

Plausible mechanisms for the boring on carbonates by microbial phototrophs

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Abstract

Photosynthetic microbes, particularly cyanobacteria, that bore into carbonates are ancient biological players in various geologic phenomena such as the destruction of biogenic carbonates and coastal limestones, the reworking of carbonate sands and the cementation of microbialites. Their signatures are important tools for paleoenvironmental reconstruction, and they play a significant role in marine aquaculture. In spite of their geologic, environmental and economic importance, the mechanism by which they are able to excavate calcareous and calcophosphatic mineral substrates remains unknown. Excavation by acidulation, commonly thought to be a possible mechanism, constitutes nothing less than an apparent paradox, in that the geochemical consequence of oxygenic photosynthesis should be carbonate precipitation, not dissolution. Three alternative mechanistic models are presented here that may allow cyanobacterial boring to proceed and be still consistent with available evidence, as well as microbiological and geologic/geochemical principles. They are based on either temporal or spatial separation of photosynthesis and respiration, and on the active extrusion of calcium ions through an active cellular uptake and transport process. From the three models, the latter is shown to be most appropriate in describing and explaining the boring phenomenon. Several experimental approaches are discussed that would be appropriate to elucidate the paradox.

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1. Introduction

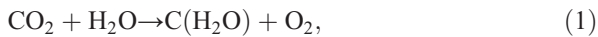
There can be little doubt that the relationship between living beings and carbonates is one of the most conspicuous interactions between the animate and the inanimate realms on the planet. Biological mediation of carbonate precipitation pervades our environment through many spatial scales and drives an important component of the geological carbon and metal cycles. We find it at the microscale in the form of subtle micritization in microbialites or as highly organized intracellular formation of beautifully sculpted coccoliths

in open ocean plankters. Mollusks and gastropods cast their shells, and corals relentlessly build massive reefs. A very diverse, evolutionarily divergent set of organisms is engaged in active carbonate precipitation, from bacteria, to algae and to animals. A record of fossil evidence, from Precambrian stromatolites to Holocene marine deposits, attests both to the continuity and to the antiquity of this relationship. It was indeed this very fossil evidence that brought about the birth of Sedimentology as a science in nineteenth century England.

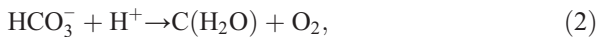
The sedimentary side of the formation/dissolution equation has received most attention by geobiologists, and much progress has been made in understanding the various environmental and cellular mechanisms

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involved in the process. Examples are known for microbially mediated precipitation of a variety of metal carbonates from carbonate and metal ions in solution. The simplest form of biotic carbonate precipitation involves the formation of microenvironments around small cells or organisms due to their metabolic activity. In these microenvironments, the concentrations of chemical species depart from those in the bulk phase in such a way that they can promote the local precipitation of carbonate. Calcification by photosynthetic cyanobacterial communities constitutes an archetypical example of this kind of indirect mechanism (Golubic et al., 2000; Merz-Preiß, 2000; Pentecost and Whitton, 2000; Arp et al., 2001). Oxygenic photosynthesis, which can be chemically abstracted for geochemical purposes as,



or, at neutral or slightly alkaline pH, as,



effectively consumes protons. This can in turn drive the thermodynamic equilibrium for metal carbonate dissolution–precipitation,



towards the solid phase. The free energy of dissolution is given by

$$\Delta G = RT \ln(\text{IAP}/K_{s0}),$$

where IAP is the ion activity product (Stumm and Morgan, 1996). If $\text{IAP} > K_{s0}$, the solution is said to be super-saturated with respect to the appropriate mineral phase and precipitation will be thermodynamically favored. Dissolution will be favorable when $\text{IAP} < K_{s0}$. Photosynthetic organisms may actually derive physiological benefits from concurrent photosynthesis and calcification (McConnaughey and Whelan, 1997). In fact, any other biological metabolism that consumes protons will have similar consequences, as is the case in sulfate reduction (Visscher et al., 2000; van Lith et al., 2003). More biologically controlled carbonate precipitation is attained by some cyanobacteria, where precise amounts of calcite crystals are used as ballast to counteract upwelling currents (Garcia-Pichel et al., 2002). The mechanism of coccolith formation in eukaryotic plankters, is biologically directed (i.e. genetically controlled) and very refined. It involves ionic transport into specific intracellular organelles, the generation of high intra-organelle supersaturation levels, and a regulation of the process through kinetic inhibition

and promotion of nucleation by polymeric templates (de Vrind-de Jong and de Vrind, 1997).

