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Microbial colonization of halite from the hyper-arid Atacama Desert studied by Raman spectroscopy

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The hyper-arid core of the Atacama Desert (Chile) is the driest place on Earth and is considered a close analogue to the extremely arid conditions on the surface of Mars. Microbial life is very rare in soils of this hyper-arid region, and autotrophic micro-organisms are virtually absent. Instead, photosynthetic micro-organisms have successfully colonized the interior of halite crusts, which are widespread in the Atacama Desert. These endoevaporitic colonies are an example of life that has adapted to the extreme dryness by colonizing the interior of rocks that provide enhanced moisture conditions. As such, these colonies represent a novel example of potential life on Mars. Here, we present non-destructive Raman spectroscopical identification of these colonies and their organic remnants. Spectral signatures revealed the presence of UV-protective biomolecules as well as light-harvesting pigments pointing to photosynthetic activity. Compounds of biogenic origin identified within these rocks differed depending on the origins of specimens from particular areas in the desert, with differing environmental conditions. Our results also demonstrate the capability of Raman spectroscopy to identify biomarkers within rocks that have a strong astrobiological potential.

Keywords: Atacama; extremophiles; Mars; biomarkers; cyanobacteria; pigments

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One contribution of 12 to a Theme Issue 'Raman spectroscopic approach to analytical astrobiology: the detection of key geological and biomolecular markers in the search for life'.

1. Introduction

The colonization of the interior of porous and translucent rocks appears to be a common survival strategy among photosynthetic micro-organisms in hot and cold deserts (see Walker & Pace 2007 and references therein). These endolithic micro-organisms find more habitable conditions within the rocks in terms of radiation shielding and access to liquid water, two of the main limiting factors for life in these harsh environments. Endolithic colonization associated with extreme conditions has been shown in environments as diverse as the Antarctic Dry Valleys (dry and cold) or the Atacama Desert (dry and hot). The latter, which was considered as the dry limit of photosynthetic activity and primary production on Earth (Warren-Rhodes *et al.* 2006), presents a clear example of adaptive colonization of the endolithic environment as a response to liquid water deprivation. The abundant and diverse endolithic colonies of photosynthetic cyanobacteria associated with heterotrophic micro-organisms within halite (NaCl) crusts found in the Atacama Desert (Wierzchos *et al.* 2006) occur in sharp contrast to the virtual absence of photosynthetic micro-organisms in the surrounding soils (Navarro-González *et al.* 2003; Connon *et al.* 2007). This is in great part due to the hygroscopic properties of halite, which result in the deliquescence and in the condensation of liquid water within the crusts at relative humidities that otherwise hinder the condensation of liquid water outside the crusts (Davila *et al.* 2008). Simultaneously, halite crusts are translucent for photosynthetically active radiation (PAR) and act as UV-light scatterers when precipitated as a mass of small crystals (Cockell & Raven 2004). The successful colonization of these types of minerals in such extreme conditions of dryness suggests that similar deposits on Mars may have also provided shelter and habitable conditions to micro-organisms in the past, and may still preserve traces of life in the form of organic biomarkers.

Our goal in this work was to search for organic biomarkers in halite crusts from the Atacama Desert, in preparation for a future life detection mission on Mars that may target these types of deposits. To that end, we employed Raman spectroscopy, a valuable analytical technique for planetary exploration because it is sensitive to organic and inorganic compounds and able to unambiguously identify key spectral markers in a mixture of biological and geological components (e.g. Villar & Edwards 2006). As a part of the scientific payload of the forthcoming probes planned within future missions aimed at searching for life on Mars (e.g. ESA's ExoMars mission), Raman spectroscopy is considered as a primary non-destructive tool to identify both organic and inorganic compounds on Mars. Raman spectroscopy is a powerful tool for the characterization of various biomarkers (especially pigments) that are produced by microbial colonies in extreme habitats as part of their survival strategy (e.g. Wynn-Williams & Edwards 2000*a*,*b*; Edwards *et al.* 2005*a*,*b*, 2006; Villar *et al.* 2005; Villar & Edwards 2006; Marshall *et al.* 2007). One group of such compounds are UV-protective pigments (scytonemin, parietin or carotenoids, for example). Wilson *et al.* (2007) successfully tested surface-enhanced Raman spectroscopy to detect scytonemin and carotenoids of endolithic cyanobacteria at nanomolar concentrations. In our previous work, we have reported on the lowest concentrations of β-carotene detectable by ordinary Raman micro-spectrometry in the evaporitic matrices, which are 0.1–10 mg kg^{-1}, depending on

