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Raman spectroscopic and scanning electron microscopic analysis of a novel biological colonisation of volcanic rocks

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Abstract

A novel type of colonisation of a basaltic rock, collected on the Arctic island of Svalbard, Norway, during the AMASE expedition in 2004, was characterised using Raman spectroscopy and Scanning Electron Microscopy (SEM). The sample contains two different types of extremophile communities, one occurring behind a radial white crystallisation and the other occurring inside a dark vacuole. Several types of minerals and microbial colonies have been identified by both Raman spectroscopy and SEM analyses. It is the first time that photosynthetic communities have been documented to colonise the inside of dark basaltic rocks. Our discovery has important implications for planetary exploration because it extends the analytical capability and our understanding of microbial rock colonisations to subaerial volcanic outcrops and has wide implications towards the search for life in extraterrestrial planets. In this work we also demonstrate that the use of different laser wavelengths for Raman spectroscopic studies and complementary microscopic analysis are critical for a comprehensive organic and inorganic compound identification.

Keywords: Spectroscopy; Astrobiology; Mars; Search for extraterrestrial life

1. Introduction

The hypothesis that life started on Earth about 3800 mya, when the Earth and Mars had similar environmental conditions, leads us to think that life could also have appeared at that time on Mars (McKay, 1997; Davis and McKay, 1996; Cockell et al., 2000; Hiscox, 2001; Holm and Andersson, 2005). It is clear that life was colonising terrestrial ecological niches and needed to adapt when the external conditions changed along with the geological planetary evolution. Life has displayed a wide range of survival strategies adapting from the most benign habitats to the most inhospitable. In benign habitats, prebiotic chemistry developed rapidly into complex organisms (Delaye and Lazcano, 2005; Hughes et al., 2004; Simoneit, 2004; Trevors, 2003; Zagórski, 2003; Raulin and McKay, 2002), creating a significant variety of species, whereas in the most adverse environments the number of species was drastically smaller and frequently limited to microorganisms as the requirements for adaptation in such extreme conditions became more and more demanding. Such extremophilic organisms, which were able to survive the steadily worsening conditions, are considered ideal terrestrial analogues to possible Mars organisms (Horneck, 2000; Onofri et al., 2004; Littlechild, 2002; Stetter, 1999; Horikoshi and Grant, 1998) and their study will give us clues for the search for life on other planets.

The survival strategies adopted by microorganisms as protection against terrestrial adverse environments extend from organic (Mueller et al., 2005; Schiraldi and De Rosa, 2002; Edwards et al., 2003a, 2004a, 2005b; Wynn-Williams et al., 1999; Wynn-Williams and Edwards, 2000) to inorganic adaptations (Edwards et al., 2004b, 2005a, 2005b; Jorge Villar et al., 2005). The production of photoprotective and accessory pigments is the first strategy and includes screening compounds against UV-radiation (such as scytonemin, parietin, calycin) (Squier et al., 2004; Edwards et al., 2000, 2003b, 2003c), antioxidants

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(carotene or rhizocarpic acid) (Edwards et al., 2003c) and accessory light-harvesting pigments which play an important role in dark, light-deficient environments (phycocyanin or phycoerythrin) (Sloth et al., 2006; Eisele et al., 2000). Finally, the use of compounds for water storage and tolerance in desiccated habitats (Wynn-Williams and Edwards, 2000) (such as hydrated calcium oxalates) has also been reported.

When survival conditions are so stressed that surface growths (epiliths) are nonexistent, microorganisms need additional protection and they migrate inside rocks. In order to survive inside porous rocks (endoliths) or inside cracks (chasmoliths), organisms may induce changes to their closest environment, such as mobilisation of minerals or crystallisation of new mineral phases.

When living several millimetres below the surface strata, the accessibility of sunlight is the determining factor for the selection of a rock matrix. Usually such rocks contain transparent minerals, such as quartz, gypsum or carbonates allowing the radiation to cross the mineral layers and reach the organisms, making chlorophyll functions possible with accessible photosynthetic active radiation, whilst the harmful destructive low wavelength UV-radiation is minimised.

The mobilisation of some iron oxides, such as haematite to the surface of rocks has previously been reported in several endoliths (Jorge Villar et al., 2005; Edwards et al., 2004b) and it has been suggested that the low wavelength radiation protective role of the Fe(III) oxides/hydroxides in this process is a crucial factor. This capability of haematite to filter UV-radiation (Pierson et al., 1993; Clark, 1998) can also been used by organisms to create a mineral screen layer on the rock surface to protect themselves against the lethal high energy, low wavelength radiation (Jorge Villar et al., 2005; Edwards et al., 2004b). Gypsum has also been cited as a UV-screening mineral and several microbial communities have been described living under gypsum layers (Cockell and Knowland, 1999; Jorge Villar et al., 2005; Edwards et al., 2005a). It has also been reported that microorganisms can also induce mineralogical changes, such as calcium carbonate polymorph or magnesite/hydromagnesite transformations (Edwards et al., 2005c).

The most common rock type where endolith or chasmolith colonisation has been described so far is sedimentary rocks, particularly sandstone layers (Friedmann et al., 1986, 1987; Jorge Villar et al., 2005; Edwards et al., 2004b). The porosity and mineralogy of these rocks allows organisms light and water to have an easy access to the interior of these rocks and most endolithic communities have been found in these types of rock. In addition, light coloured rocks, such as marbles or aplites (Jorge Villar et al., 2003), that lack porosity but which have many cracks and fissures can be invaded by organisms, which find the necessary conditions for their survival in fractures.

Although some epilithic organisms have been found living on dark coloured rocks or in external vesicles in terrestrial basalts (Holman et al., 1998) or marine basalts (Thorseth et al., 1992, 1995; Fisk et al., 1998, 2004; Storrie-Lombardi and Fisk, 2004a, 2004b), we present here a complementary spectroscopic and microscopic analysis of endolithic and chasmolithic communities that colonised the inside of a subaerial dark coloured basaltic rock. Based on the classical definition for endoliths (living inside porous rocks) or chasmoliths (living inside cracks in rocks) the organisms analysed in this study were not true endolith or chasmolith per se. The first extremophile community lives behind a radial white crystal (calcite); this type we have termed it 'chasmolith.' The second extremophile lives inside a vacuole in the same black host rock and is termed here 'endolith.' Both vacuole and interface rock-radial mineral crystallisation found here show a thin mineral layer deposited over the fresh rock. This mineral layer could be related to either biotic and/or abiotic weathering processes (Thorseth et al., 1992, 1995; Furnes et al., 2001; Storrie-Lombardi and Fisk, 2004a, 2004b).

The system described here is significant because it opens a new door for the examination of preserved and continuing life inside volcanic rocks and it is therefore relevant in the search for extraterrestrial life in volcanic outcrops during planetary exploration, particularly on the surface of Mars.

Many volcanic episodes have been described on the surface of Mars, from the Noachian period (4600–3500 mya) until recent times. The most important volcanic areas are the Valles Marineris, Tharsis region and Elysium Planitia (Komatsu et al., 2004; Hynek et al., 2003; Fuller and Head, 2002). Valles Marineris is a rift structure which started to be formed in the late Noachian or early Hesperian (this period extends from 3500 to 1800 mya) and some structures can be explained as having originated from possible sub-ice volcanic activity. The characteristic shield volcanoes in the Tharna area and Elysium Planitia are described as of the effusive type; in these regions the volcanic period could have extended from the late Noachian or early Hesperian to Amazonian eras (Chapman and Tanaka, 2001, 2002).