But organisms are also active at the erosional side of the cycle. Grazing on surface biofilms by hard-toothed higher animals and invertebrates (Shachak et al., 1987), as well as by the growth of chasmolithic and cryptoendolithic (Friedmann and Weed, 1987) microbes can result in significant physical erosion of limestones. Some organisms will tend to dissolve carbonates chemically (Ehrlich, 1996) by virtue of their metabolic activity. Aerobic heterotrophic bacteria, fermenting, sulfide-oxidizing and nitrifying bacteria, can dissolve acid-labile minerals due to the production of acid as by-product of metabolism (carbonic, organic, sulfuric and nitric acids, respectively). In the case of aerobic heterotrophs, it is possible to switch their naturally acidulant metabolism by supplying preferentially organic sources that yield alkali upon catabolic processing, such as amino acid rich protein hydrolysates; they will then tend to precipitate carbonates. In a more directed mode of dissolution, higher organisms, such as sponges (Hatch, 1980) and polychaetes (Haigler, 1969) are also known to actively bore into carbonate substrates, in a process of directed excavation. But by far the most common, widespread and environmentally significant of carbonate borers are microorganisms: fungi (Burford et al., 2003), microalgae and cyanobacteria (Golubic, 1969) that actively dissolve carbonate substrates, excavating microscopic galleries as they grow within them (Fig. 1). While the boring mechanism of any of these organisms remains unknown, the production of acid equivalents has often been suggested (Haigler, 1969; Golubic et al., 1984).

Thus, it should be evident that a synthetic understanding of the mechanisms that underlie the interactions between carbonates and living organisms has the potential to benefit interpretive models in a range of biological, environmental and geological disciplines. But to achieve such mechanistic understanding requires necessarily an interdisciplinary approach that draws from, and is consistent with, basic mineralogical, geochemical, and biological principles. It requires the experimental testing of such models under controlled, simplified conditions in the laboratory. It also requires that the models can be scaled up to explain a wealth of geological and biological observations in nature.

2. Diversity and relevance of carbonate-boring microorganisms

Carbonate microborers are found among filamentous or pseudofilamentous forms of both eukaryotes (fungi,

