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Did silicon aid in the establishment of the first bacterium?

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Abstract: Silicic acid increased numbers of both aerobic and facultatively anaerobic bacteria in ultrapure water incubated under strict oligotrophic conditions; soil extracts acted as the bacterial inoculum. The results are discussed in relation to the possibility that silicic acid, produced by the hydrolysis of silicates on the early Earth, could have stimulated the growth of the first bacterium, thereby allowing it to become established in the then prevailing conditions (presumed to be oligotrophic).

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Introduction

While much has been written concerning the evolution of life, less attention has been devoted to the question of how the first bacterium became established on the early Earth.

Life may have originated indigenously on Earth or have arrived from elsewhere. If life did arise on Earth, it seems unlikely that the first organism would have been as complicated as a fully-fledged modern bacterium. At some point however, the first protobacterium would have evolved and would have needed to establish itself. Similarly, if life arrived from elsewhere as a proto-life form, or as an already established bacterium, it would also need to become established in a terrestrial setting. It is assumed here that, in both cases, the protobacterium would have been a heterotroph, rather than a chemoautotroph.

It is generally believed that the protobacterium, having developed, or arrived, on Earth would increase rapidly and quickly colonize the planet. Such assumptions are based on the rapid doubling times seen when bacteria are grown in nutrient-rich laboratory media. However, the establishment, and subsequent spread, of exogenous life might have been more precarious than is generally assumed, simply because the prebiotic Earth may have been nutrient-limiting (Cairns-Smith 2001). Moreover, until about 3.8–3.9 Gya the planet was subjected to intense cometary bombardment (late heavy bombardment, LHB, Nisbet & Sleep 2001). From the time of the Moon-forming collision, at about 4.2 Gya until the end of the epoch of the LHB, the prebiotic Earth would have been periodically stripped of its oceans and volatiles, thereby making it a distinctly oligotrophic environment. Since carbon was limiting in such an environment bacteria adapted for growth in low concentrations of available carbon, i.e. oligocarbotrophs, would have been selected for and anything that promoted growth in such an environment would obviously have been seized upon by the protobacterium.

Silicon compounds have been shown to stimulate bacterial growth in nutrient-rich conditions (Wainwright 1997); such growth stimulation, occurring under oligotrophic conditions might have helped to establish the protobacterium on Earth. In order to confirm this possibility, we investigated the effect of silicic acid on bacterial growth under oligotrophic conditions; our findings are discussed in relation to the establishment of the protobacterium on the early Earth.

Materials and methods

Inoculum and incubation system

A suspension of soil from either an agricultural loam soil or a deciduous woodland soil (under *Acer pseudoplatanus*) acted as the bacterial inoculum. Soil (1 g in triplicate) was added to ultrapure water (upw, 100 ml) adjusted to pH 6.8 with Sorensen's phosphate buffer (which is C- and N-free). The suspension was then diluted 1:10 and 0.1 ml was added to pre-sterilized uncovered, plastic tubes containing either sterile upw (15 ml), sterile upw containing washed and heat-treated silicic acid powder (0.1 g, BDH). Potential inorganic nutrients were removed from the silicic acid with three washes of sterile, distilled water and 0.1 mM HCl, the washed powder was then heated in a muffle furnace at 400 °C for 4 h in order to remove any carbon contamination.

Incubation under strict oligotrophic conditions and culture of bacteria

For incubation under aerobic conditions, the plastic tubes were placed in an autoclaved metal gas jar (pre-washed using 50% v/v H_2SO_4). Air was sucked into the jar (for 30 min),