2. Methods

2.1. Specimen

The specimen under study was a black volcanic rock (Fig. 1), of about 7 × 7 cm in size, collected at about 1000 m altitude at 80° N on Spitzbergen Island (Norway) during the AMASE 2004 expedition (Arctic Mars Analogues Svalbard Expedition 2004). The sample was collected from a Miocene flood basalt on Scot-Keltie Fiellet ridge and consisted of a black basaltic rock that exhibited a radial growth of a white mineral (identified as calcite—see below); the first extremophile community, the chasmolithic colonisation was detected beneath these crystals (Fig. 2). In addition, several vacuoles in the volcanic host rock were detected and some of them were connected to the exterior by small orifices. Inside some of these vacuoles (maximum diameter about 1.5 cm, orifice of less than 1 mm) endolithic communities were discovered (Fig. 3).

2.2. Raman spectroscopy

A Renishaw InVia Raman spectrometer with a coupled Leica DMLM microscope has been used to collect the Raman

Fig. 1. Basaltic rock with extremophile colonisation. The external sample surface shown has been exposed to environmental conditions under the Arctic climate at about 1000 m altitude on Svalbard, Norway.

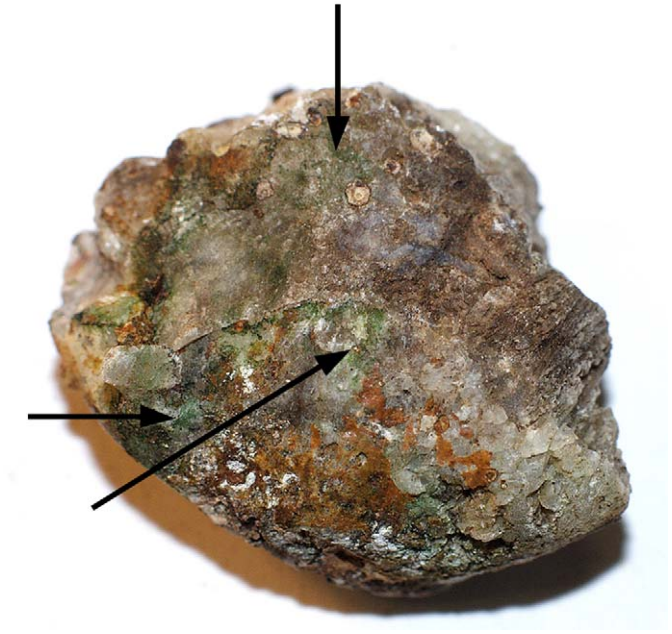

Fig. 2. Internal surface of the white radial crystallisation (calcite) where different colourations are related to different minerals and to the chasmolith colonisations (shown by arrows).

spectra. The sample was analysed using 785 nm (near infrared) and 514 nm (green) laser wavelengths, with ×20 and ×50 objective lenses, giving sample footprints of about 10 and 5 μm, respectively. Between 20 to 70 accumulations, each of 10 s exposure time were used to improve the signal-to-noise ratios of the recorded spectra. The laser powers range from 0.5 to 5 mW and 0.5 to 50 mW for organics and minerals, respectively, when the laser used was 785 nm while at 514 nm laser excitation

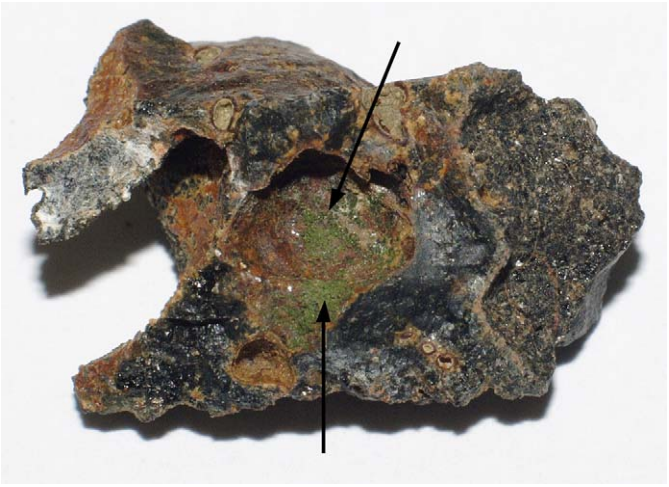

Fig. 3. Vacuole in the basaltic rock where the endolith colonisation (shown by arrows) was detected.

the power used ranged between 0.5 and 2.5 mW. Samples were analysed without pre-treatment.

2.3. Scanning electron microscopy of host rock bacterial communities

The morphology and distribution of mineral phases and microbial communities in both the chasmolith and endolith were imaged using a LEO 1530 Field-Emission Gun Scanning Electron Microscope (FEG-SEM). In addition, qualitative elemental analyses were carried out using an energy dispersive spectrometer (EDS) system that was attached to the FEG-SEM. After Raman analyses, the samples were placed on a stub and coated with 3 nm platinum layer. Imaging was carried out with an accelerating voltage of 3 kV and a working distance of 3–5 mm while EDS spot analyses were carried out at 10 keV.

3. Results and discussion

3.1. Raman spectroscopy

The mineralogy of the host rock as well as the microbial communities has been studied with both laser excitation wavelengths (Table 1). The results obtained show important differences in the ability of the apparatus to identify the relevant inorganic and organic compounds. Whereas most minerals as well as chlorophyll could be identified from their Raman spectra at 785 nm excitation, the 514 nm wavelength was very sensitive to carotenes but not to chlorophyll, which could not be detected in the analyses with the green laser. Furthermore, because of the background fluorescence emission which can swamp the weaker Raman spectra, several minerals could also not be identified using the green laser. The special sensitivity exhibited towards the carotenes, when the 514 nm excitation was used can be ascribed to a resonance Raman scattering which enhances the Raman spectral bands by several orders of magnitude over normal Raman scattering.

The Raman spectrum of the white radial crystals (Fig. 4) shows bands at 1086, 712, 281 and 154 cm^{-1} which were unambiguously assigned to calcite. Below these crystals (Fig. 2),

Table 1
Organic pigments and minerals detected by EDS, and 785 and 514 nm laser wavelengths in the chasmolith and endolith communities

Compounds	Chasmoliths			Endoliths			Characteristic Raman signatures (cm^{-1})
	785 nm	514 nm	EDS	785 nm	514 nm	EDS	
Organic matter			X			X	
Chlorophyll	X			X			1438, 1326, 1285, 986, 915, 517
Carotene							
Lutein	X	X		X	X		1525, 1156, 1002
β-Carotene		X		X	X		1513, 1156, 1005
Astaxanthine					X		1508, 1155, 1005
c-Phycocyanin				X	X		1642, 1629, 1580, 1467, 1369, 1272, 1110, 814, 664
Minerals							
Calcite	X		X	X			1086, 712, 281, 156
Apatite	X		X			X	963, 587, 444
Haematite	X			X			223,291, 405, 608
Lepidocrocite	X			X			343, 379, 500
Anatase			X	X			141, 194, 395, 512, 636
Quartz			X	X		X	201, 263, 354, 394, 463, 693, 795
Anglesite				X			979
Strontianite				X		X	1067
Cerussite				X			1048
Feroxyhyte				X?			280, 412, 686
Iron oxides			X			X	
Aluminosilicates			X			X	
NaCl			X				
Gypsum			X			X	1135, 1007, 669, 621, 492, 413

Note. We have also added the characteristic Raman bands for an unambiguous spectral identification.

