Biologically–induced elemental variations in Antarctic sandstones: a potential test for Martian micro-organisms

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Abstract: Lichen-dominated cryptoendolithic communities from the Dry Valleys of Antarctica have been the subject of much research over recent years owing to their potential as analogues of Martian life forms. Their ability to mobilize iron compounds and organize themselves into distinct coloured biotic zones suggests that they may alter the chemistry of their host rock. By conducting a major, minor and trace element study utilizing inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectrometry (ICP-MS) techniques, we have been investigating the relationship between the microbes and the chemistry of the sandstones. Different layers within a suite of sandstones collected from six localities in the Dry Valleys have been analysed to establish if or how the microbes influence or directly affect the chemical composition of the rocks. Background petrographic studies have shown significant differences in mineralogical maturity between rocks colonized by the communities and those that are not, and the chemistry results have shown significant elemental disparity between colonized and uncolonized rocks. By obtaining accurate percentages of the minerals present in each sample the differences in elemental concentrations could be construed to be caused by the differences in mineralogy between samples. The nature and extent of the concentration differences has led to the conclusion that either the cryptoendolith communities are able to alter their host rock by the solubilization and mobilization of elements that are then subsequently removed or that the organisms are simple opportunists that can only successfully colonize rocks that provide the ideal substrate, being mineralogically mature with ample pore space and less concentrated in the elements tested for.

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Introduction

To ascertain if life exists, or may have existed in the past, on the Martian surface and whether it flourished within certain biological niches, the evolution and adaptation of life in the Antarctic environment is of particular interest. Unforgiving and extreme conditions of low temperatures, low humidities, diurnal freeze–thaw cycles even during daylight hours, low annual precipitation, desiccating winds, high sublimation and evaporation, high incidence of solar and especially ultra-violet radiation together render most of inland Antarctica unfavourable to higher forms of plant and animal life (Campbell & Claridge 1987); similar conditions are assumed to pertain at Mars' surface. Microbial life, however, survives successfully in the few, isolated pockets not continually covered by snow and ice. The McMurdo Dry Valleys of Southern Victoria Land are of special interest, where basic ecosystems with organisms showing remarkable adaptations have been discovered. Friedmann (1982) first described the variety of micro-organisms that live in narrow zones under the surface of rocks in the Dry Valleys, colonizing extended areas and producing considerable biomass.

Endolithic organisms fall into two types: chasmoendoliths, living in rock fissures and cracks, and cryptoendoliths, inhabiting structural cavities of porous rocks. Endolithic microbial communities have a photosynthetic primary producer, so translucent rocks through which sunlight can penetrate are their only suitable substrate. They also favour colonization of rocks which either have a porous structure or are weathered and permeated by fissures, as they are reported not to penetrate the substrate by solution. Lichen-dominated ecosystems with green algae and cyanobacteria are the most widespread of the microbial communities in the mountainous desert areas of the McMurdo Dry Valleys (Ocampo-Friedmann & Friedmann 1993). For example, cryptoendolithic communities form conspicuous multicoloured zones under the surface of the Beacon Sandstones. All zones are produced by filamentous fungi (mycobionts) and unicellular green algae (phycobionts), which together form a symbiotic lichen association. Cryptoendolithic lichen form an upper

Table 1. *Samples and field localities*

Sample	Date collected	Locality	Grid reference	Alt. (m)
TP1	01/12/96	Timber Peak, East Plateau	74° 10′ S 162° 30′ E	2800
EB1	23/12/82	East Beacon Mountain	77° 50′ S 160° 52′ E	2200
BP1/2/3	15/12/95	Battleship Promontory	76° 55′ S 160° 55′ E	1000
TP43	04/01/96	Timber Peak, East Plateau	74° 10′ S 162° 30′ E	2800
VH59	28/12/95	Vulcan Hills	73° 40′ S 163° 38′ E	2900
MM45	04/01/96	Mount MacKintosh	74° 40′ S 161° 45′ E	2100

zone, which can be further differentiated into an upper black zone and a lower white zone (Friedmann *et al.* (1988) and see Fig. 2). Two different structures may form the black zone. In one arrangement, dark brownish, greyish or greenish masses of fungal hyphae enclose groups of algal cells. The hyphae often form small, spherical, lobe-like structures, which have been described as 'presquamule-like bodies' (Friedmann 1982; Friedmann *et al.* 1988). Alternatively, the black zone may contain non-lichen-forming pigmented fungi, which occur in association with the cryptoendolithic lichens and are thought likely to be parasymbionts (lichen parasites) (Friedmann *et al.* 1988), but have also been postulated to be a free-living active fungus (Sterflinger 2000). The white zone appears only to be formed by lichens, but may also contain colourless soredia (Friedmann *et al.* 1988).

The stress tolerant nature of these micro-organisms, particularly that of the cyanobacteria, which possess a remarkable suite of survival mechanisms, has led to the suggestion that they may have been the type of micro-organisms that could have flourished at the surface of an early Mars (Wynn-Williams & Edwards 2000). Cryptoendolithic lichens have mastered a unique adaptive feature that enables them to change their morphology in order to colonize the sandstone substrate. In 'typical' lichens, the fungal hyphae form a coherent mat-like structure, or pseudotissue. However, in the Antarctic sandstones, because the cryptoendolithic niche is only accessible to organisms that are able to penetrate the narrow system of the porous rock, the mat-like structure cannot form. Instead, the lichens have adopted a modified morphology, consisting of loose filaments and cell clusters that grow between and around the crystals of the rock (Friedmann 1982). On occasion, exfoliative weathering of the substrate can cause the lichens to become exposed at the surface, in which circumstances the lichen may continue to grow on the surface of the rock, changing morphology again by reverting to the 'typical' mat-like structure. The ability of the mycobiont to change its growth from mat-like to filamentous while maintaining organization within definite layers, and to be able to reconvert to mat-like when conditions so permit, is a remarkable adaptive achievement (Friedmann 1982). Cryptoendolithic communities of the Antarctic desert are probably amongst the slowest growing organisms on Earth: microbial growth inside the rock and resulting rock weathering occur on the same time scale (10^3–10^4 years), so the biological and geological time scales overlap (Sun & Friedmann 1999). Exfoliation events (which result in loss of biomass) are thought to be triggered when the microbial biomass reaches the carrying capacity of the cryptoendolithic habitat and microbial growth is continuous until this point is reached (Sun & Friedmann 1999).