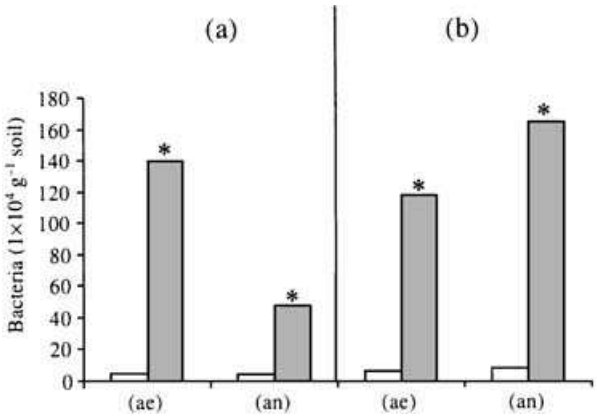

Fig. 1. Numbers of bacteria (in excess of inoculum value) in ultrapure water (white) and upw containing silicic acid (shaded) amended with (a) a solution of an agricultural loam and (b) a deciduous soil solution, incubated under aerobic (ae) and anaerobic (an) conditions. *The amended value differs significantly from the unamended value, $p=0.05$.

after first passing through Dreschler bottles containing first sulphuric acid (50% v/v, to remove airborne organics) and then sterile upw. The anaerobic jar was then sealed and incubated at 25 °C for 7 days. Aliquots (0.1 ml) from each plastic tube was then spread on to the surface of plate count Agar (five per sample) and the plates were incubated aerobically at 25 °C for 4 days.

The effect of silicic acid on the number of facultative anaerobic bacteria was determined by sealing the anaerobic jar as before but including a catalyst (Oxoid, BR42) and an Oxoid H_2 and CO_2, gas-generating kit (Oxoid, BR38); anaerobic conditions being confirmed using an anaerobic indicator (Oxoid, BR55). After incubation at 25 °C for 7 days an aliquot from each tube was transferred to plate count agar and incubated aerobically at 25 °C for 4 days.

Determination of soluble silicon

Soluble silicon was measured colorimetrically by adding the following to 1 ml of sample: ammonium molybdate (2 ml 10% w/v); ascorbic acid (2 ml, 5% w/v); oxalic acid (1 ml, 10% w/v) and HCl (5 ml, 1:1 dilution of concentrated acid). After incubation at room temperature (15 min), the absorption of the resultant blue colour was measured at 600 nm. A standard curve was prepared using EIL standard silicon solution (BDH).

Results and discussion

Bacterial numbers in the solution, amended with both soil extracts, increased only slightly above the inoculum value in the absence of added silicic acid. This trend occurred independent of whether the solutions were incubated under aerobic or anaerobic conditions (Figs 1a and b). The heterotrophic bacteria population of both soils clearly have only a limited ability to grow under the strict oligotrophic conditions imposed. The addition of silicic acid led to substantial and significant increases in the numbers of both aerobic and facultatively anaerobic bacteria (Figs 1a and b). Since silicon amendment increased bacterial numbers from both soil inocula the effect was not restricted to a single soil bacterial population. The experiment was conducted twice, with essentially the same trends occurring in both experimental runs; the stimulatory effects were also seen when the buffer was excluded, showing that it was not caused by the presence of phosphates.

The fact that the air entering the incubation system was scrubbed free of carbon, by passage through sulphuric acid, means that any bacteria developing in the nutrient-free solutions employed would have been unable to use atmospheric gases and volatiles as carbon sources; they must therefore have relied upon carbon sources present in the soil inoculum to support growth. Although the silicic acid used here was in the form of an insoluble powder, some dissolution occurred in the solutions amended with agricultural and deciduous soil extracts which, respectively, contained 26 and 35 $\mu g\ ml^{-1}$ of soluble silicon; such dissolution to ionic silicon is likely to be essential if the element is to play a role in bacterial metabolism.