the particular matrix, excitation wavelength and the number of Raman bands registered (Vítek *et al.* 2009*a*,*b*). Recently, haloarchaeal communities entrapped in fluid inclusions of laboratory-made halite crystals were successfully analysed by Raman spectroscopy (Fendrihan *et al.* 2009). Here, following Darwin's interest in exploration of life and its adaptations (Darwin 1859), we report the successful application of Raman spectroscopy to the study of endolithic microbial colonization of natural halite crust from the different zones of the hyper-arid core of the Atacama Desert.

2. Material and methods

(*a*) *Samples and study area*

Halite samples depicted in figure 1*a–g* were obtained from three different areas of the Atacama Desert (figure 2). The samples were collected from 'salars' within the hyper-arid core, which have a large spatial distribution. Halite crusts have characteristic irregular shapes, formed by wind action and partial dissolution and reprecipitation of evaporitic deposits. Two samples (YUN-01 and YUN-02) were taken from the Yungay area (24°05′53″ S; 69°55′59″ W)—where mean annual precipitation is less than 1 mm yr^{-1} (McKay *et al.* 2003). This area is located 60 km from the coast at an altitude of 962 m and lies between two mountain ranges—the coastal mountains to the west (1000–3000 m high) and the Domeyko Mountains to the east (about 4000 m high). The coastal mountains block most of the marine fog from reaching Yungay, except in very rare episodes. The other sampling sites are located north of Yungay. Sample CDLR (20°20′35″ S; 70°01′11″ W) was obtained from a small field of halite crusts located to the east of the coastal town of Iquique, approximately 12 km inland. Finally, three samples (SG-01, SG-02 and SG-03) were obtained from Salar Grande (north: 20°55′44″ S; 70°00′48″ W and south: 21°08′54″ S; 70°01′04″ W). Samples were collected during a field expedition in April 2008, and the samples were stored in the dark, at approximately 20 per cent relative humidity, and 22°C, until Raman analysis (January 2009).

In all sampling places, the air temperature and air relative humidity data were collected during 12 months from May 2008. To this purpose, we used temperature (T) and relative humidity (RH) sensors with data loggers (Onset, HOBO Pro v2). The RH/T sensors were placed on the soil adjacent to the crusts in the shadow. The temperature registered from these sensors will therefore be a function of the air temperature and the radiation heat from the soil. According to information from different sources, no rain was observed in sampling areas from 2007. The T/RH data from sampling zones are shown in table 1.

(*b*) *X-ray diffraction analysis*

The mineralogical composition of the halite rocks was determined by X-ray powder diffraction measurements using a Bragg-Brentano theta/2theta PANalytical X'Pert PRO alpha1 diffractometer, CuK(α) radiation and a D-500 X-celerator detector (Siemens, Karlsruhe, Germany). Halite rock samples were taken from the colonization zone. Rock samples were dried at 60°C and powdered in agate mortar.