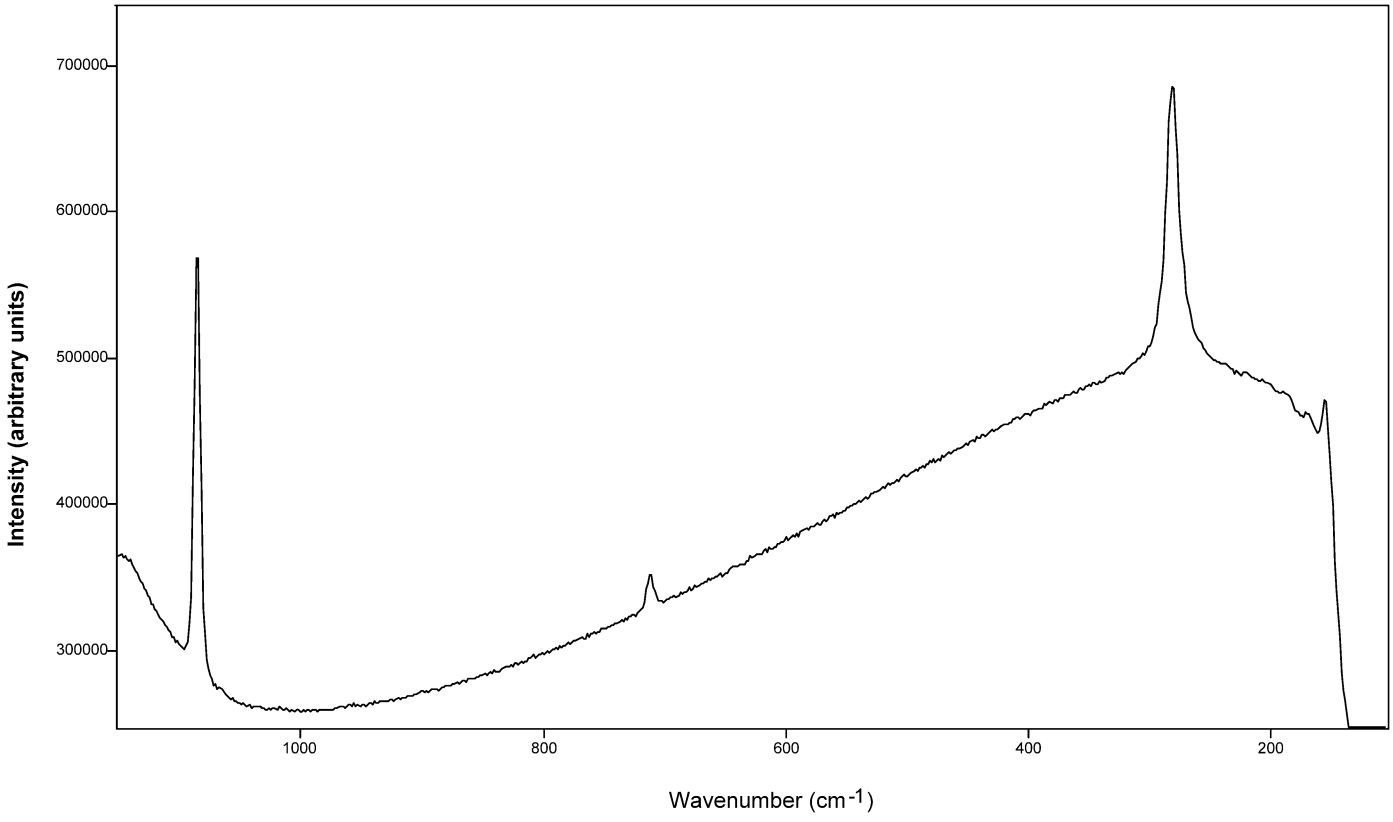

Fig. 4. Raman spectrum of the radial white crystallisation showing the characteristic bands of calcite (785 nm laser excitation).

several analyses were carried out and the spectra give Raman bands at 963, 587 and 444 cm^{-1} assigned to apatite, a calcium phosphate mineral; bands at 223, 291, 405 and 608 cm^{-1} were attributed to haematite (Fe_2O_3) whereas the signatures at 343, 379 and 500 cm^{-1} have been assigned to lepidocrocite (gamma-FeOOH).

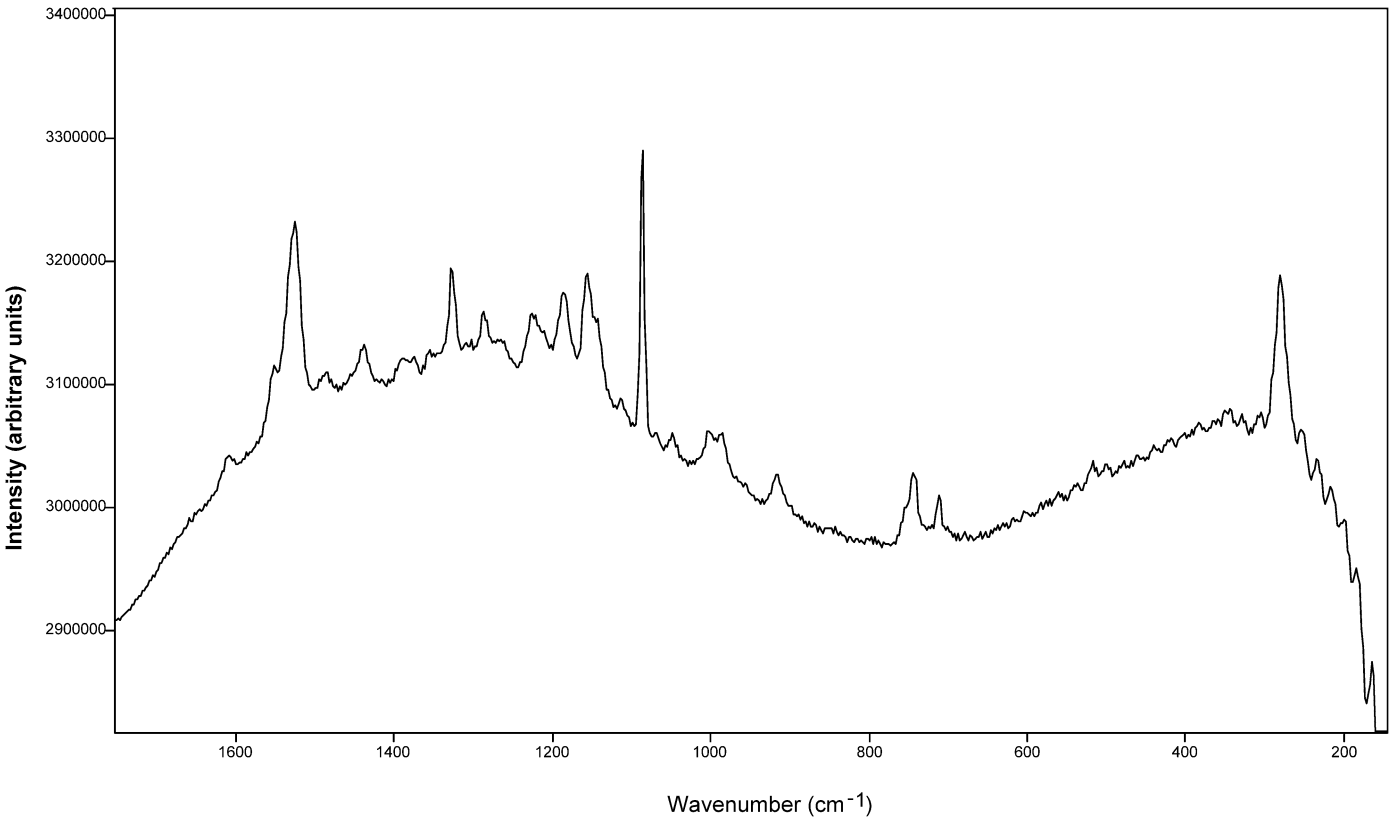

Fig. 5. Raman spectrum collected with 785 nm laser wavelength of the organic area of the specimen in Fig. 3. Signatures of calcite, carotene and chlorophyll are clearly differentiated.

In spite of the relatively large number of analyses (approx. 30) of the chasmolith community carried out using the 785 nm laser excitation, few Raman spectra with organic signatures were identified (Table 1). In some places, bands of a carotenoid at 1525, 1156 and 1002 cm^{-1}, which could be attributed to lutein, were found. Elsewhere, the signatures for chlorophyll (1438, 1387, 1326, 1285, 1225, 1048, 986, 915, 743 and 517 cm^{-1}) were clearly visible, sometimes together with the Raman bands of calcite (Fig. 5). However, with the 514 nm wavelength excitation carotenes were easily detected because of the resonance Raman effect but no other organic signatures were observable in the Raman spectra using this wavelength.

