Cryptoendolith colonization of diverse substrates (1): cultivation and characterization

Conference or Workshop Item

CRYPTOENDOLITH COLONIZATION OF DIVERSE SUBSTRATES (1) : CULTIVATION AND CHARACTERIZATION

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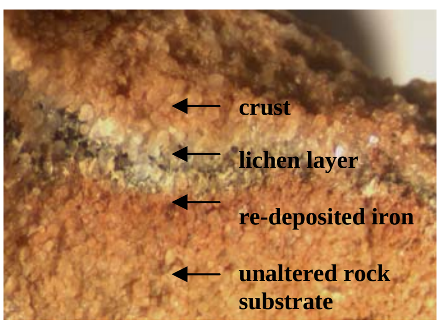
Introduction: Cryptoendoliths are microorganisms that have found refuge from the hostile environmental conditions of the Dry Valleys of Antarctica in the interstitial spaces of porous rocks. The rock substrates provide conditions that are suitable for life which contrast sharply with the abiotic rock surface [1]. Endolithic communities are thought to have evolved when changes in environmental conditions rendered rock surfaces inhospitable (e.g. owing to glaciation) and the organisms withdrew into protective rock niches [2]. These microbial habitats are thought to represent the last footholds of life in a gradually deteriorating environment [1], and as such serve as models for certain exobiological scenarios of the putative Martian biosphere.

The multi-coloured layers under the surface of the rocks (fig.1.) are produced by filamentous fungi and unicellular green algae, which also form symbiotic lichen associations [2].

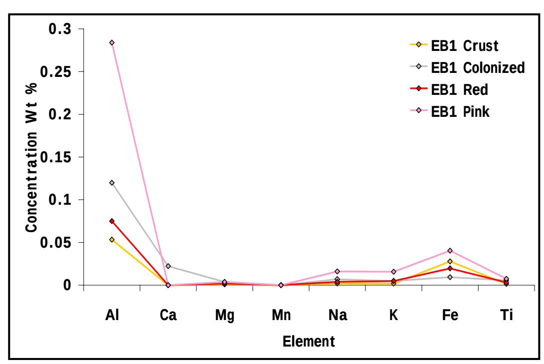
Figure 1: Cryptoendolithic community layers in the profile of a sandstone



Previous Work: Cryptoendolithic lichens interact with the substrate they inhabit by producing oxalic acid which has a two-fold affect: (1) they leach iron-bearing minerals from their inhabited zone and re-deposit them a few millimetres below causing the substrate here to appear a darker red; and (2) oxalic acid also acts to dissolve the cementing substance between the crystals in the colonized zone leading to the cyclic exfoliation of the surface crust [3]. Our previous work has investigated the mineralogical and geochemical characteristics of a suite of colonized and un-colonized sandstones in order to determine any further microbe-substrate interaction. A major, minor and trace element study was undertaken utilizing ICP-AES and ICP-MS techniques. The samples were collected by a British Antarctic Survey expedition to Terra Nova Bay and the McMurdo Base during the Antarctic summer of 1995-1996 [4]. The results showed that significant disparity in elemental concentrations was evident between colonized and un-colonized samples. However, mineralogical analysis revealed that the differences were likely to be a primary feature of the different rock types rather than a result of cryptoendolith action. There were distinct differences in mineralogical maturity between the samples and each fell into one of three categories; mature (colonized), intermediate (colonized), and immature (un-colonized).

To establish whether the differences had a biological or mineralogical control we conducted a further study involving the comparison of the different layers within individual samples (e.g. colonized and un-colonized). Several layers can make up these rocks, a siliceous crust, colonized upper lichen zone, a micro-algal zone, a red layer formed of re-deposited iron compounds and the deeper rock substrate, which is presumably unaltered. (fig. 1). It is therefore easy to differentiate between layers that are colonized and those that are not. Chemical and mineralogical analyses were conducted on each of the layers within samples of different mineralogical maturity. The results of both the chemical and mineralogical study revealed that compositional differences occurred between colonized and un-colonized layers within the same samples (e.g. fig. 2.). These results indicate that cryptoendoliths may weather the layers they inhabit through their production of oxalic acid. This may be in order to access essential nutrients and/or perhaps to increase the habitability of the layer.