Cryptoendolithic lichens interact with the substrate they have colonized by producing oxalic acid (Johnson & Vestal 1993). They can mobilize iron compounds, which results in their inhabited zone being leached of iron-bearing minerals, causing the thin crust above this zone, and rock substrate a few millimetres below it, to appear darker owing to re-deposition of iron at these levels, leaving a white zone in the rock (Sun & Friedmann 1999). The oxalic acid also dissolves the cementing substance between the crystals in the colonized zone leading to exfoliation of the surface crust and resulting in loss of biomass (Sun & Friedmann 1999). The oxalate ion is one of the most abundant ligands detected in solutions interacting with mineral surfaces, and studies have focused on oxalate-promoted mineral weathering (Barker *et al.* 1997). Weathering reactions (rock and mineral dissolution) are increased by oxalic acid in many other systems such as soils, beach sand, sandstone buildings, monuments and historic sites, quartz, feldspars, various oxides and aluminosilicates (Johnson & Vestal 1993). It is important in creating the pore space in deep subsurface sandstone for hydrocarbon reservoirs (Johnson & Vestal 1993).

The activity and organization of the endolithic communities involving their use of oxalic acid suggests that these communities could interact further with the substrate. We, therefore, have been investigating the possible effect these microbes have on the overall chemistry of the sandstones they inhabit. Utilizing inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectrometry (ICP-MS) techniques a major, minor and trace element study of the different layers within the sandstones has been undertaken to establish if, or how, these microbes may further influence or directly affect the chemical composition of the rocks. Our interest lies in whether these microbial communities can alter the chemistry of more hostile substrates to favour their survival. Aluminosilicates and silicates are easily degraded by fungi as silicate minerals are readily attacked by the oxalic acid they produce (Sterflinger 2000). Although the communities have been reported not to penetrate the substrate by solubilization and favour colonization of rocks which have a pre-existing porous structure (Friedmann 1982), they have the means for mineral dissolution and could therefore inhabit a less porous substrate and modify it to create more favourable conditions (a motive). Life in the extreme has had to evolve and adapt to overcome the hostile
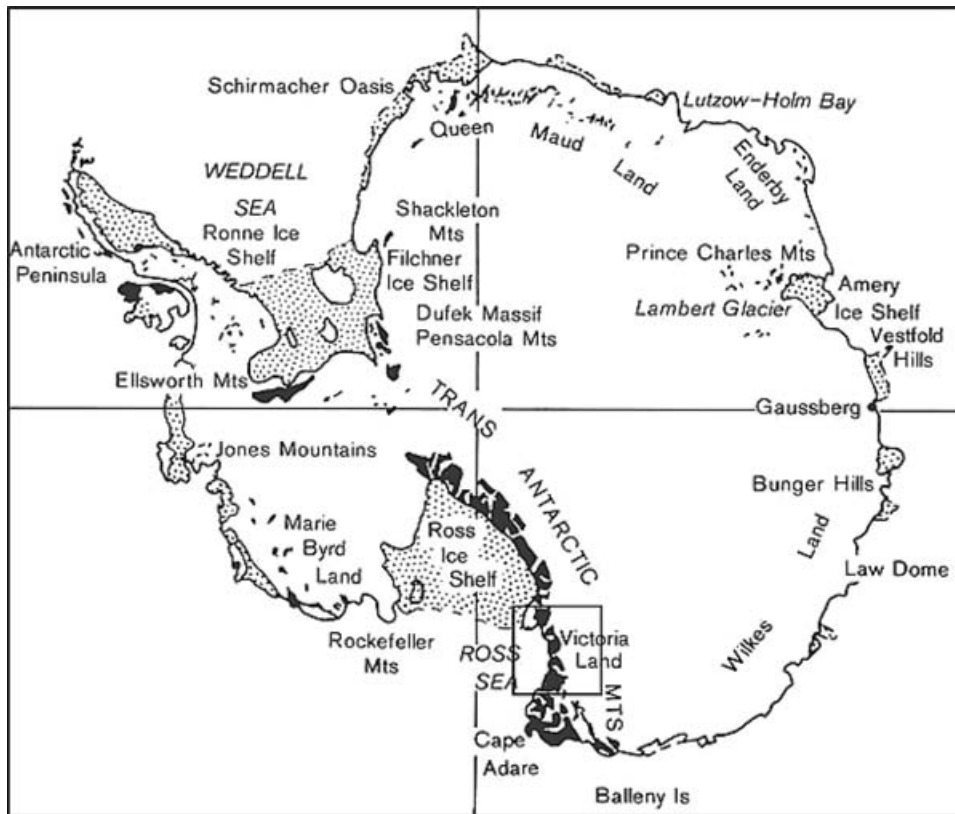
Fig. 1. The distribution of ice-free areas in Antarctica, with black areas representing the localities where bare ground occurs (from Campbell & Claridge 1987). The boxed area is the general location from which the materials were obtained.

constraints of its environments. The detection of elemental variations and the nature and extent of any elemental disparity could add value to the potential role of cryptoendolithic communities as suitable analogues of 'Martian' micro-organisms and as biomarkers when considering future *in situ* analysis of Martian surface materials and Mars sample return rocks.