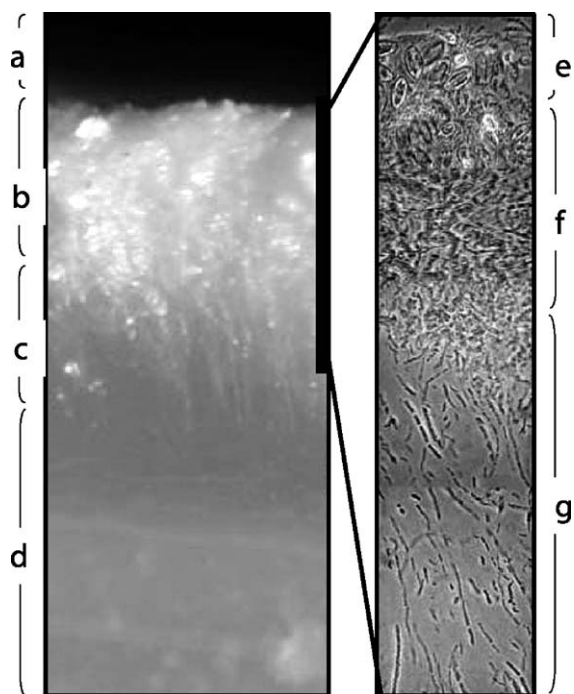


Fig. 1. Cyanobacterial communities boring on a seashell. Left: a macrophotographic view of a seashell from Puerto Peñasco (Sonora, Mexico) freshly broken in a plane orthogonal to the surface. Changes in refractive index caused by extensive infestation and bioerosion by boring cyanobacteria are evidenced as a highly reflective upper layer (b), optically very different from the macroscopically smooth carbonate. (d) In the transition zone (c), single vertical boreholes are visible. Right: photomicrograph (phase contrast) of the cyanobacterial community after fast chemical decalcification with EDTA (Wade and Garcia-Pichel, 2003), where the phototrophic microbes are visible. A biofilm with cyanobacteria and diatoms (e) colonizes the degraded surface; below it (f) actively boring large-celled cyanobacteria (*Hyella* spp and others) constitute the bulk of the euendoliths, and at the boring front (g) filamentous forms (*Plectonema terebrans*) initiate the bioerosion process. The depth of the photomicrograph is 500 μm .

green and red algae) and prokaryotes (cyanobacteria). They are abundant in both marine and freshwaters, and excavate calcareous and calcophosphatic substrates, including bone, shells, skeletal carbonate, limestones and dolostones (Campbell, 1983). Since their discovery (Bornet and Flahault, 1889), a robust database of microborer taxa and of their ecological distribution has been gathered through decades of descriptive work (Golubic, 1969; Perkins and Tsentas, 1976; May and Perkins, 1979; Budd and Perkins, 1980; Le Campion-Alsumard et al., 1995; Ghirardelli, 2002). In spite of the well-documented shortcomings of using merely morphological taxonomy (see Chacón et al, 2006-this volume), it is clear that cyanobacteria tend to dominate intertidal and shallow-water carbonate-boring assemblages, and that red and green algae are typically more

prominent in deeper euphotic waters, whereas fungi tend to be independent of light exposure. Euendoliths, as they are also called, and their microborings, fossilize well and their record is abundant. Fossil, bona fide cyanobacterial euendoliths have been found in stromatolites of the Mesoproterozoic (Zhang and Golubic, 1987) some 1500 Myr old. By the time of the Neoproterozoic ca. 700–800 Myr ago, entire assemblages of euendoliths existed penetrating silicified ooids (Knoll et al., 1986; Knoll et al., 1989), their traces of infilled microborings in ooids, being almost as ancient: 570–700 Myr (Campbell, 1982). Analysis of trace-fossil assemblages is now commonly used to reconstruct paleobathymetry (Vogel et al., 1987; Glaub, 1999; Vogel et al., 2000) and has been used as a paleoindicator of coral growth (Elias and Lee, 1993).

In temperate to tropical shallow marine waters, submerged carbonates are readily and heavily colonized with steady states reaching as much as 50% of the exposed solid surface (Fig. 2), and with full colonization times in the order of months (Gektidis, 1999). In the case of photosynthetic microborers, complex communities with several different species may develop, excavating towards the interior, which are limited in

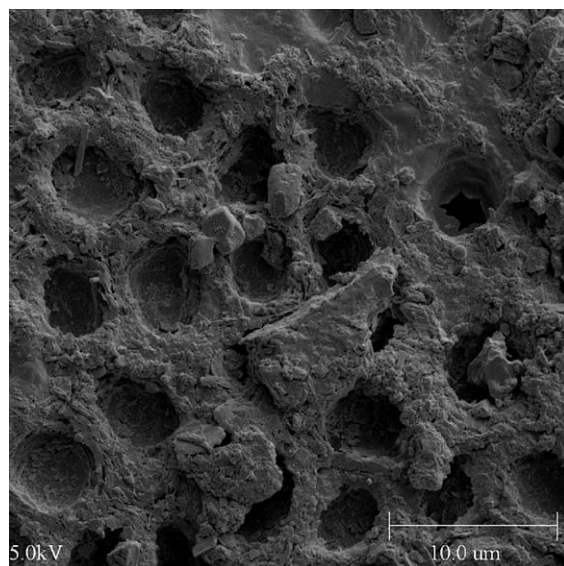


Fig. 2. SEM image of an infested shell, fractured parallel to the surface plane and gold coated, showing the high population density of microborers, and the tight fit between cyanobacterial trichomes and boreholes. In the sample organic components were not removed, and a multitude of tightly packed boreholes is visible. Each hole is filled with a trichome, from which only the interior part of the last cell's wall is visible, so that the tunnels appear to end in the trichome cross-walls, unless it has broken during preparation (as in the upper, right-hand cell).