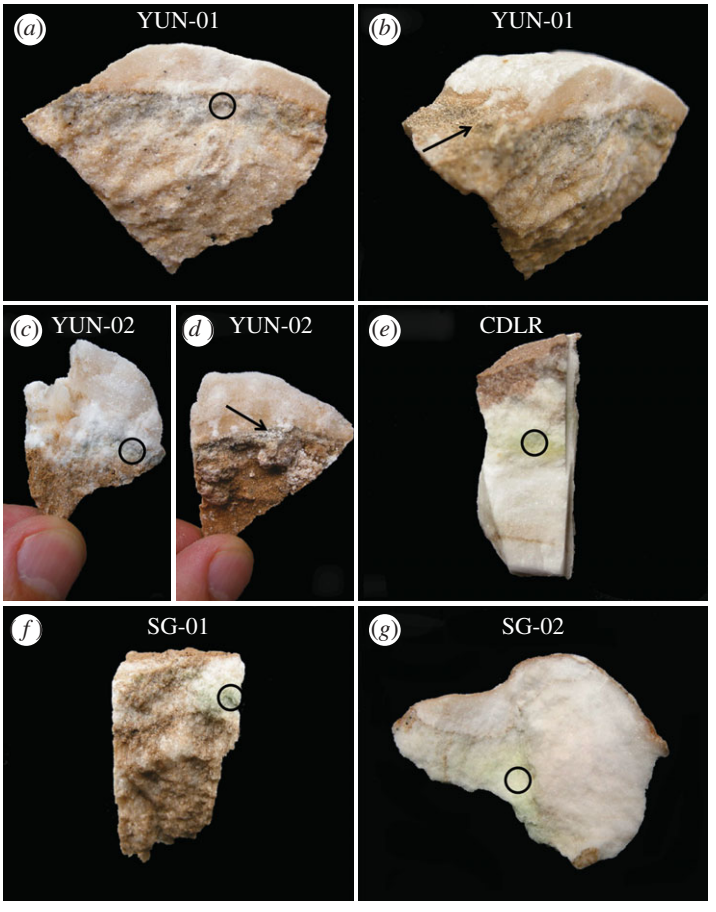

Figure 1. Pictures of the samples studied: the circles point to zones of colonization where Raman measurements were made. Note that, in the samples shown in (*e*), (*f*) and (*g*), the greenish colour indicates the presence of photosynthetic micro-organisms. Mineralogy of rocks beneath and above these layers has been studied as well. The arrows in (*b*,*d*) indicate the Raman measurement area in the dark stripes at the sample surface.

(*c*) *Raman spectroscopy*

Raman analysis of the samples was performed on a Renishaw InVia Reflex Raman micro-spectrometer. Excitation was provided by the 785 nm line of a diode laser. A 20× and 50× lens objective (Leica) was used. Typically, 15 s scans were accumulated 20 times using 15–30 mW laser power for analysis of organic compounds, with reduced irradiance to prevent molecular decomposition, and up to 150 mW for inorganic mineral grains. The zones analysed by the Raman spectrometry are indicated in figure 1. Zones of colonization have been studied preferentially; nevertheless, spectral signatures of the host rock surrounding the colonization patterns have been examined as well. Measurements were replicated at different points to be sure of the representative spectral features of the studied zones related to microbial activity.

All spectra are presented in raw form, except the sulphates.