Materials and methods

Specimens

The suite of rocks analysed here are Devonian age (395–345 Ma) quartzite sandstones of the Beacon Supergroup. The cryptoendolithic habitat is widespread throughout the Beacon Supergroup, extending from Victoria Land through the Transantarctic Mountains, East Antarctica (Russell *et al*. 1998). The samples were collected by a British Antarctic Survey expedition to Terra Nova Bay and McMurdo Base during the Antarctic summer of 1995–1996 (Edwards *et al*. 1997), from six different localities (Table 1 and Fig. 1). Four of the samples contain cryptoendolithic communities, whilst two (MM45 and VH59) do not; these samples were used as negative controls for the study. Although the distinct vertical sequence of the black, white and green zones is generally constant in cryptoendolith samples, any one or two of these zones may be absent. This set of colonized samples all contained the white zone, but BP2 (Fig. 2) was the only sample to have the black and white zone and BP3 the only sample to have the white and green zones.
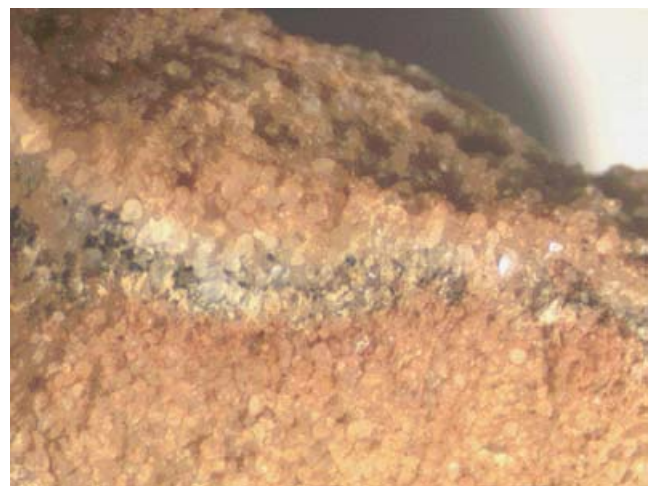

Fig. 2. Colonized Beacon Sandstone sample BP2 showing the lichen zone.

No samples here contained all three of the typical zones. In this initial study samples from each locality have been selected for analysis. Details of the samples and their localities are given in Table 1.

Optical microscopy

The nature and characteristics of the sandstone samples were determined by optical microscopy of uncovered polished
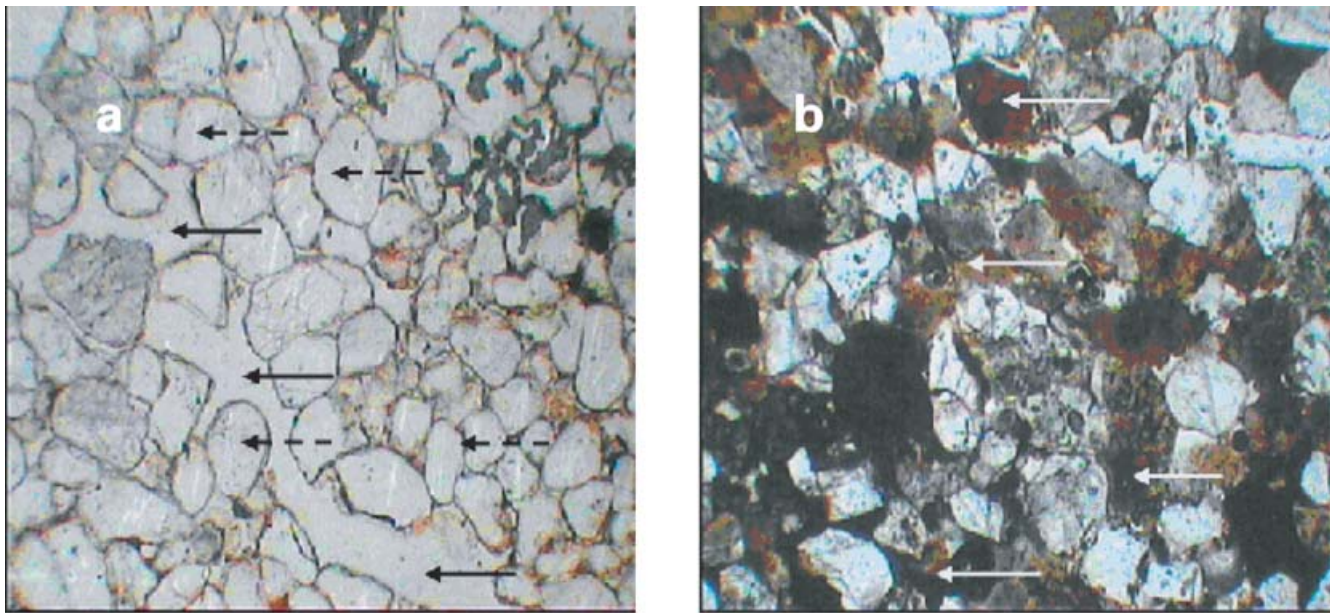
Fig. 3. Beacon Sandstones in thin section (PPL) at a magnification ×25: (a) colonized sandstone showing pore space (solid arrows) and well-rounded grains (dashed arrows) indicative of maturity; (b) uncolonized sandstone showing lack of pore space and dark staining (white arrows) indicative of mineral breakdown.

thin sections produced to a thickness of 30 μm. As well as the mineralogy of the sample, the textures (grain size, shape, porosity) were determined. Approximate modal abundances of minerals in each of the samples were estimated, to ascertain the overall nature of the sandstones (e.g. altered, unaltered).

Electron microscopy

Following optical characterization, high-magnification images of the specimens were obtained by scanning electron microscopy (SEM). The Philips XL30 is a conventional, multi-function, multi-user scanning electron microscope. It has optimum performance for both imaging and micro-analysis of conductive and/or coated specimens. A piece of each sample was broken off and mounted on aluminium Cambridge pin stubs (12 mm). After drying overnight, they were coated in 95% Au and 5% Pd to a thickness of 20 nm in a sputter coater (Cressington 208 HR) and analysed with the Philips XL30 operating at 5 and 7 V (working distance 10 mm).