their extent presumably by the depth of light penetration into the typically diaphanous substratum (Fig. 1). For most geologically relevant mineral substrates (limestones, biogenic carbonates) light attenuation is due to a combination of direct absorption by impurities and multiple scattering due to their microcrystalline habit, and results in effective photic zones in the order of millimeters, just like in many loose sedimentary substrates such as sands or soils (Garcia-Pichel and Bebout, 1995). Once the light compensation depth for photosynthesis is reached, boring either stops or proceeds parallel to the surface. The continued bioeroding effect of these microorganisms, thus requires the sustained abrasion of the weakened surface.

Several long-term geological phenomena have been attributed to the action of microborers. The erosive morphogenesis of coastal limestones (Purdy and Kornicker, 1958; Schneider and Toruski, 1983; Trudgill, 1987) is one such phenomenon. A second one is the destruction of coral reefs and other biological carbonates (Le Campion-Alsumard et al., 1995; Ghirardelli, 2002). Agassiz himself, reportedly (Duerden, 1902) wrote that the evidence available was "...enough to satisfy [himself and others] that boring algae played a prominent part in the disintegrating effect of corals...", and that he had "...not failed to notice the existence of these parasite vegetable organisms in the coral reefs [he had] visited". Yet a third geological consequence is the destruction of sedimentary particles, which occurs through a process of micritization of ooidal carbonate sand grains (grain obliteration) by the pervasive and repetitive cycles of boring and re-precipitation within boreholes (Al-Thukair et al., 1994; Golubic et al., 2000). Finally, a recently observed additional effect of microbial euendoliths is the cementation of loosely bound carbonate grains in coastal stromatolites by a process of fusion as carbonate precipitates around boring cyanobacteria crossing between grains (MacIntyre et al., 2000). Several studies (Tudhope and Risk, 1985; Hoskin et al., 1986; Peyrot-Clausade et al., 1995; Vogel et al., 2000) have attempted to gauge the long-term rates of microborer carbonate dissolution, the so-called "bioerosion" process. Using exposure of artificial substrates in natural settings, and in spite of the diversity in locality and substrate, erosion rates in those studies were found consistently high, ranging between 20 and 570 g CaCO₃ m⁻² yr⁻¹. Such rates are more than sufficient to account for the geological phenomena attributed to the action of microbial boring in the long term. At such rates, unimpeded microborer attack will promote recess of porous limestones, with dry density around 2100 kg m⁻³, by meters per century, or remove the equivalent

depth of fine coral sand. If the bored surface is subjected to grazing, the combined biological effect of borer and grazer can enhance bioerosion rates by more than an order of magnitude to tens of kilograms per square meter per year (Pari et al., 1998). There is perhaps no more convincing account of the environmental relevance of microborers than one expressed in morbidity terms: more than 50% of individuals of the intertidal South African mussel *Perna perna* typically show signs of mortality due to attack by phototrophic euendoliths (Kaehler and McQuaid, 1999).