Figure 2: Major elemental concentrations within separate layers in mature colonized sample EB1



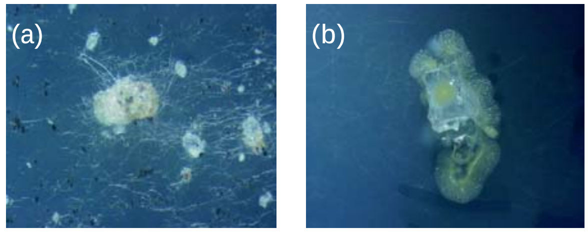
Current Analysis: Most lithobionts (rock-inhabiting microbes) have been classified into types, however intermediary forms exist e.g. *Ostreobium sp*., a green algae that inhabits corals. These organisms can behave as euendoliths (rock-borers) and penetrate carbonate structures but also colonize pre-existing structural cavities [5]. Though cryptoendolithic communities are reported not to penetrate the substrate by solubilization and favour the colonization of rocks which have a pre-existing porous structure they have the means for mineral dissolution. If the essential porosity is not available can cryptoendoliths behave like euendoliths and inhabit more diverse substrates then weather them to improve their habitability?

Our current study is investigating the extent of the weathering capabilities of cryptoendolithic communities to determine whether they are used in colonization e.g. does the ability to weather rock substrates equal the ability to colonize more hostile substrates? As pioneering colonists of diverse substrates in extreme environments, their role as analogues of Martian microorganisms would be greatly enhanced. The study involves cultivation of microbial consortia from Antarctic sandstone habitats and then their re-colonization of a variety of selected substrates. These substrates will include a range of rock types including sandstones of varying mineralogical maturity and porosity, basalts, fractured granites and a Martian meteorite.

Cultivation of Cryptoendoliths: Samples were flame sterilized to eliminate exterior contamination. Each sample was then cracked open using a flame-sterilized chisel. Grains from individual layers (e.g. colonized and un-colonized) from the interior of each sample were scraped onto Petri dishes containing BG-11 agar and PYGV agar using a flame-sterilized blade. Whole pieces of the rocks taken from the interiors of larger samples were place in sterile jars containing liquid media BG-11 and SNAX both commonly used to cultivate cyanobacteria. Petri plates and jars were incubated at 4^{o}C under constant illumination. After one week under these conditions fungal mycelia with spores were observed to be growing from around sandstone grains scraped from the "colonized" layers of several samples (fig. 3. a). Fungal growth occurred on both BG-11 and PYGV agars. Spherical structures growing out from around grains indicated the growth of bacteria colonies derived from the grain surface (fig.3. b). The colonies were prepared on a wet slide and observed under a phase-contrast microscope. They consisted of rod-shaped bacteria which were motile and could be seen to be dividing by binary fission. Observed bacterial colonies and fungal mycelia were separated and placed on agar slants to isolate pure cultures. After eight weeks bacterial colonies were also observed growing out from several rock samples immerse in both the BG-11 and SNAX media.

We are currently verifying the identity of the cultured microbes and characterizing the constituents of the cryptoendolithic communities using both culture-based and culture-independent molecular phylogenetic analyses. This is being conducted by examining small sub-unit (SSU) rRNA genes amplified from community DNA using the techniques outlined in [6] and [7].

Figure 3: Cultured microbes (x 6.5) (a) fungi growing from around sandstone grain; (b) bacteria colony emerging from around grain



Discussion: In cultivating these cryptoendolithic communities potential problems arise, like contamination. Steps to limit contamination have been taken throughout the study e.g. sterilization and DNA sequencing will allow characterization of the microbes that have been cultured. Another problem may be in only successfully cultivating parts of the cryptoendolithic community. The microbes may only recolonize as a consortium. To date we have not observed any of the cultured fungi or algae growing together in lichen association. If the microbes recolonize "out of symbiosis" then they may not interact with the substrate in the same way as true cryptoendolithic consortia.

Implications: If cryptoendolithic organisms, cultivated in this study, are shown to be able to colonize samples by biogenic weathering then they are likely to be able to adapt relatively rapidly to changing environmental conditions without adaptive evolutionary changes. Such ability has important implications for the survival of micro-organisms close to the Martian surface.

References: [1] Friedmann E.I. and Ocampo-Friedmann R. (1984) *Origins of Life, 14, 771-776* [2] Friedmann E.I. (1982) *Science, 215, 1045-1053* [3] Sun H.J. and Friedmann E.I. (1999) *Geomicrobiol. J. 16, 193-202* [4] Edwards H.G.M. et al., (1997) *J. Raman Spectrosc., 28*, 685-690. [5] Golubic S. (1981) *J. Sed. Pet., 51, 0475-0478* [6] Hugenholtz P. et al., (1998) *J. of Bacteriology 180, 366-376* [7] de la Torre J.R. (2003) *App. Environ. Microbiol., 69, 3858-3867*