Two different carotenes, one with the strongest bands at 1524, 1157 and 1006 cm^{-1} (probably lutein) and second one with signatures at 1513, 1156 and 1005 cm^{-1} (probably beta-carotene) (Fig. 6), have been identified in the chasmolith community using the 514 nm laser excitation. Beta-carotene was not identified here using the 785 nm laser excitation, probably because the organisms that produce this compound appear in too low proportion for the detection capability of this laser.

The studied vacuole presents a richer and more varied mineralogy (Fig. 7, Table 1). Some spectra show the characteristic Raman bands of anatase (TiO_2) at 141, 194, 395, 512 and 636 cm^{-1}; quartz (SiO_2) at 201, 262, 354, 394, 463, 693, 795, 1063, 1086 and 1160 cm^{-1} and calcite (1086, 712, 281 and 154 cm^{-1}). A Raman band at 979 cm^{-1} was assigned to anglesite (a lead sulphate) and the bands at 1067 cm^{-1} and 1048 cm^{-1} to strontianite (strontium carbonate) and cerussite (a lead carbonate), respectively.

In addition, inside the vacuole several iron oxide phases were identified by Raman spectroscopy. The signatures at 223, 290, 408 and 608 cm^{-1} are characteristic of haematite, whereas the bands at 343 and 379 cm^{-1} were assigned to lepidocrocite. We have also identified rather broad bands at 280, 412 and 686 cm^{-1}. Based on this Raman signature the bands could be assigned to feroxyhyte (delta-FeOOH) or possibly another oxide/hydroxide of Fe(III), but the occurrence of feroxyhyte is less probable due to the low stability of this mineral at ambient oxidised conditions. The assignment of these bands is still in debate.

Several spectra of the endolithic community have been recorded using the 785 nm laser excitation (Table 1). The two carotenes previously identified in the chasmolithic community (Fig. 3) have also been identified in the endolith; again, the most common carotene shows Raman signatures at 1525, 1155 and 1002 cm^{-1} (lutein) (Fig. 8). Furthermore, additional bands of chlorophyll and carotene were observed in all spectra in the endolith sample.

When the carotene spectrum shows signatures at 1514, 1155 and 1003 cm^{-1} (probably beta-carotene) bands at 1642, 1629, 1580, 1467, 1369, 1272, 1236, 1110, 969, 814, 664, 506, 356 and 211 cm^{-1} appear too (Fig. 9) and they have been assigned to c-phycocyanin, a pigment which works as an accessory light-harvesting molecule and which assists in collecting as much light as possible in a dark, relatively light-impermeable vac-

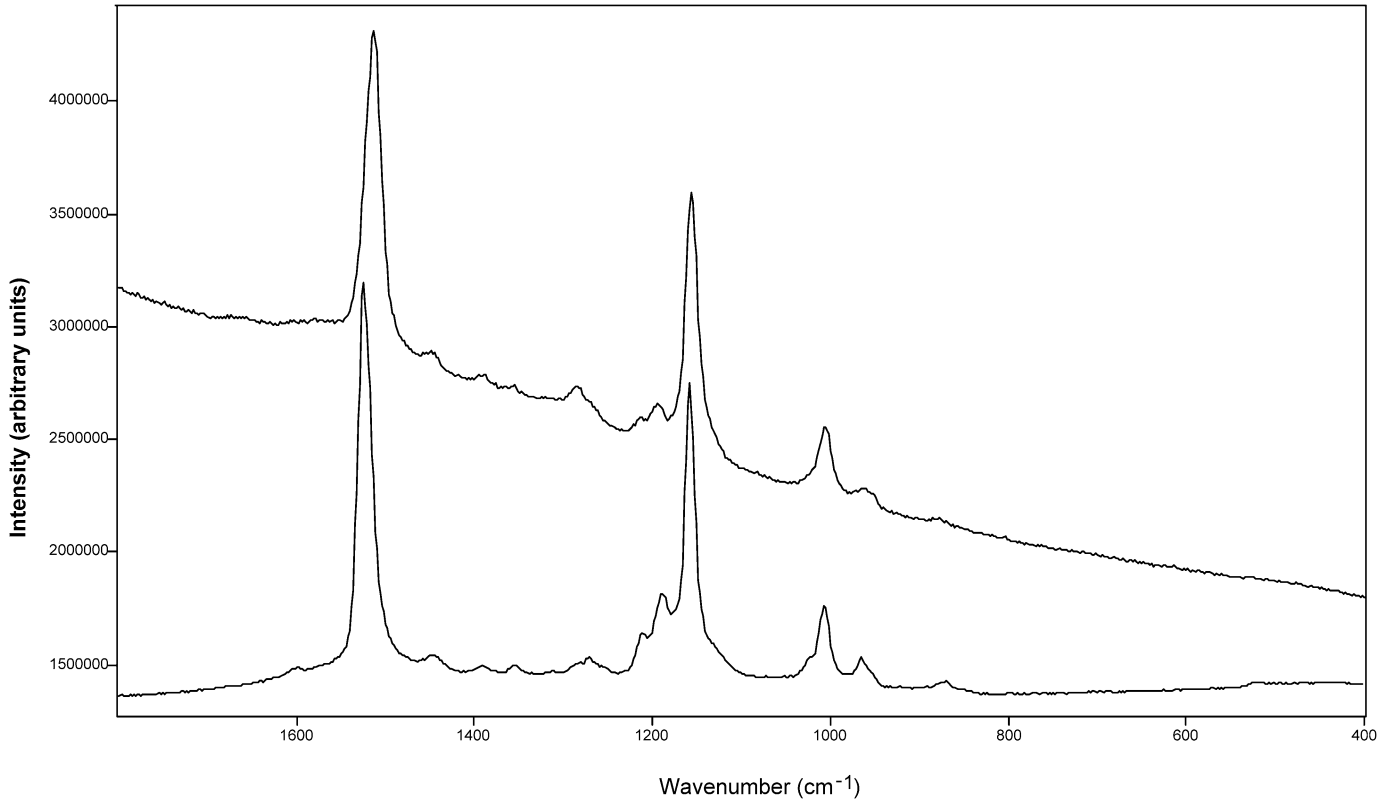

Fig. 6. Raman spectra collected using the 514 nm laser excitation showing the different carotenes found in the vacuole and identified by the shift in the highest wavenumber Raman band, at 1524 and 1513 cm^{-1}. It should be noted that no bands of chlorophyll are visible.