3. Boring cyanobacteria: an apparent geomicrobial paradox

Surprisingly, and for all of the microborer's relevance as proven agents of geological transformations, as ecological keystone species, as well as for their value as paleoenvironmental indicators, the mechanism that enables directed carbonate dissolution remains to be understood in any detail. In fact, no single study has attempted to investigate this phenomenon under controlled laboratory conditions using cultivated microbes and well-characterized substrates. Using natural populations of cyanobacteria (*Hormatonema*, *Hyella*) boring on Iceland Spar coupons, Golubic (Golubic, 1969) could demonstrate that the tunnel direction is determined by the planes of crystal cleavage and twinning. In such highly crystalline substrates, the tunnel then resembles a sequence of negative microcrystals (Fig. 3). This is consistent with the presence of a focused

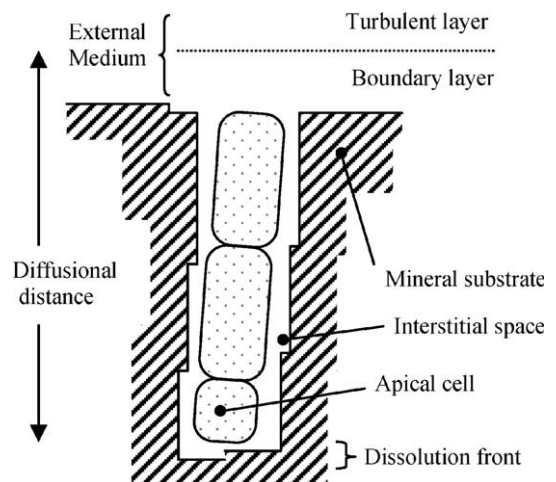


Fig. 3. Abstracted cross-section of a multi-cellular microborer/substrate system, depicting physically and biologically important components.

dissolution front around the apical cell. Early electron microscopic analyses of the microtopography of *Hormatonema* boreholes in natural limestone (Alexanderson, 1975) indicated that the process is not a straightforward extracellular dissolution at the organismal scale. The presence of extracellular organelles postulated by Alexanderson to explain the characteristic pinnacled etching patterns found on the borehole walls, however, seems now unfounded in view of a lack of corroborative biological evidence in decades of ensuing studies. The simplest mechanistic explanation, that of localized excretion of acids (Schneider and Le Campion-Alsumard, 1999), is certainly not geochemically attractive for the case of cyanobacteria and microalgae. As seen in Eqs. (2) and (3), the unavoidable consequence of their autotrophic metabolism is the alkalinization of the medium, with the most common indirect effect being carbonate precipitation, not dissolution. The mere excretion of small-molecular-weight organic acids (products of carbon fixation), as opposed to a direct extrusion of protons, is metabolically unsustainable. This is because the maximal number of carboxyl moieties in such an acid (1 in formic, 2 in oxalic, 3 in citric...) corresponds stoichiometrically to the moles of CO₂ taken up from the medium and, thus, to the protons already consumed. The same holds true for the alleged involvement of heterotrophic bacteria hosted within the borehole that would respire organic excretion products from cyanobacteria; stoichiometrically, there will be less acid produced than protons consumed in the overall (phototroph+heterotroph) process, since the transfer of carbon between organisms cannot be 100% efficient. In addition to these metabolic considerations, two independent, if circumstantial, pieces of evidence

speak against the acid-dissolution model. First, in the light of recent studies on dissolution kinetics of carbonates (Fredd and Fogler, 1998), Alexanderson's (1975) observation of pinnacled etching patterns in the borehole walls of microcrystalline substrates gains a new light, and is more consistent with dissolution occurring under neutral to alkaline pH, and not acidic pH (Fig. 4). Second, the fact that calcophosphatic substrates such as bone and, particularly, dentine, which are much more stable to acid attack than carbonates, are also readily colonized by boring cyanobacteria (Davis, 1997), has yet to be contended with.

Thus, the fact that some cyanobacterial species do concurrently photosynthesize and dissolve their calcareous surroundings represents in this regard nothing less than an apparent paradox.