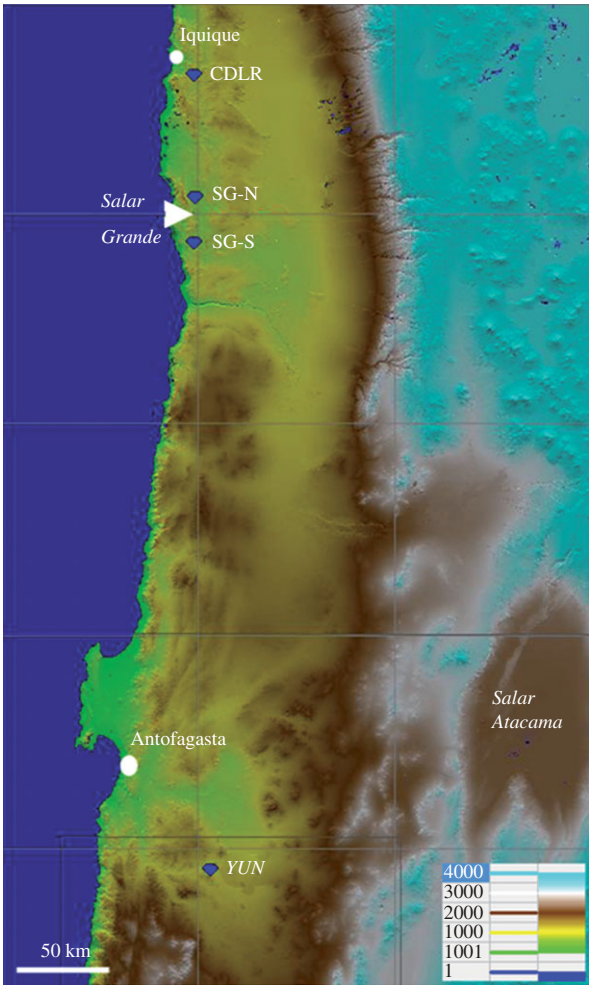

Figure 2. Relief three-dimensional map of the Atacama Desert region obtained by the SRTM (NASA Shuttle Mission) programme with places of halite rock sample collection indicated by blue diamonds.

Table 1. Microclimatic data of temperature (°C) and relative humidity (%) of the air in the sampling sites recorded from May 2008 to May 2009; s.d., standard deviation; n.d., not determined.

	temperature °C yr^{-1}				relative humidity % yr^{-1}			
site	mean	max.	min.	s.d.	mean	max.	min.	s.d.
Yungay	17.83	46.51	−8.00	12.30	36.93	91.90	1.40	23.32
Salar Grande North	20.24	44.57	4.97	9.07	53.97	93.01	2.57	23.26
Salar Grande South	16.28	36.23	5.03	5.76	64.74	89.56	9.11	14.82
Iquique	17.15	35.64	2.56	6.75	n.d.	n.d.	n.d.	n.d.

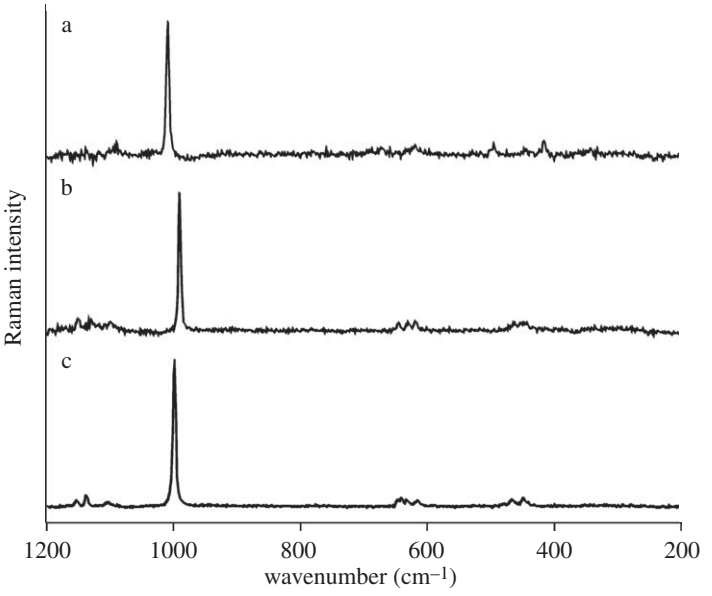

Figure 3. Raman spectra (baseline corrected) of sulphate minerals identified as trace compounds within studied halite samples. a, Gypsum ($CaSO_4{\cdot}2H_2O$) from the CDLR sample; b, thenardite (Na_2SO_4); c, glauberite ($Na_2Ca(SO_4)_2$), both from Yungay samples.