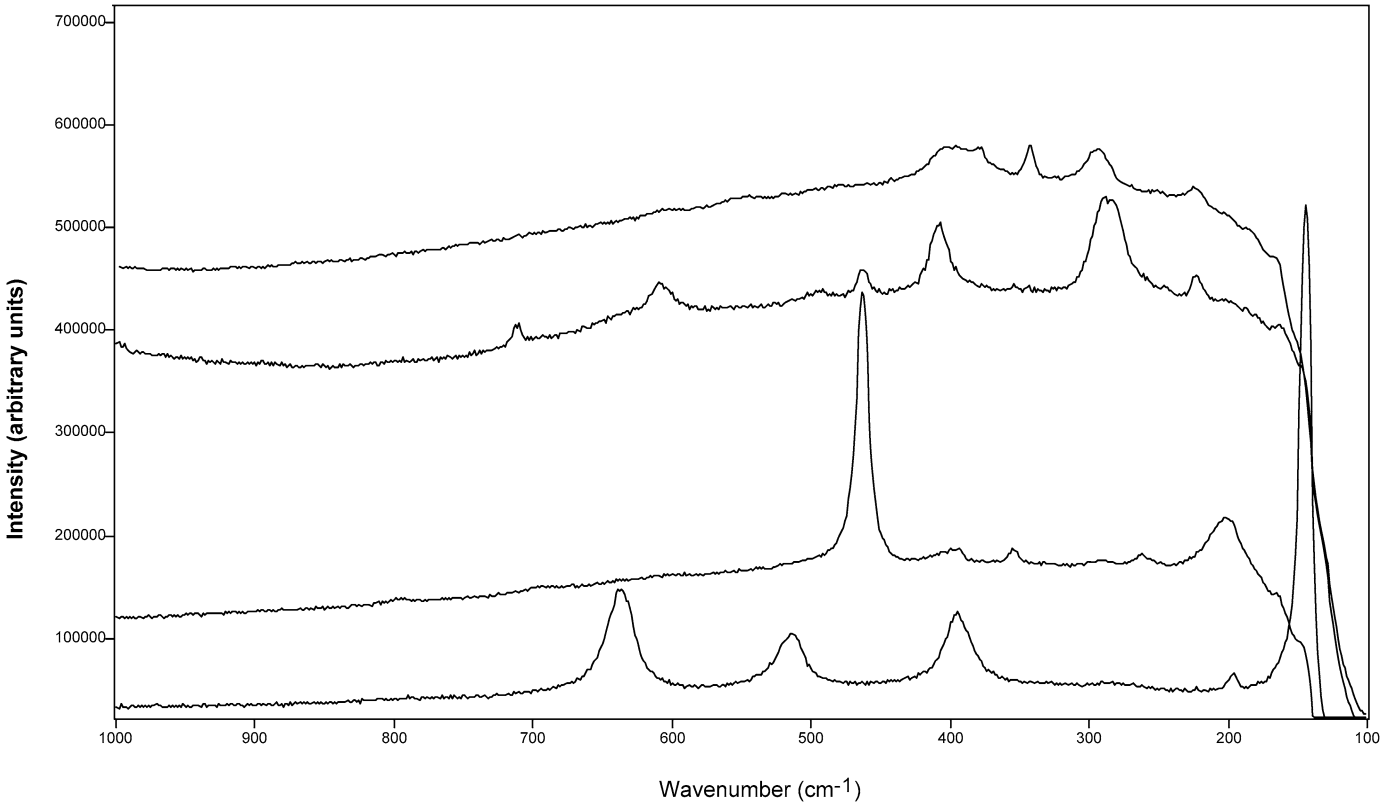

Fig. 7. From top to bottom, Raman spectra of lepidocrocite and haematite; haematite with a weak signature of quartz and calcite signatures at 713 and 281 cm^{-1}; quartz and, finally, anatase. All these spectra have been collected from the vacuole.

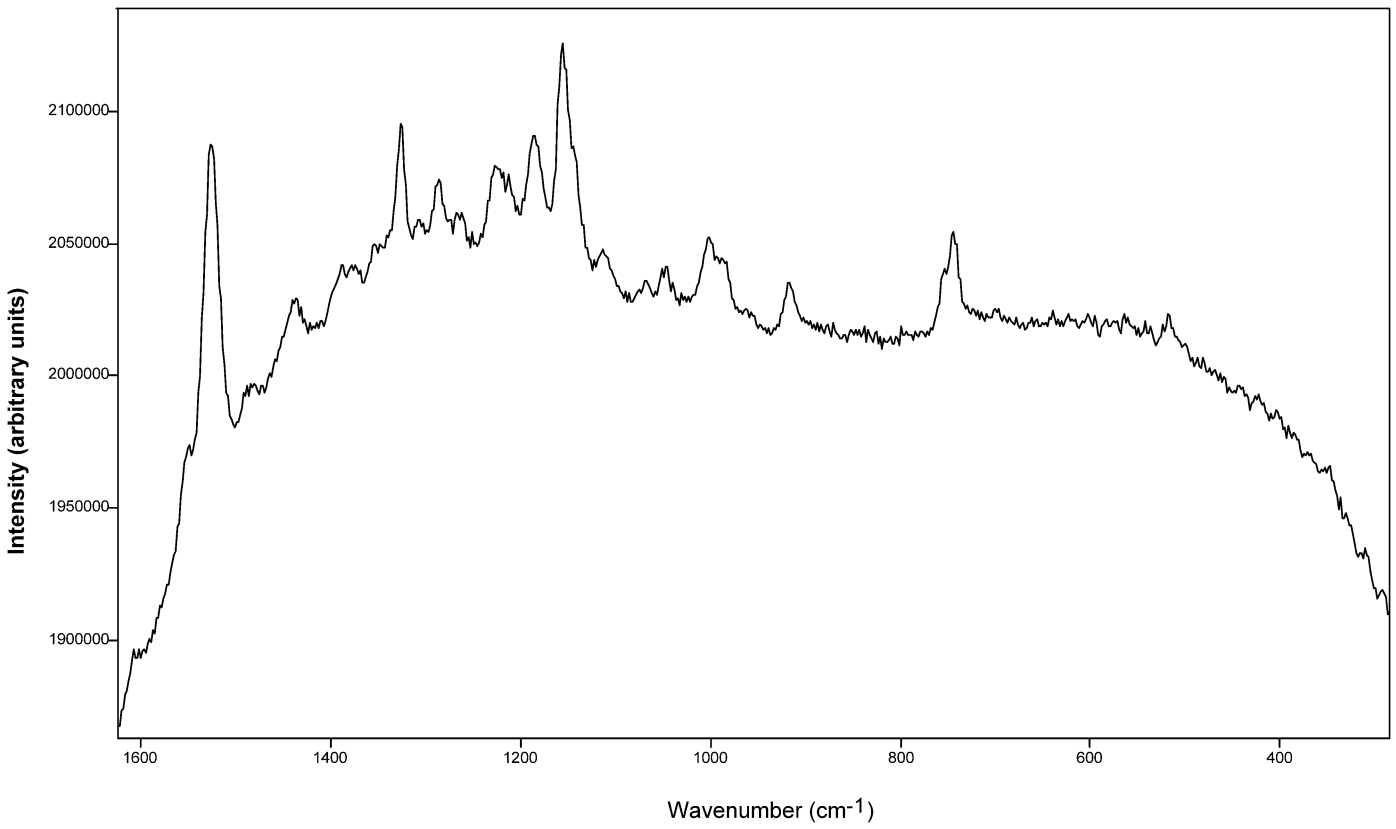

Fig. 8. Raman spectrum of the vacuole colonisation showing bands of carotene (lutein) and chlorophyll collected with the 785 nm laser excitation wavelength.