4. Plausible mechanisms for cyanobacterial boring

Obviously, models other than the excretion of acids need to be brought forward that offer a plausible alternative, while being consistent with available observations, physiological and geochemical principles. The nature of any such mechanism is constrained by the following necessary conditions:

- i) The dissolution process, under many of the geochemical conditions in which it has been described, occurs in waters saturated or supersaturated with respect to calcite and aragonite, and is thermodynamically unfavorable. Excavation must be thus at the cost of cellular energy. The need for an energy input is also evidenced by the rapid infestation of relatively insoluble biogenic fluorapatite substrates. For the organism, this

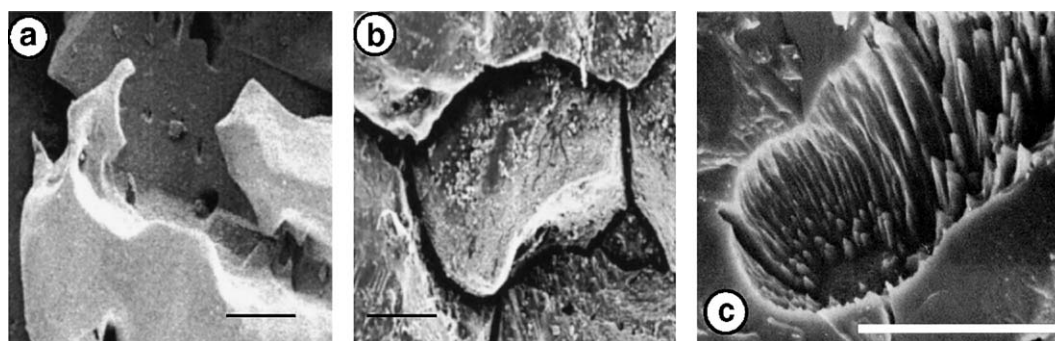


Fig. 4. SEM views of the surface of calcite etched by chemical dissolution at pH 4 (a), and pH 12 (b) in the presence of a calcium chelator (EDTA) according to Fredd and Fogler (1998), and the etched interior of a cyanobacterial borehole on calcite (c) according to Alexanderson (1975). Scales: 2 μ m (a, b) and 10 μ m (c). The pinnacled biological etching patterns are similar to those obtained by chelator dissolution, and unlike those obtained at high proton concentration. Photographs modified from their original, with permission.

expenditure may take the form of ATP usage, proton motive force generation, or excretion of organic products.

- ii) The carbonate-dissolving mechanisms must be localized, and spatially restricted at the leading end of the cyanobacterial filament or apical cell, since no “pits” are produced but true tunnels, typically with the “negative” shape of the borer's morphology.
- iii) The mechanism must allow for the conservation of mass and electrical charge, either through active transport or passive diffusion.

According to Le-Châtelier's equilibrium, one can dissolve carbonate by increasing acidity, by pulling on the carbonate ions or by pulling on the metal ions (see Eq. (3)). While the latter has not really been considered, it is as real a possibility as dissolution by acidulation. Additionally, it is in principle possible to change environmental pressure and temperature conditions so that the solubility of carbonate may be affected, but the variations needed to affect the process significantly are well beyond the capabilities of microbial metabolism. We advance three theoretically possible physiological mechanisms that could enable cyanobacteria to excavate carbonates, while circumventing limitations imposed by their metabolism. These are listed and explained below, in an order of apparent complexity.

4.1. Mechanism 1: temporal separation of photosynthetic and boring activities during the daily cycle

Here, boring activity would simply be relegated to the nighttime (typically some 8–12 h), when cyanobacteria turn to the oxidation (or fermentation) of intracellular glycogen accumulated during the daytime (Garcia-Pichel, 2000). In this scenario, release of products of heterotrophic metabolism, namely CO₂ and/or organic acids (mainly formic and lactic) would promote carbonate dissolution in the same manner proposed for most acid-producing microorganisms, the open space being occupied immediately by the fast expansion of apical cells. This model requires necessarily an internal cell-to-cell transport of carbon so as to concentrate the extrusion of acid equivalents around the apical cell and promote the formation of a tunnel, as opposed to a pit. The microbiological principles behind this mechanism are not without parallel, since such temporal separation of mutually exclusive metabolic activities has been demonstrated for *Oscillatoria* and other cyanobacteria in the case of nitrogen fixation (Stal and Krumbein, 1987; Berman-Frank et al., 2001) many