3. Results

(*a*) *X-ray diffraction and Raman analysis of inorganic minerals*

The X-ray diffraction analysis of salt crusts reveals that halite is the major element (97–99%) of all studied samples. Slight differences in mineralogical composition were observed in trace minerals among the samples. The composition of trace minerals in the samples was the following: YUN contains gypsum, anhydrite, quartz, cristobalite, glauberite, feldspars and illite/mica. These trace minerals occur mainly below the zone of colonization and are in accordance with the Raman analysis that has detected poorly distributed grains of quartz, glauberite, feldspars and phyllosilicate minerals. Moreover, efflorescence of thenardite (Na_2SO_4) was recognized on the surface of the halite crust by Raman analysis. Carbonates were not identified within the samples from Yungay. In samples from Salar Grande, gypsum, haematite and anatase were identified; CDLR samples contain gypsum, quartz, calcite and feldspars, respectively. Raman spectra of sulphate minerals identified within studied samples are depicted in figure 3, while other trace minerals are shown in figure 4.

(*b*) *Raman spectroscopy of zones related to endolithic microbial colonization*

(i) *Yungay samples*

Halite samples from Yungay (YUN-01 and YUN-02) revealed characteristic zones of endolithic colonization, as can be seen in figure 1*a–c*. A few millimetres below the sample surface, a pale grey-to-green colonization layer is present. At

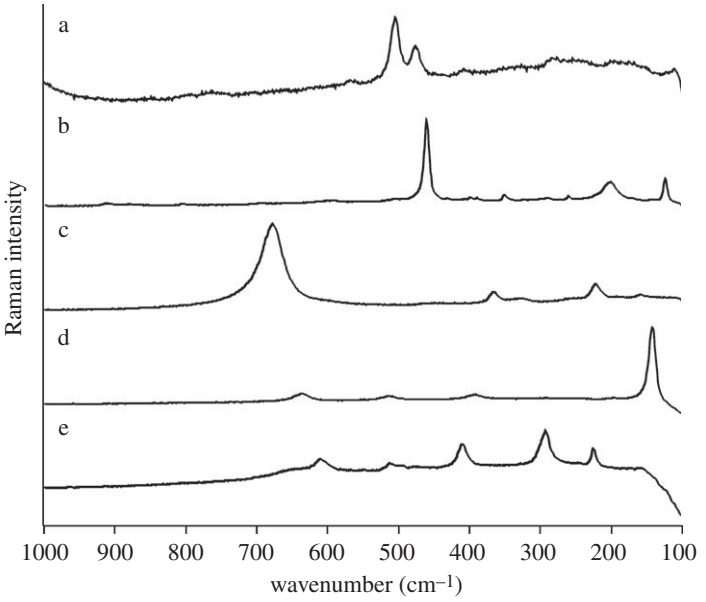

Figure 4. Raman spectra of other inorganic trace minerals detected in halite samples. a, Feldspar; b, quartz; c, not specified phyllosilicate mineral; d, anatase; e, haematite.

high magnification, this layer is resolved as dark spots corresponding to aggregates composed of cyanobacteria and associated heterotrophic micro-organisms, as described by Wierzchos *et al.* (2006). Occasionally, the colonization layers can be traced up to the surface. These features are clearly visible as dense and narrow stripes on the surface of the halite crust, and are markedly different from the interior colonized zone, which is more diffuse. Just organic remains without living cells are considered to form these stripes at the rock surface. Raman spectroscopic analysis of the samples from Yungay revealed organic biomolecules in both—the colonized zone in the interior and the dark stripes at the surface, as can be seen in figures 5 and 6. A strong signal of scytonemin with characteristic Raman bands at 1595 and 1554 cm^{-1} owing to ν(CCH) modes and 1173 cm^{-1} owing to vibration of the ν(C=C−C=C) system (Edwards *et al.* 2000) was detected in the colonized zone of both samples. Other scytonemin bands of medium to weak intensity are also present, as evident in figures 5 and 6 (for detailed assignments, see the paper cited above). The spectral features revealed within these zones are generally almost identical to the Raman signal of the dark stripes at the surface. We also detected minor signals of carotenoid associated with scytonemin. This is evidenced by the presence of Raman bands at 1518, 1155 and 1000 cm^{-1}. These Raman bands appear owing to in-phase ν(C=C) and ν(C−C) stretching vibrations of the polyene chain, and a very weak corroborative band around 1000 cm^{-1} corresponding to the in-plane rocking modes of the CH_3 groups attached to the carotenoid polyene chain coupled with C−C bonds. Additionally, a strong signature at 1322 cm^{-1} is assigned to the combination of chlorophyll and scytonemin signal. In sample YUN-02, a more diffuse, light-toned greenish part of the colonization zone also revealed different spectral signatures. These

