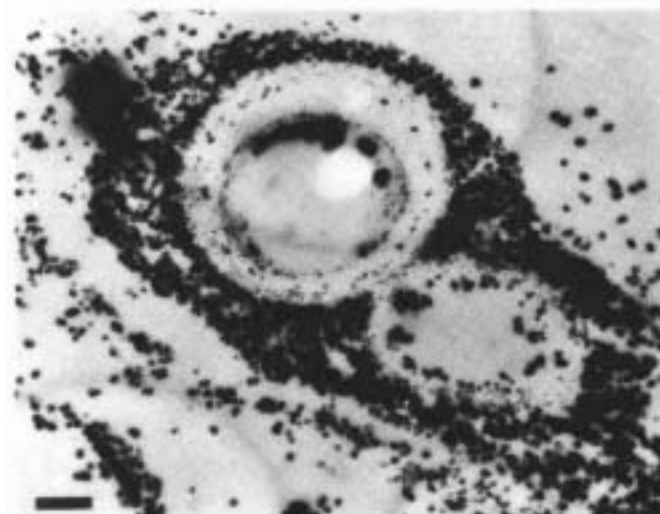
FIG. 4. Transmission electron micrograph of two bacteria (stained) completely encrusted by iron phosphate grains of chemical composition similar to that of the mineral strengite. Scale bar = **300 nm.**

with the periplasmic space and encompassing capsule (Fig. 2; see Beveridge 1989 for details about these bacterial structures). SAED analyses confirmed that the polyphosphate granules were typically amorphous in structure (as are most biologically produced phosphates, Brown 1982), and EDS analyses of these same intracellular deposits detected trace amounts of Fe, Si, Ca, Ti, and Al (Fig. 3A). In addition to the polyphosphate granules, numerous iron phosphate grains were found associated with the periplasmic space and the extracellular capsules of bacteria in the biofilms; some cells were completely encrusted by the mineral grains (Fig. 4). These authigenic mineral precipitates had an amorphous structure, as indicated by SAED (i.e., no diffraction patterns were discernible). In EDS spectra, these aggregates exhibited a Fe:P ratio compositionally similar to the mineral strengite ($FePO_4 \cdot 2H_2O$) (Fig. 3B). This correlates well with the expected stable solid phase predicted under the low pH conditions, created by the production of organic acids in the biofilms (Cole and Jackson 1950; Stumm and Morgan 1981).

Discussion

The formation of authigenic mineral phases has been proposed as a two-step sequence where, initially, the fixation of soluble metallic ions to bacteria arises through a stoichiometric interaction with the anionic surfaces of the cell wall (Beveridge and Murray 1980; Beveridge and Fyfe 1985). Constituent carboxyl and phosphoryl groups interact electrostatically with available cations, and are major sites for metal deposition (Beveridge and Murray 1980; Ferris and Beveridge 1984). In addition, microorganisms in biofilms form extracellular sheaths or capsules comprising acidic polysaccharides whose molecular components are similarly reactive and consequently accumulate metals around the cell (Ferris and Beveridge 1985). Once bound to the bacteria, the metals may then serve as nucleation sites for the formation and growth of authigenic mineral phases. The end result is a mineralized cellular matrix that contains detectable concentrations of metal ions (as fine-grained minerals) that are not easily solubilized.

FIG. 3. EDS spectra of (A) the polyphosphate granules within the cytoplasm, (B) the iron phosphate grains within the periplasmic space and encompassing capsule, and (C) the mineralized capsule. Cu peaks are from the supporting TEM grid and the Cl peak is due to contamination from the Epon.

For the epilithic Arctic bacteria, the reactive components of the cell clearly provided a unique environment for the deposition of iron and other soluble cationic metal species. This was confirmed by the strong iron signals detected by EDS of the capsular areas in which extensive phosphate deposition was not evident (Fig. 3C), and is consistent with other work that shows that biofilms are particularly potent scavengers of iron (Ferris et al. 1989). The abundance of adsorbed iron suggests mobilization of this metal from the rock substratum, a process that is brought about principally by organic acids secreted by the lichens (Jones et al. 1980).