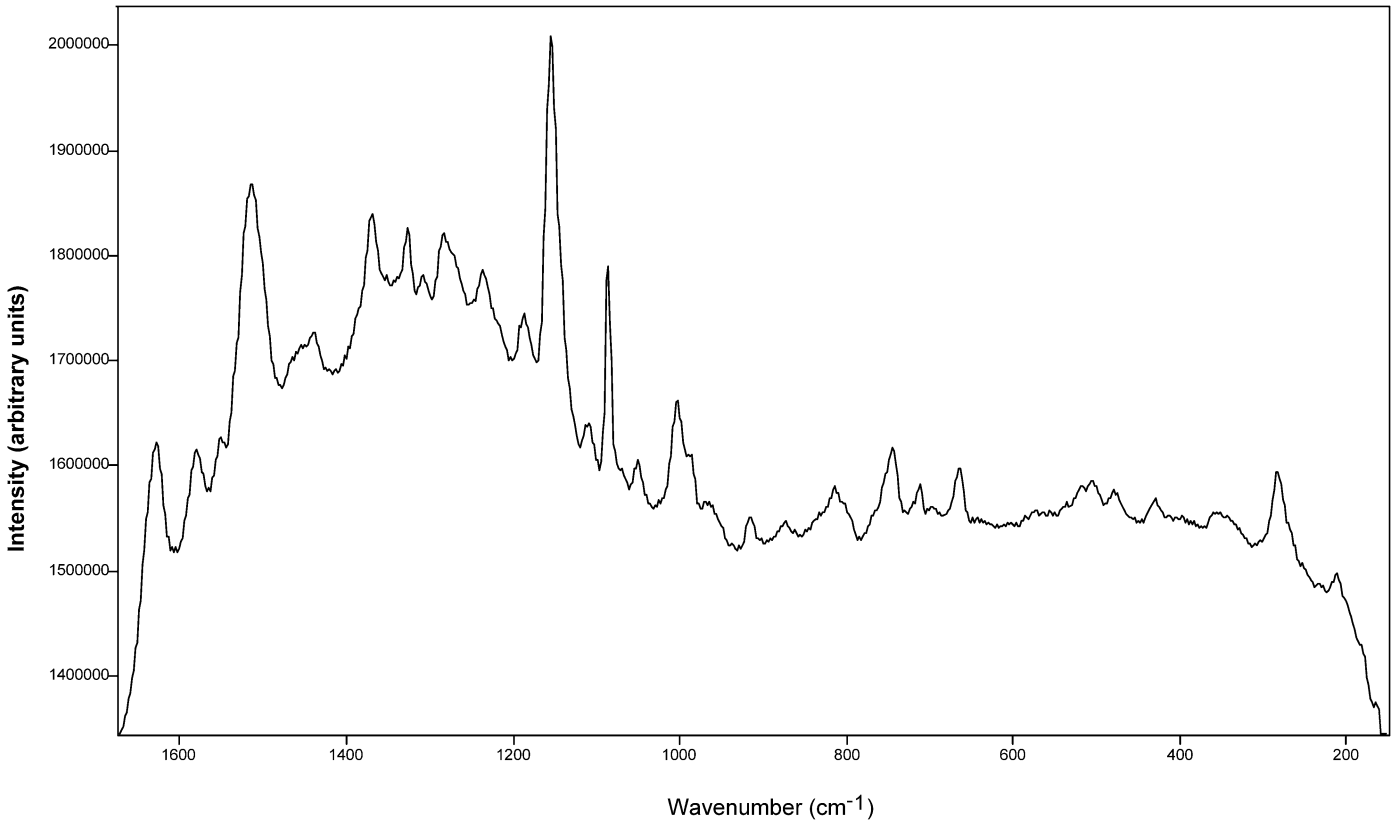

Fig. 9. Raman spectrum of carotene and c-phycocyanin collected from the endolithic community inside the vacuole using the 785 nm laser excitation.

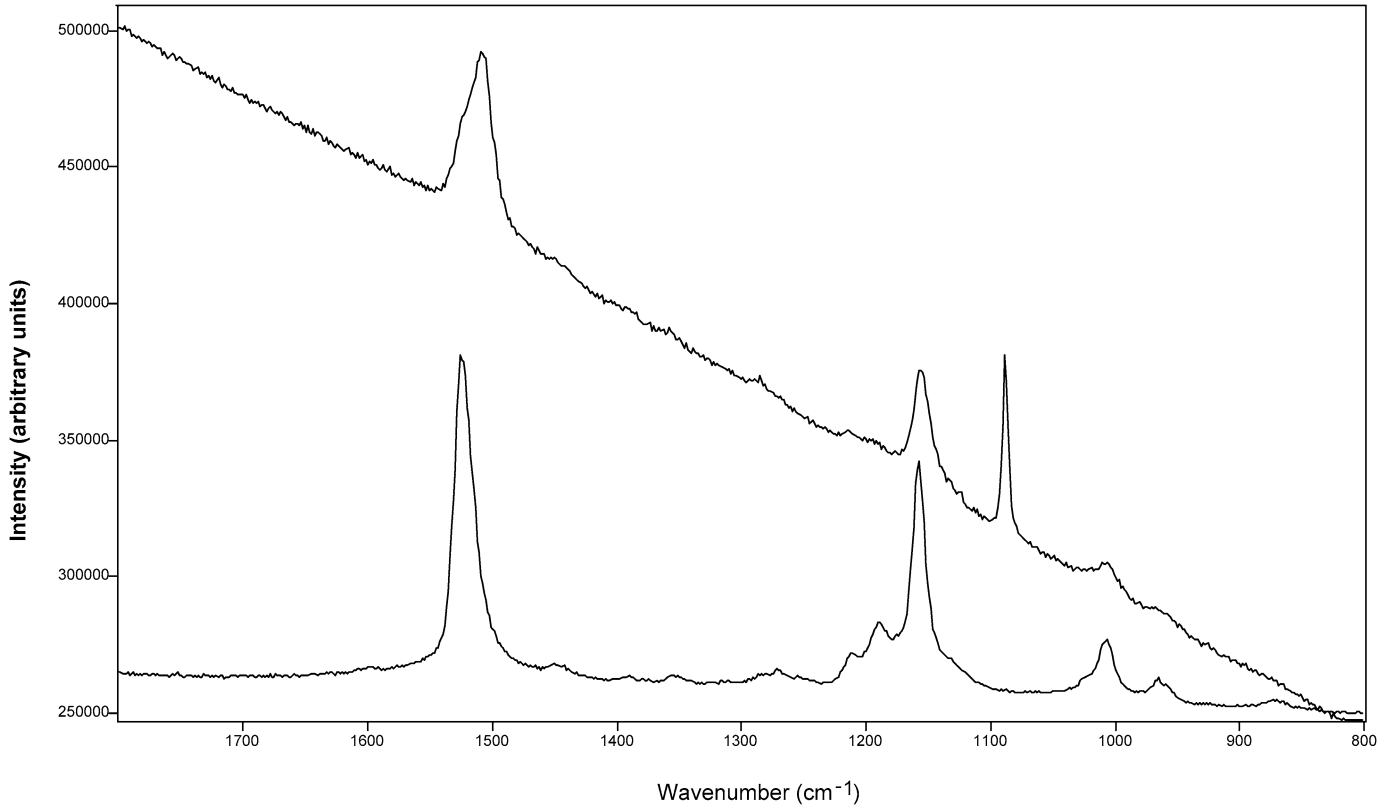

Fig. 10. (Top) Raman spectrum of carotene (probably astaxanthine) and lutein (seen as a shoulder); (bottom) lutein Raman spectrum. Both spectra have been collected on the endolith community using the 514 nm laser excitation wavelength.

uole. This pigment appears together with chlorophyll but it has never been found associated with the carotene lutein that is represented by the bands at 1524, 1155 and 1001 cm^{-1}.

Using the 514 nm laser, three different carotenes were identified in the endolith community. These are the two carotenes previously identified with the 785 nm excitation wavelength and a third compound with bands at 1508, 1155 and 1005 cm^{-1} These bands represent a carotenoid with a long 12 C=C chain length, such as astaxanthine. Despite of the numerous analysis carried out on the endolith relatively few spectra show this type of carotene (Fig. 10), which is easily recognisable because of the low wavenumber of one of the main bands; this can be attributed to the low density of this type of organism or pigment in this sample and it explains why it was not detected using the 785 nm laser excitation.