A polished thin section of each specimen was examined using a Jeol 5900LV SEM operating at 20 kV and 1 nA with a working distance of 10 mm. This second phase of electron microscopy was carried out to determine accurate percentages of each mineral phase present in the different sandstones. To achieve this we mapped the major elemental composition of characteristic areas within each specimen. The percentage of each mineral phase present was then determined by phase map analysis using Oxford Instruments Inca microanalyser software.

Chemical analysis (ICP-AES and ICP-MS)

The major, minor and trace element chemistries of the specimens were measured by ICP-AES using a JY24 Sequential Spectrometer and ICP-MS using the VG PlasmaQuad PQ2STE at Kingston University. Grains were hand-picked from each layer of the sandstone samples, homogenized using an agate pestle and mortar, weighed and then digested in HF-$HClO_4$-NHO_3 acids. All reagents used were Aristar and 18 MΩ deionized water was used throughout. A standard reference material, SARM1 NIM-G (granite), was also prepared along with two blanks (HF-$HClO_4$-NHO_3).

ICP-AES and ICP-MS test procedures. Calibration standards, BCR1 (basalt); MESS2 (marine mud); STM1 (nepheline syenite); AGV1 (andesite); W2 (dolerite); ACE (granite); AWI1 (shale); KH2 (limestone); and JLS1 (Japanese limestone) were run through the ICP-AES procedure to calibrate the instrument. The blank and SARM1 NIM-G granite were then run through the instrument followed by each of the samples consecutively. For ICP-MS analysis, the solutions were further diluted by a factor of 100. Calibration standards used were multi-element solution Claritas PPT (1, 2 and 4) at 10 ppm. The multi-element solution was made to 100 ppb of 10 ml. The standards were run through the ICP-MS, with three tests per sample. The standard SARM1 NIM-G was run followed by the samples and each was tested three times.

Results

Petrologic analysis

The petrographic study by optical microscopy of the colonized samples BP1-3 and EB1 showed that they were mineralogically very mature quartzite sandstones. They were fairly homogenous and predominantly quartz with some clay. These samples were well sorted and well rounded and ranged from fine to coarse sand (Fig. 3(a)), with average grain diameters of between ~0.16 and 0.8 mm. They had abundant

pore space, with occasional quartz cement. Non-endolith bearing samples MM45 and VH59 were significantly different from the colonized samples, being mineralogically immature, VH59 particularly so. They contained a much greater proportion of feldspar and clays, had less rounded and more angular clasts, and pore space was difficult to determine but appeared almost absent. Extensive mineral breakdown was evident, explaining the dark nature of the rocks in thin section (Fig. 3(b)).

Between the end-members of the uncolonized MM45 and VH59 samples and the colonized rocks BP1-3 and EB1 lie TP1 and TP43. These are colonized sandstones and, although mature mineralogically, had higher feldspar content than the other colonized samples and evident mineral weathering was in still in process, though not to the degree of the uncolonized samples. The Timber Peak samples appear to be the medial representatives of mineralogical maturity in this suite of sandstones.

Scanning electron microscopy

Electron microscopy revealed the presence of eukaryotic communities. Cryptoendolithic lichen were identified in all the samples (except the controls MM45 and VH59). The lichen identified in the black-pigmented zone of BP2 corresponded to the morphology of those described by Friedmann (1982), i.e. loose filaments and cell clusters growing around the quartz crystals of the rock substrate (Fig. 4(a)). The white zone, found in each of the samples, comprised fungal hyphae filaments forming loose woolly webs around crystals of the substrate. Algae were not as readily identified, and have been previously reported as being sparse in this zone (Friedmann 1982). The dark pigmented zone distinct in BP2 (Fig. 2) was absent from the other samples and some of the hyphae imaged in the white zones appeared to be free living, i.e. not forming a symbiotic lichen association with algae.

Some hyphae bodies were found encrusted with a precipitate. This was observed in samples TP43 and TP1 (Figs 4(d), (e)). Fungi known to synthesize oxalic acid are frequently found encrusted with crystalline oxalates (Barker *et al.* 1997). Calcium oxalate precipitation on fungal mycelia is observed through all taxa of the fungal kingdom (Sterflinger 2000), and oxalic acid is produced by cryptoendolith lichen (Johnson & Vestal 1993), so oxalate crystals are assumed to be the material encrusting the hyphae pictured here. Algal cells, not associated with fungal hyphae, were identified and imaged in samples EB1 and TP43 (Fig. 4(f)) with similar encrustations.

Phase map analysis

Each of the four phase maps in Fig. 5 corresponds to one site of interest from each sample representing different mineralogical maturity, i.e. a very mature mineralogical sample (BP2), an intermediate sample (TP1), an immature sample (MM45) and a very immature sample (VH59). The maps show the proportional percentage of each phase present and are colour coded for clarity (Fig. 5 and Table 2). The table shows the total percentage of each mineral phase in the whole sample, taken from the average of the phase over five sites of interest.

Phase map analysis has shown significant disparity in mineral composition between the different samples. The analysis confirmed the colonized samples BP1-3 and EB1 as mature mineralogically. Figure 5 shows mature sample BP2 with, on average, ~77% quartz, ~10% pore space and kaolinite and illite clays comprising the remaining percentages of ~7% and ~4%, respectively (Table 2). The other colonized samples, BP1, BP3 and EB1, are similar to BP2, with ⩾77% quartz, ~10% pore space and the remainder divided between kaolinite and illite. TP1, shown in Fig. 5, differs from the other colonized samples, comprising, on average, only ~46% quartz, with ~8% pore space and, in addition to kaolinite clays, alkali feldspars comprising ~38% of the total. Despite mineralogical differences this sample still has ample pore space and is colonized by cryptoendoliths. The other sample from Timber Peak, TP43, had a very similar mineralogy to TP1. Uncolonized samples VH59 and MM45 are both immature with a greater percentage of feldspars and clays, illustrating mineral breakdown. MM45 contains an average of ~20% quartz with ~54% alkali feldspars. Along with kaolinite and illite, this sample has pore space comparable to BP2 at greater than 10%. In contrast, VH59 contains only ~9% quartz and is mainly K feldspar (~53%). Unlike any of the other samples, there is a calcium carbonate cement that runs through the sample in large veins and comprises ~19% of the total sample. The pore space here is minimal, just 2% overall.