cyanobacterial physiological processes are regulated by internal molecular clocks (Golden and Canales, 2003), and transcellular organic carbon transport mechanisms are known to exist in filamentous heterocystous cyanobacteria (Haselkorn, 1978). Chemically, it also requires the passive transport by diffusion of carbonate and calcium ions away from the dissolution front, which due to their membrane-impermeability, must occur through the interstitial, extracellular space. This mass-transport requirement imposes a time constraint on the effectiveness of such phototrophic/heterotrophic transitions as an excavating mechanism. The mean square diffusional time is expressed by $t=x^2/2D$, where x is the distance, and D the diffusion coefficient (Crank, 1975). If D is around $2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, as is typical for small molecules, and $x=500\text{--}1000 \text{ }\mu\text{m}$ (the sum of typical length of boreholes plus the thickness of a typical benthic boundary layer through which ions need to cross to reach the bulk phase), then the associated mean diffusional times for small molecules in the microborer/substrate/medium system are in the order of minutes; this represents the minimal time frame needed for any switch between heterotrophic and phototrophic metabolism to become efficient in excavation in that charge equilibration and the relaxation of ionic concentration gradient would be allowed. Evidently a switch following daily patterns will accommodate it. However, the temporal separation mechanism has some shortcomings. It makes the boring activity dependent of the length of the night period, a prediction for which there is no observations in Nature, and it also does not satisfactorily explain boring on calcophosphatic substrates.

4.2. Mechanism 2: spatial separation of photosynthetic and boring activity

In this scenario the photosynthetic activity of cyanobacteria should be restricted to the cells closer to the opening of the tunnel, while the distal cells would be mostly respiring. This would naturally create acidity at the interstitial space of the leading end of the tunnel and promote localized dissolution. Such mechanism would also require a net intracellular transport of organic carbon down the filament, to sustain the respiratory activity and growth of the apical cells. Diffusion of carbonate and calcium up the interstitial space in the tunnel could in principle account for long-term mass balance. Spatial separation of mutually exclusive photosynthesis (in normal, vegetative cells) and nitrogen fixation (in specialized heterocysts) is a well-known adaptation in some cyanobacteria, and it is not rare for apical cells to display special adaptations not found in

the rest of the trichome (Garcia-Pichel, 2000). This model presents some apparent microbiological and geochemical complications. It makes the initial stages of boring problematic, for example, since in a short filament or single cell there may not be enough spatial coverage to create an efficient separation. Additionally, partial or total loss of photosynthetic activity during cell differentiation is typically accompanied by loss of unnecessary pigmentation, as seen for example in the yellowing of heterocysts, and this has not been reported in any boring cyanobacterium. Further inconsistencies may be related to mass transfer from the dissolution front towards the outside, when carbonate and calcium ions would have to cross areas of the interstitial space that are flanked by actively photosynthesizing cells, and that are thus probably already locally highly supersaturated with respect to calcite (Eqs. (2) and (3)). This may create problems by promoting precipitation there, particularly due to the effect of calcium. On the other hand, it is a possibility that incoming bicarbonate may act as a source of inorganic carbon for the cells there, and by maintaining a concentration gradient between terminal and distal areas of the interstitial space, in fact increase the efficiency of mass export. A simple comparison of the relative concentration of organic C in cells vs. inorganic C in the carbonate substrate, demonstrates that this effect would not suffice. Cyanobacteria have a buoyant density of some 1.008 g/ml, of which 80% is water and 20% dry mass. Of the latter, about 1/2 is attributable to C, so that, approximately, the volume occupied by cells in a borehole contains 0.10 g C/ml. The carbonate substrate may have densities between 2.1 and 2.7 g/ml, of which, for a calcium carbonate, 12% is C mass, or 0.25 to 0.31 g C/ml. Thus, in substituting a certain volume of substrate with cyanobacterial volume we still have an excess of 0.1–0.2 g of C that needs to be exported. In any event, while C uptake may alleviate a potential re-precipitation problem, and bicarbonate would not be a major ionic species in apatite-like substrates, I regard these geochemical problems as a weak point in the spatial separation model.