When the spectra recorded at 514 nm laser excitation show the characteristic signatures of beta-carotene, additional broad bands at 1667, 1587 cm^{-1} and a very broad band with several shoulders at 1441, 1382, 1354, 1324 and 1289 cm^{-1} together with the bands at 962 and 812 cm^{-1} are also clearly visible in some spectra. These could indicate the presence of c-phycocyanin although the assignments are somewhat speculative at this stage

3.2. Microscopic imaging and EDS results

The FEG-SEM images of the chasmolith sample, clearly shows the presence of three distinct microbial communities in this sample (Figs. 11a–11c). A large filamentous assembly was found in front of the calcite crystal (Fig. 11c) and two communities (one coccus and one smaller vibrio type) were found behind and on the side of the radial calcite crystal (Figs. 11a and 11b, respectively). In all cases EDS analysis confirmed that these structures were organic in nature (see EDS spectrum (d) in Fig. 11). In addition, EDS analysis also confirmed the presence of a variety of mineral phases that have been identified during the Raman analysis, such as apatite (Ca–P–O peaks) or iron oxides (Fe–O peaks). However, in addition, the EDS analysis showed the presence of peaks that could be assigned to other minerals, i.e., anatase (Ti–O), quartz (Si–O peaks), aluminosilicates (Si, O, Al, K, Na, Mg peaks), NaCl and gypsum (Ca–S–O peaks). These minerals were not identified by Raman probably due to fluorescence effects.

When the endolith sample in the vacuole was imaged using the FEG-SEM (Figs. 12a–12d) up to 4 different microorganism types could be identified. Close to the orifice, a mixed community consisting of a filamentous and a coccoid shaped organism was detected (Fig. 12a), while inside the vacuole two other organisms (a vibrio and a coccus) were clearly visible (Figs. 12c and 12d). These corroborate the results from the Raman data in the endolith. Based on the Raman bands characteristic of organic compounds the presence of three to possibly 4 different organisms can be stipulated, with the organisms in the centre exhibiting additional light harvesting pigment signatures. From the EDS analysis of the organisms, large carbon peaks were seen for all organisms. When the minerals were analysed we

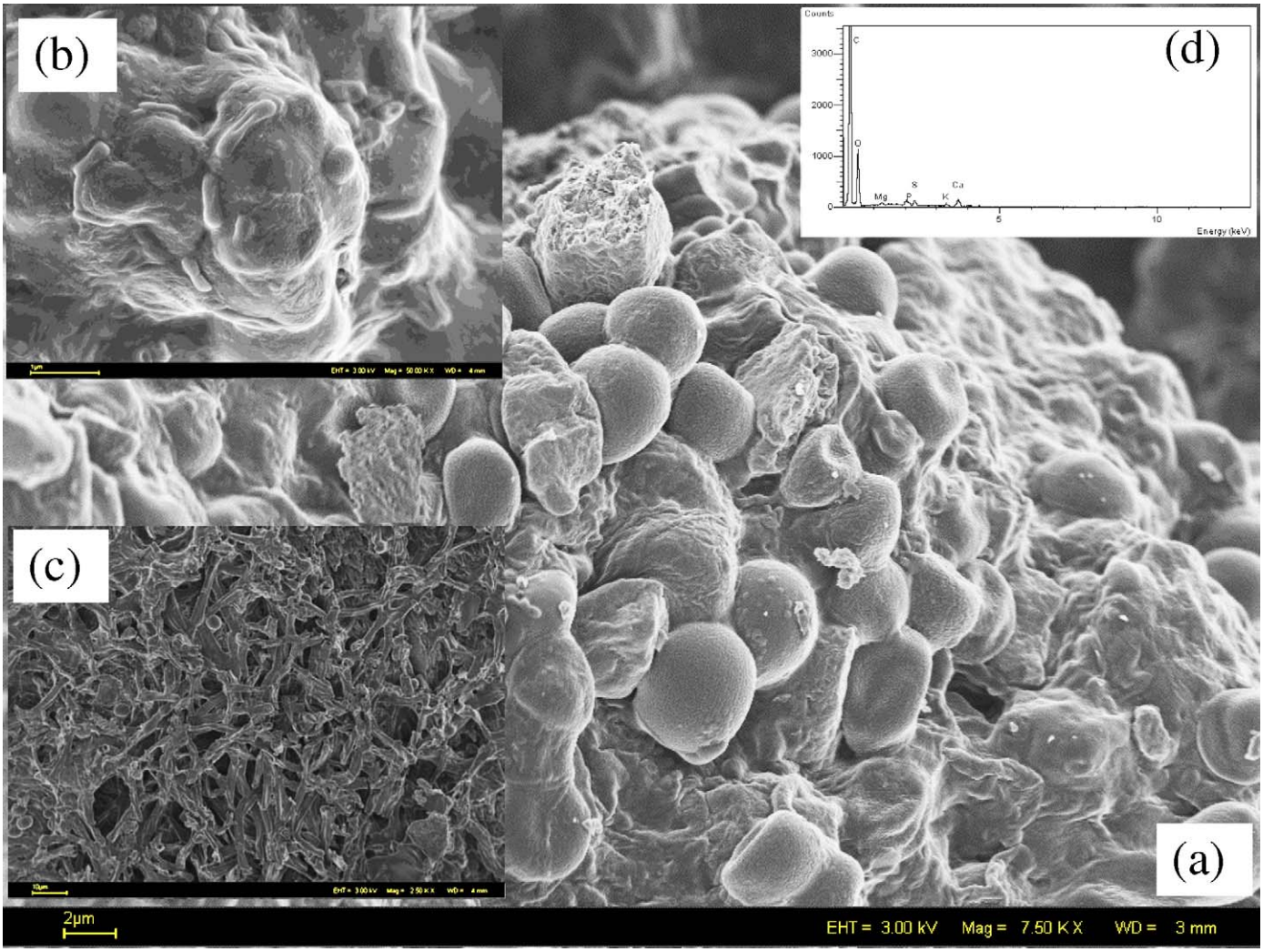

Fig. 11. FEG-SEM images of the 3 different microbial types and an associated EDS spectrum from the chasmolith. (a, b and c) Scale bars are 2, 1 and 10 µm, respectively; (d) EDS spectrum collected on the biofilm shown in (a) clearly showing the C peak.

could again confirm the presence of gypsum, apatite, quartz, aluminosilicates, iron oxides as well as strontianite; in the EDS analysis no lead containing minerals were found.

4. Conclusions

There are references in the literature about organisms living on lava substrates (Thorseth et al., 1992; Fisk et al., 1998; Storrie-Lombardi and Fisk, 2004a, 2004b) but what we present here are extremophile organisms living inside dark lava vacuoles and behind light minerals associated with such basaltic dark rocks. Both these features could serve as a protective habitat against extreme environmental conditions. Here we present for the first time data pertaining to the presence of chasmolithic and endolithic organisms based on Raman spectroscopic and high-resolution SEM microscopic analyses that show the presence and persistence of microbial colonies in dark coloured rocks containing one or more photosynthetic organisms. Until now, there have been references in the geological literature pertaining to endolith and chasmolith communities inside porous sedimentary rocks and cracks, respectively and on the surface of dark lava (Jorge Villar et al., 2003, 2005; Friedmann et al., 1987). In addition, all literature data point towards such communities inhabiting only light-coloured and/or transparent rocks. However, this work opens a new door for the search for extremophilic organisms living inside dark volcanic rocks and thus may provide a novel insight into the adaptation of organisms to light-deprived environments by the production of additional light-harvesting pigments.