Chemical analysis

Major, minor and trace element abundances from ICP analyses are given in Fig. 6. It is immediately apparent that there are significant differences in the concentration of elements between colonized samples and uncolonized samples. In the majority of cases, major elemental concentrations are greater in uncolonized than in colonized samples by on average a factor of 11 and range from as high as a factor of 20 to as low as a factor of four (Fig. 6(a)).

Minor element data (Fig. 6(b)) also show higher elemental concentrations in uncolonized compared to those in colonized samples. The ICP-MS results show that the concentrations of elements are generally higher in uncolonized than in colonized samples by a factor of about seven, ranging from as high as a factor of 26 to as low as a factor of two.

Discussion

It has been well documented that cryptoendolithic organisms favour the colonization of translucent rocks for the benefit of their photosynthetic primary producer. The ready transfer of fluids in the cryptoendolithic habitat is also important in terms of the organisms' metabolic activity, where optimal activity for photometabolism occurs between 80 and 100% water content (Vestal 1988). The ability to take up water quickly and efficiently in an otherwise dry environment is a vital requirement for cryptoendoliths, and so a porous and
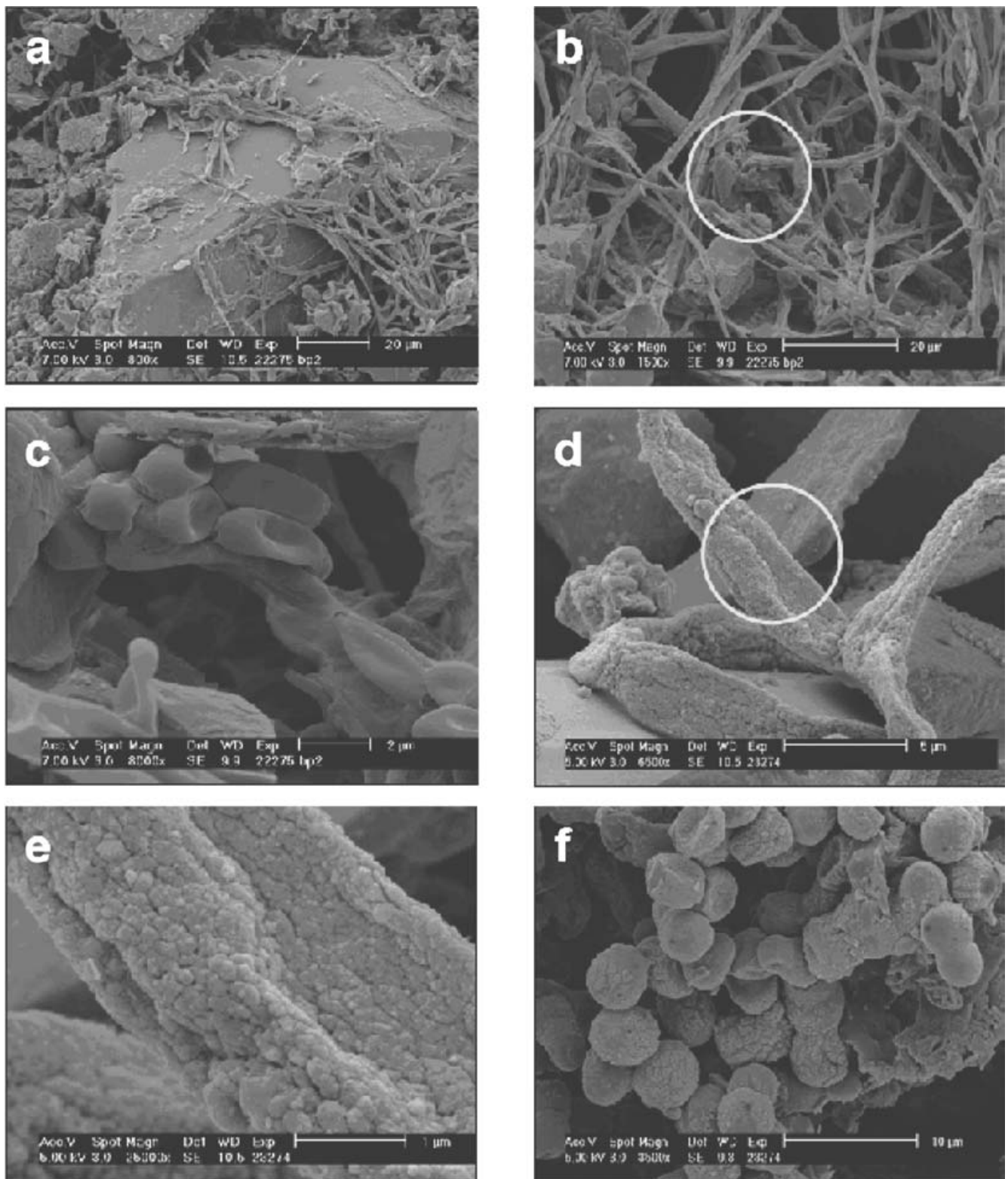
Fig. 4. Philips XL30 SEM images of cryptoendolithic communities: (a) cryptoendolithic lichen growing between and around a crystal in the rock substrate ×800; (b) fungal hyphae and algal cells forming symbiotic lichen association; (c) hyphae forming presquamule-like bodies circled in B shown at higher magnification ×8000; (d) calcium oxalate precipitate on fungal mycelia; (e) precipitate on hyphae circled in D at ×25 000 magnification; (f) phycobiont cells shown without fungal (mycobiont) hyphae association.
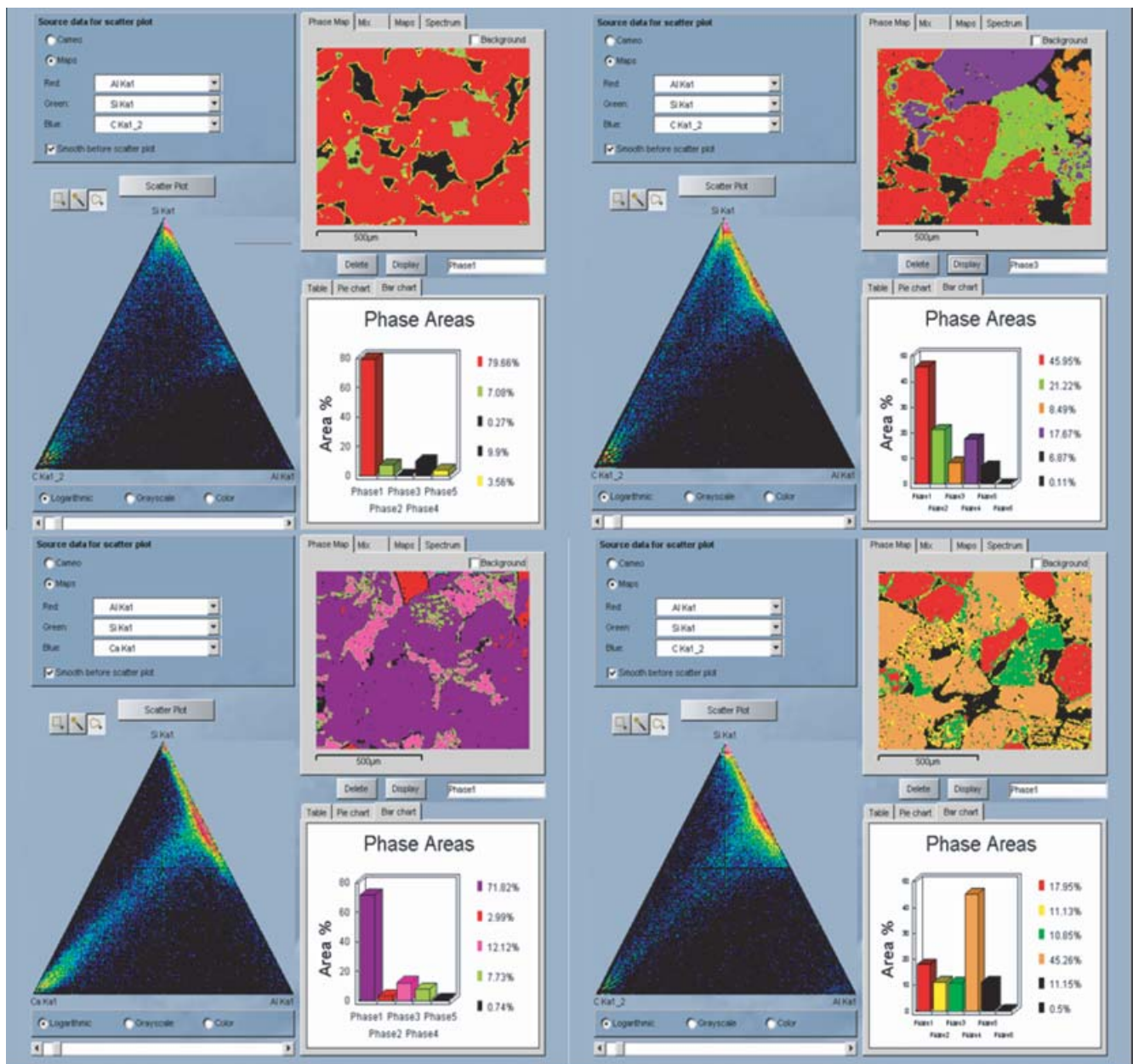
Fig. 5. Phase maps showing the difference in mineral makeup. Phase colours are indicated in Table 2. Clockwise from top left: BP2 (mature), TP1 (intermediate), MM45 (immature) and VH59 (very immature).