Once solubilized, the metallic ions would be transported along the rock surface in a continuous liquid-phase water film (Ugolini and Grier 1969). Because freezing would concentrate the metals in the unfrozen fluid phase, the electrolytes are progressively pushed towards natural boundaries, such as rock faces, where they are brought into chemical contact with residing biological material. The biofilms consist not only of the microorganisms themselves but also their extracellular polymers, which increase the viscosity of the immediate fluid phase and should act as a natural antifreeze agent. Therefore, as the external aqueous phase freezes and as the metal ions are forced toward rock boundaries, it is most likely that these ions are concentrated and immobilized within the biofilm. In addition, the darkly pigmented cells are more effective in absorbing light, thereby increasing their microenvironmental temperature and concomitantly discouraging rapid freezing of the biofilm.

Results from metal uptake studies with isolated bacterial cell walls and natural biofilms indicate that the adsorption of iron from solution, by binding to cellular components, is often sufficient to induce a transformation to an insoluble hydroxide form ($Fe(OH)_3$) (Ferris et al. 1986). This is similar to the secondary weathering products formed by lichens growing on basalts (Jones et al. 1980). In 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 iron phosphates were probably precipitated when dissolved phosphate reacted with cellularly bound iron (Jacobsen 1978), as might be expected given the large surface area and high adsorptive affinity of amorphous iron hydroxide for large quantities of phosphate (Parfitt et al. 1975). Because the iron hydroxide surfaces are hydrous in structure, the resultant iron phosphates were typically amorphous. Examples of iron phosphate reactions are abundant in nature, with the availability of iron hydroxide surfaces significantly decreasing the concentrations of phosphate ions in oxidized lake and marine sediments (Jensen et al. 1992; Jensen and Thamdrup 1993).

A key question in this study is, What is the source of the abundant phosphate? Not only do abundant polyphosphate granules forrn, but there is an excess of the ions to chemically bind with the fixed Fe. Whole-rock analysis by XRF indicated that there was a minor amount of phosphorus within the granodiorites, chemically bound in the form of apatite (Table 1). Although apatite is generally an insoluble form of phosphorus at neutral to alkaline conditions (Stumm and Morgan 1981), acidic conditions (e.g., as created by microbial production of organic acids) will solubilize the mineral phase (Fenchel and Blackburn 1979). Therefore, through solubilization, the rock substratum can serve as a primary source of dissolved phosphate for the epilithic microbial communities.

TABLE 1. **Whole-rock analysis**

Oxide	Wt.%
SiO_2	67.85
Al_2O_3	15.31
Fe_2O_3	4.91
MgO	0.91
CaO	3.68
K_2O	2.24
P_2O_5	0.16
Na_2O	3.41

Aside from the rock substratum, another major source of phosphate may be acquired from the bacterial cells themselves. Bacteria posses two phosphate-rich lipid bilayers containing either phospholipid or lipopolysaccharides (Beveridge 1989), and it is possible that a proportion of the phosphate was derived from the bacterial membranes or their precursors. In addition, while phosphorus is an essential nutrient for microbial growth (Fenchel and Blackburn 1979), and bacteria are able to temporarily store this valuable element excessively (compared to their external environment) in the forrn of polyphosphate granules, especially if they are growing under nutrient limitation in the presence of PO_4 (Beveridge 1989), they are only a transient sink for P. When they die and are decomposed by heterotrophs, part of the previously fixed P might become assimilated by other microorganisms, part might eventually become particulate refractory organic P compounds, and the remainder may be released as dissolved inorganic P (Gachter and Meyer 1993). Some of this dissolved inorganic P may later re-precipitate as a mineral phase, such as the $FePO_4$ described in this study. Invariably, this phosphate may serve as a second pool from which the microorganisms can further draw upon to satisfy their nutritional requirements. Additional sources also include aerosol dust composed of clay minerals, sea salts, soot, and other combustion products (Tazaki et al. 1994), as well as guano deposits (Ugolini and Grier 1969).

The results from this study provide a unique insight into the biogeochemical cycling of PO_4 in the Arctic. Diverse microbial communities were shown capable of growing under the extreme climatic conditions and low nutrient availability of the polar environment by developing a relatively closed recycling mechanism that restricted the immediate loss of phosphorus from the biofilm. Nutritional requirements of the microorganisms were actively maintained through a complex process of phosphate solubilization from the granodiorite to the formation of more soluble authigenic phosphatic forms in the cytoplasm, periplasm, and extracellular capsules of the microorganisms. Therefore, in the Arctic, as in surface environments throughout the world, biofilms have a profound influence on their surroundings and play a highly active role in the global metal cycle.

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