From our study we can infer that there are at least three (possibly 4) different microorganism communities living inside the vacuole. These organisms are clearly differentiated via the molecular Raman spectra of the different carotenes they produce and have been confirmed by microscopic imaging and EDS analysis. Moreover, only the organisms living inside the vacuole which is producing beta-carotene seems to need an additional light-harvesting pigment, such as c-phycocyanin. The most probable explanation is that due to the small hole in the vacuole only tiny amounts of sunlight can reach the interior cavity and therefore, only organisms that were capable of adapting to these conditions by producing extra light harvesting pigments could survive. It is remarkable that no other carotene has

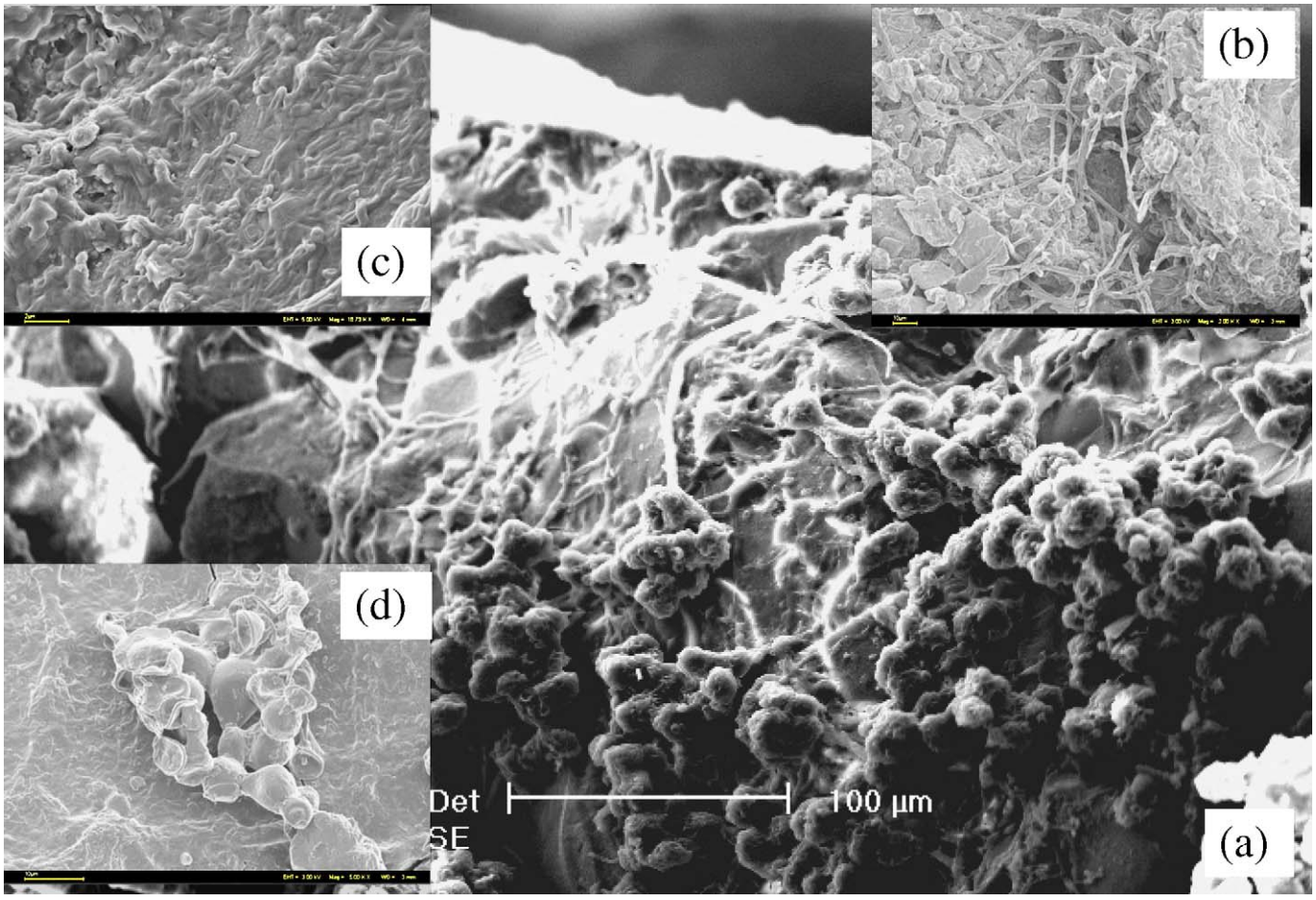

Fig. 12. FEG-SEM images of three, possibly four, distinct organisms observed in the endolith associated with the vacuole sample. (a) and (b) represent microorganisms localised close to the orifice showing large filamentous bacteria and (a) also shows aggregated, possibly fungal, type colonies; bars are 100 and 10 µm, respectively; (c) and (d) resemble yeast/coccoid and vibrio type organisms that were located in the interior of the vacuole; bars are 2 and 10 µm, respectively.

been found related with c-phycocyanin among the organisms analysed in this study although chlorophyll was found along side all the carotenes.

From the Raman data only two different types of organisms were clearly identified in the chasmolithic community. These require no extra light-harvesting pigment yet carotene and chlorophyll were found associated with these organisms. This can be explained on the basis that the white translucent radial calcite crystal permits enough sunlight to reach the surface of the sample and thus the organisms can produce enough chlorophyll for their metabolism without resorting to the synthesis of additional pigments. The microscopic and EDS analysis confirmed the presence of three different type of organisms in the chasmolith, but as can be seen in Fig. 11b, two of these organisms occur only in very close association with each other (possibly even symbiotic) and thus the Raman spectra for the organic compounds in these communities may be a combination of signals from both colonies.

The most common organism community in both the chasmolith and the endolith situations produces a carotene with a 10 double-bonded C=C chain (1525, 1157 and 1006 cm^{-1}) which can be assigned to lutein. In addition, for this community chlorophyll was detected but no accessory pigments were found. The organism community with a longer conjugated chain, such as beta-carotene, are less common and even rarer are the organisms with the longest conjugated carotene chain (1508 cm^{-1} assigned to astaxanthine) equating with 15 C=C bonds. All these carotenes have been identified because of the use of the 514 nm wavelength Raman laser excitation. The confirmation of the presence of specific carotenes in low proportion makes the use of the green laser vital for their detection through the operation of the resonance Raman effect.

The combination of Raman analysis using lasers with different wavelengths with the imaging capabilities of the high-resolution microscopy and the associated EDS analyses proved to be powerful tools for the study of organisms living inside rocks with almost no manipulation of the sample. The non-destructive characterisation of the samples with Raman spectroscopy was followed by a high-vacuum analysis of the same sample using the FEG-SEM instrument. In both samples the Raman and microscopic/EDS data were complementary, showing the presence of several organisms. In the Raman data only the use of both 785 and 514 nm laser wavelengths provided a comprehensive set of organic molecule spectra identifiable by their Raman signatures. For the study of the inorganic phases, the 785 nm laser was found to be more useful, whereas for

the detection of carotenes the 514 nm laser excitation was more sensitive because of the resonance Raman effect. The unambiguous identification of chlorophyll and other organic compounds (specifically also the light-harvesting pigments) was easier when the laser excitation wavelength selected was 785 nm.

With our present work we have demonstrated that for a more comprehensive analysis of mineralisation and organisms the use of both laser wavelengths and complementary microscopic analysis is critical. The clear link between the organics identified in the Raman spectra using both types of laser excitations with the morphological and spectroscopic confirmation of microorganisms using FEG-SEM shows that such a combined approach provides a sound and novel protocol for organic compound identification, particularly related to carotenoids appearing in low concentration.

The results of the present study not only provide the first combined molecular spectroscopic and elemental analysis of a novel extremophilic system which has not been recognised hitherto, but they are important for the current projects that are being proposed for miniaturised instrumentation for the search for life-detection signals in extraterrestrial exploration missions. Raman spectroscopy is being recommended for adoption on the instrumentation suite of planetary landers and rover vehicles in conjunction with other analytical techniques (among them also more high-resolution microscopic techniques); the relative ease of presentation of the samples to the Raman spectrometer, involving little or no sample preparation is a major factor in the consideration of Raman analyses to be undertaken as prime events, followed by more destructive analytical experiments on the same specimens. This work confirms this protocol and has also provided a new terrestrial situation for the testing of prototype miniaturised Raman spectrometers on field expeditions. The primary colonisation of lava rocks is an important biogeological scenario, which has clear implications for the detection of life on other planets, since extinct or extant volcanic activity is often identified from satellite orbital cameras. Hence, a better understanding of the survival strategies being adopted by extremophiles in terrestrial volcanic regions is vital for our ability to process data that will emerge from extraterrestrial experiments.

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