permeable substrate is a prerequisite, and rocks that have a porous structure are thus favoured over those with low porosity. Cryptoendolithic lichen leach out iron, which is then redeposited both above and below the inhabited layers, indicating that iron compounds are carried by water in two directions: downward when snow melts on the surface and upward when capillary water rises as a result of evaporation (Friedmann 1982). Again, a porous and permeable substrate is a necessary requirement for such processes.

Phase map analysis provided an accurate technique for determining the percentage pore space in each sample, as well as modal mineralogy. The maps (Fig. 5) showed that there were significant differences in mineralogy between colonized and uncolonized samples, with endolithic communities limited to substrates that have attained a high level of maturity (and so porosity). The well-rounded and well-sorted grains in samples BP1–3 and EB1 have attracted cryptoendolithic organisms into the large interstitial spaces in the sandstones. The arrangement of particles has given the samples high porosity, the pores are wide and fluids are therefore free to move, making the samples permeable.

The colonized samples from Timber Peak (TP1 and TP43) have a modal mineralogy intermediate between the two extremes of mineralogical maturity (Fig. 5). Percentage pore space is similar to the other colonized samples but quartz content is lower, while alkali feldspar content is greater, and

Table 2. *Phases identified in the four varying samples showing the total percentage of each phase in the whole sample taken from an average of the phase over the five sites of interest*

Sample	BP2 average%	TP1 average%	MM45 average%	VH59 average%
Quartz (red)	77.12	46.31	20.86	9.62
Na feldspar (orange)	0.00	6.23	45.45	0.00
K feldspar (purple)	0.00	32.21	8.87	53.82
Kaolinite (green)	7.26	8.92	13.06	10.50
Illite (yellow)	4.26	0.00	7.01	0.00
Pore space (black)	10.23	8.40	10.43	2.09
Calcite (pink)	0.00	0.00	0.00	19.36
Total%	98.87	103.11	101.34	95.39

closer to that of the uncolonized samples. The lack of suitable porosity and permeability in sample VH59 indicates that this sample, being mineralogically immature, is unfavourable for cryptoendolith communities. However, the uncolonized MM45 is also a mineralogically immature rock, yet it has a porosity comparable to the most mature rocks, for example BP2. The fact that it is not colonized could be for several reasons, including: (1) despite being porous, the immature substrate is not suitable for the micro-organisms; (2) the orientation and locality of the outcrop the sample was collected from may have been unfavourable. It is clear from these results that porosity is not the sole criterion for rock colonization by micro-organisms.

It is known that oxalic acid produced by microbes leaches metals from the lichen-dominated zone, resulting in mobilization of major and minor elements; formation of oxalate complexes may also be important in keeping amorphous metal oxides and silicates from enveloping the microbiota (Johnson & Vestal 1993). Soluble oxalate complexes are carried from the lichen zone into deeper zones in the rock by water from snow melt or up towards the surface by capillary action as the rock dries, thus depleting the lichen zone of major elements (Johnson & Vestal 1993). On the face of it, our un-normalized major, minor and trace element data (Fig. 6) revealed sizeable differences in the concentrations of elements between colonized and uncolonized samples, and seem to indicate that the cryptoendolithic micro-organisms had altered the chemistry of their host rocks. However, from this dataset, we cannot determine whether this depletion is secondary, i.e. the result of cryptoendolith action, or whether it is a primary feature of the different rock types. In order to take account of inherent differences in mineralogy between the rocks from different locations, we looked in greater detail at the major elemental compositions of the individual layers in each sample. Results are shown in Fig. 7.

Major element data indicate that there are slightly lower concentrations in the lichen-inhabited zones. Indeed, most minor and trace elements are also lower in concentration in inhabited layers when compared to other layers in the samples. This relationship suggests that the organisms solubilize, use and remove elements from the inhabited zones. The samples from Timber Peak (TP1 and TP43) have
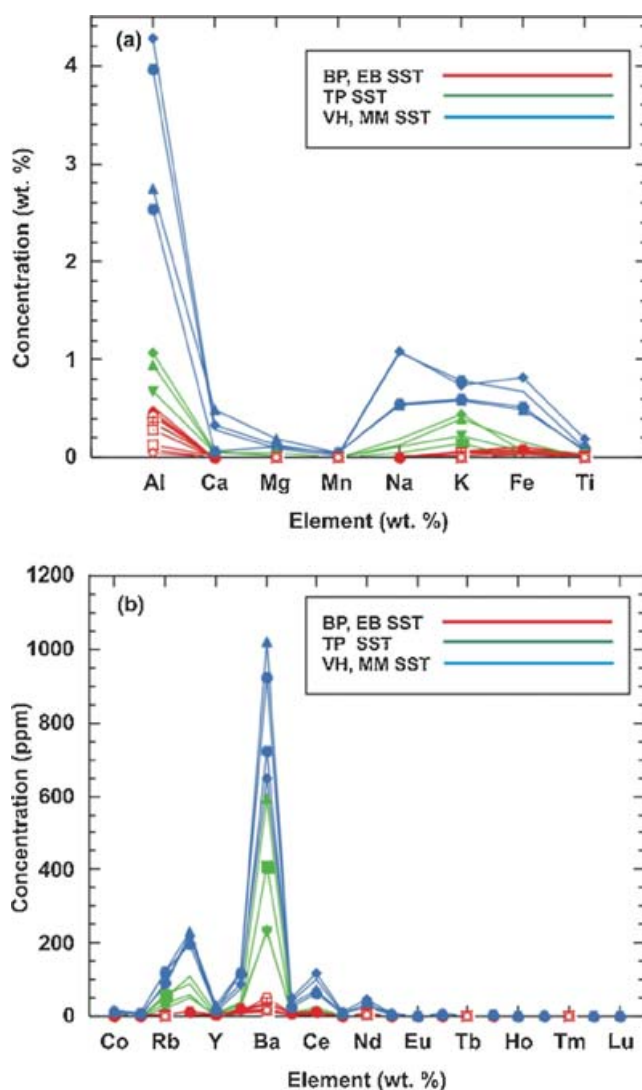

Fig. 6. Elemental concentrations in colonized sandstones (red and green) compared to uncolonized sandstones (blue): (a) major elements measured by ICP-AES; (b) minor element concentrations measured by ICP-MS.

higher elemental concentrations compared to their colonized counterparts (Figs 6(a), (b)); phase map analysis (Fig. 5) shows that the Timber Peak samples have different mineral abundances from the other colonized samples. The results may indicate that the micro-organisms undertake mineral weathering in order to change the substrate to one that is more favourable. The Timber Peak samples may represent a median stage of a biogenic weathering process, not yet having reached the mineralogical maturity of the other colonized sandstones when they were collected.