4.3. Mechanism 3: the calcium pump

An alternative mechanism may be based on active transport of Ca^{2+} through the cyanobacterial filament so that low concentrations of free Ca^{2+} in the interstitial space at the end of the tunnel are created, thus locally decreasing IAP below levels that would make dissolution thermodynamically favorable. In this scenario the released carbonate ions could be (partly) taken up as

bicarbonate and used for photosynthesis and partly would diffuse outwards. This mechanism would require necessarily the presence of Ca^{2+} uptake mechanisms across the apical cell membrane, transport between cells, and export at the trailing end, so that a net mass transport away from the tunnel can occur efficiently. Knowledge about calcium homeostasis must be taken into account in building functional models invoking long-range calcium transport. In cyanobacteria, as in all organisms, intracellular calcium, $\text{Ca}^{2+}(\text{i})$, is tightly regulated at the cost of energy through independent processes of calcium uptake and efflux (Smith and Wilkins, 1988; Pandey et al., 1999). The normal levels of $\text{Ca}^{2+}(\text{i})$ are maintained very low, 0.1–0.2 μM , to prevent toxicity to the cell metabolism, but transient levels may rise to 5 μM in cyanobacteria (Torrecilla et al., 2001). If external Ca^{2+} concentrations are higher than that, as is typically the case, calcium uptake may involve low passive permeability and/or Ca^{2+} -sensitive trans-membrane channels. Efflux (typically against a concentration gradient) can be mediated by $\text{Ca}^{2+}/\text{H}^{+}$ antiporters or by Ca^{2+} -ATPases, powered by proton motive force of energized membranes or by intracellular coupling with ATP hydrolysis, respectively. Thus, evidence exists for the presence of the building blocks needed for the calcium pump mechanism. A functional mechanism would require their disjunct, polarized localization at opposite ends of the cyanobacterial filament. The calcium pump mechanism seems to me the most attractive of all models, since it would allow dissolution to proceed at high interstitial pH. In fact, this is really the only model that is consistent with the coincidence in microetching evidence (see Fig. 4) found by Alexander (1975) and by Fredd and Vogler (1998), which points to a dissolution mode by cation removal (as occurs with addition of chelators at high pH). It is also consistent with the known range of bored substrates, which share only Ca^{2+} as the common denominator (as, for example, in hydroxylapatite and calcite). It also alleviates the problem of re-precipitation of carbonate because transport of the cation occurs intracellularly, effectively isolated from the interstitial space, where the local IAP would not be raised over background levels, even though pH and HCO_3^- concentrations would.

5. Prospects and outlook

The discussion brought forward here makes it evident that an effort involving experimental approaches is necessary to test those models. However, I do not fail to appreciate that, in reality, a combination of such

mechanisms is possible, and perhaps the most efficient means of boring. These are not mutually exclusive processes.

But relatively simple experiments using cultivated isolates of boring cyanobacteria under controlled laboratory conditions should reject or support them. For example, in an experiment testing the rate of boring or infestation under varying light/dark periods for growth, “model 1” predicts a response curve with a maximum, since there should be no boring in constant darkness (no energy available) and no boring under constant illumination (no necessary dark period available). In the same experiment, models 2, and 3 predict a monotonously increasing rate of boring with increased length of the light period. Model 3 predicts that there should be no boring on non-calcic carbonates, whereas models 1 and 2 predict that boring efficiency should be a function of the acid-sensitivity of the mineral substrate. A comparison of boring activity and rates on selected mineral substrates should shine light on the physiological approach used by the cells. More sophisticated experiments may directly and in real time allow us to monitor the process of excavation. However, the most significant hurdle to attain this goal is the apparent absence of cultivated cyanobacteria that actively bore under laboratory conditions. Lab-boring cultures have been reportedly obtained, but many of the strains are no longer available. Those deposited in culture collections have apparently lost their ability to bore, at least in our hands. Efforts should thus be directed towards the isolation and maintenance of cultures under conditions that require the boring capacity for survival.

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