The composition of the bacteria present in the soil inocula was not determined, although it was clear that a mixture of colony types was isolated and that a single species was not selected for. All of the bacteria were heterotrophs; the fact that, after exposure to strict oligotrophic conditions, they grew on carbon-rich media shows that they were facultative in this respect. The ability of the protobacterium to grow as a facultative oligotroph would have been essential as it would allow for the utilization of any large amounts of carbon nutrients once they became available; obligate oligotrophy, in contrast, would have proved disadvantageous to the rapid establishment of the protobacterium. It is also important to note that silicon also stimulates microbial growth under nutrient-rich conditions (Wainwright 1997; Wainwright *et al.* 1997), and so may have helped establish the protobacterium, even if the early Earth had not been predominantly oligotrophic.

The ability of the protobacterium to grow as a facultative anaerobe would similarly have been advantageous to the protobacterium, as it would have allowed for the rapid colonization of oxygen-rich habitats once they became available.

Our use of modern soil bacteria as the inoculum might be considered artificial relative to studies on the establishment of the first bacterium. However, it seems unlikely that a heterotrophic, protobacterium would have possessed a metabolism that differed significantly from that of modern heterotrophic, soil bacteria; silicon would probably have had a similar stimulatory effect on growth had the protobacterium been a heterotrophic thermophile.

These results suggest that the silicon present as hydrolysable silicates on the early Earth would have stimulated the growth of the protobacterium had the prevailing nutrient conditions been oligotrophic. There would certainly have been no shortage of silicon on the early Earth, as the Earth cooled and the lighter silicate crystals flooded the surface.

High-energy comet and asteroid impacts evaporating primitive oceans during the LHB would also have been particularly suitable form providing an instantly hydrolysable source of silicates.

Silicon could have also had an important effect on the establishment of the protobacterium had it arrived on Earth from elsewhere, assuming: (1) that the protobacterium arrived in a carrier that was itself an oligotrophic environment and (2) that it landed into an oligotrophic environment. Most types of carbonaceous meteorites (chondrites) are also known to contain carbon (around 3% w/w) mostly in the form of bacteria-available organic molecules; since such organics are present in very low concentrations such meteorites are characterized as being oligotrophic environments (Mautner *et al.* 1995); it is also noteworthy that meteorites contain nitrogen and other potential bacterial nutrients, including vital biological trace elements (Wasson 1974). The hydrolysis of silicon, the predominant component of carbonaceous chondrites, would have led to the release of soluble silicon, which could potentially have led to significantly stimulated bacterial growth; such a growth stimulation would have been particularly important during the critical breakout from the carrier into the Earth environment.

The fact that silicon is an ubiquitous element throughout the universe (Evans 1994) means that life will gain a 'kick start' by indigenous silicon on its arrival on a new planet. However, since transportation systems such as carbonaceous meteorites, comets or clumps of interstellar dust, are likely to already be rich in silicon compounds (e.g. olivine, Hoyle & Wickramasinghe 1991) such organisms need not have relied upon the target environment being silicon-rich.

Our results suggest that whenever water-soluble silicon becomes available on Earth, from extraterrestrial sources, the potential arises for a stimulation in bacterial growth to occur, even when nutrients are very scarce. Such stimuli may have been repeatedly produced by cometary bombardment during the LHB epoch between 4.2 and 3.8 Gya. Although it is unclear how silicon compounds stimulate bacterial growth (Wainwright 1997), such stimulation may be critical to the establishment of bacteria in novel planetary environments. Although fungi have been ignored in relation to panspermia, it is worth noting, however, that fungal growth under oligotrophic conditions is also stimulated by silicic acid (Wainwright *et al.* 1997).

Since soil and most water bodies are regarded as being oligotrophic, silicon-related stimulation of microbial growth may also be important in present-day Earth environments, as well as being crucial to the establishment of any bacteria-like life forms that arose on Earth.

In conclusion, Darwin's 'little pond' may have been warm and inviting, but also lacking nutrients to support rapid growth of the first bacterium; silicon compounds, which were periodically replenished by cometary bombardment, might have stimulated the growth of the protobacterium (under such oligotrophic conditions) and aided its critical establishment, independent of whether it developed here on Earth or arrived from elsewhere.

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