How long would it take for cryptoendoliths to weather a rocky substrate? As they are reported as being possibly the slowest growing organisms on Earth, it would likely be synonymous to the timescales related to the growth of the microbial communities inside the rock and the rock weathering which leads to exfoliation events, which as shown by Sun
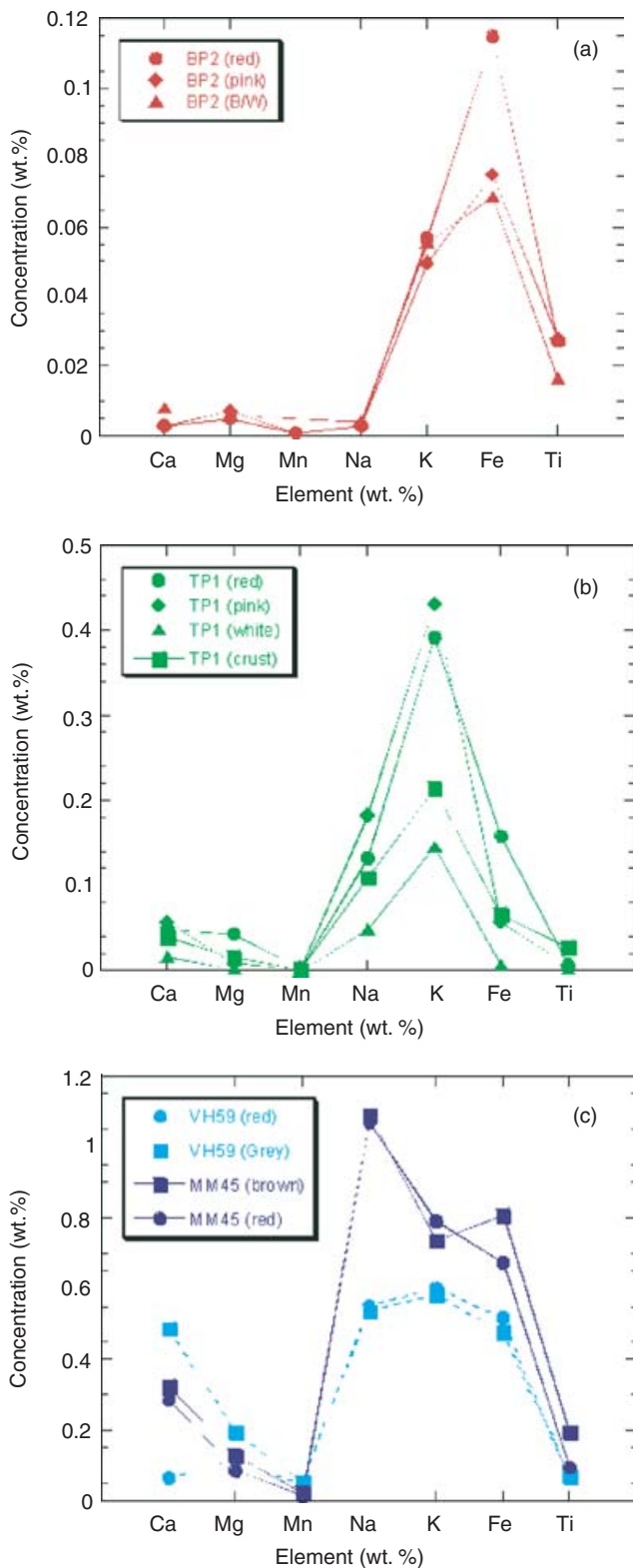
& Friedmann (1999) occur on the same timescale of around 10^3–10^4 years. We cannot, as yet, attempt to answer these questions; the next stage of our study will be to compare the effects of endoliths on rocks from hot deserts, as well as an attempt to measure timescales of chemical alteration through cultivation of the microbial communities on sandstones.

Conclusion

This preliminary study of the effect that cryptoendolithic micro-organisms have on the chemistry of the rocks they inhabit has yielded some interesting results. However, as is the case with many preliminary studies, new questions have arisen. We have found that there are significant elemental variations in different layers from sandstones collected from the Dry Valleys region of Antarctica. We have also found that there is a direct correlation between the maturity of sediments and the extent to which they are colonized by micro-organisms: mature, porous sandstones are colonized, whilst immature sandstones of low porosity are not. However, it is not yet clear whether these differences are a result of secondary alteration by endoliths 'digesting' the rocks or are a reflection of primary differences in mineralogy of the sandstones. The current situation could be cast in terms of the eternal 'chicken and egg' conundrum: which came first, the microbes or the mineralogy? Did the microbes alter the rocks so that they were suitable to colonize or were the rocks sufficiently porous that they attracted micro-organisms to 'move in'? Our current thinking is that the significant elemental disparity between the mature colonized, intermediate colonized and immature uncolonized specimens may not simply be explained just by organism activity, and that overall differences in mineralogy provide a stronger argument.

However, if differences in element concentrations between samples are caused by the micro-organisms, then the fate of these elements needs further investigation. In a cold and dry environment these elements would not be readily washed out of the substrate, although over lengthy timescales they could be removed by periodic snow melt. Alternately, if the elemental differences are a result of mineralogical differences, chemical, rather than biological, weathering may be the alteration process, which would also be a slow procedure. The Timber Peak samples may have been at an earlier stage of this weathering process, but were still suitable for colonization, having the pore space required by the colonists. Another possibility is that the organisms themselves play a part in the physical weathering process as well as the chemical. As pioneers, endoliths might be able to infiltrate substrates that are not ideal but have adequate pore space, then once established they may be able to adopt a euendolithic role and alter the substrate to increase its habitability.

The chemical and mineralogical study conducted here has paved the way for further research and analysis on the effect these micro-organisms may or may not have on the chemical composition of the rocks and what mineralogical controls (if any) govern whether a rock is suitable for

Fig. 7. Major element concentrations in separate layers. (a) BP2. The black and white zone is the lichen-colonized zone, the red zone the layer of iron deposition and the pink zone the lower remaining layer of sample (refer to Fig. 2). (b) TP1. Here the white layer is the colonized one. (c) VH59 and MM45. All layers are uncolonized.

colonization. The samples that we prepared were all taken from close to the outer edge of each rock – we did not sample at depth within the material. Thus we are not yet able to make a 'like with like' comparison within individual specimens. This series of analyses will be part of our follow-up studies. The value of cryptoendolithic communities as biomarkers for future *in situ* analysis of Martian surface materials and Mars sample return rocks would be greatly enhanced if their influence on the chemistry of their host rock could be proved. Much research lies ahead on cryptoendolithic communities and, from the behaviour, characteristics and influences of these terrestrial extremophiles, an important astrobiological profile may be compiled so that it can be used as a guide for future exobiological research.

Acknowledgements

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