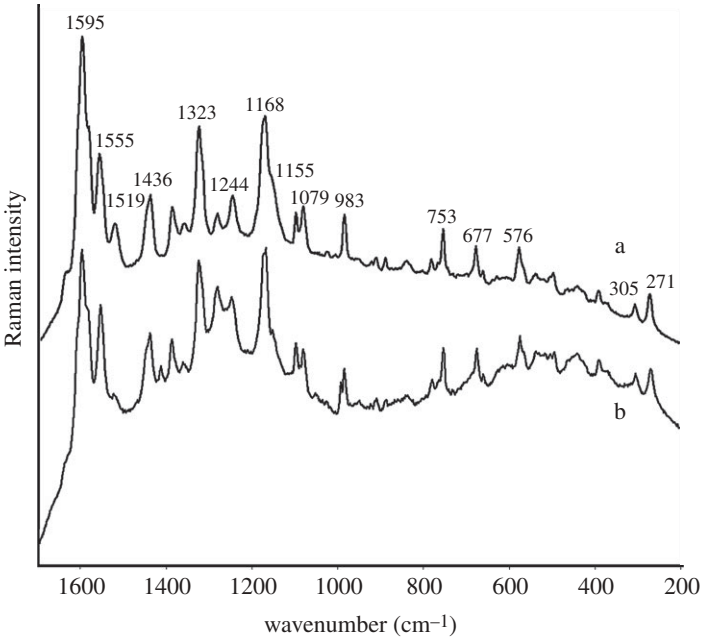

Figure 5. Spectra obtained on the dark-green colonization layer in the halite from a, the Yungay area (YUN-01) and b, dark stripes at the sample surface showing similar spectral signatures pointing to scytonemin, carotenoid and chlorophyll.

parts are localized below the scytonemin-rich zone, further from the surface. The carotenoid signal is much stronger relative to scytonemin in this area. A strong broad band at 1324 cm^{-1} is assigned to chlorophyll.

(*c*) *Salar Grande samples*

We have not found any dependence of the colonization spectral features on the particular locality in Salar Grande (north and south). The halite samples from the Salar Grande contain clearly distinguishable greenish colonization zones (figure 1*f*,*g*). In the SG-01 sample, the colonization zone forms a narrow layer a few millimetres thick, with an intense green colour. Additionally there is a more diffused and dispersed pale green colonization. The colonization zone of the SG-02 sample has a diffused manner (figure 1*g*). In sample SG-03 from the Salar Grande (sample not shown), two different types of colonization were observed—a diffuse zone, pale green in colour, similar to those described above, and a more constrained grey band, resembling those observed in the samples from Yungay. The spectral signatures obtained from the greenish zones of all three samples (figure 7, spectra a and b from the SG-03 sample and c and d from the SG-01 and SG-02 samples, respectively) revealed the presence of a carotenoid compound. Relatively broad ν(C=C) bands in the case of the SG-01 and SG-02 samples centred at approximately 1519 cm^{-1} may be a composite of particular peaks in the range 1515–1524 cm^{-1} as a result of a mixture of different carotenoids or some minor changes in the carotenoid molecule. Other carotenoid bands are located at 1155 and 1003 cm^{-1}. It is probable that the

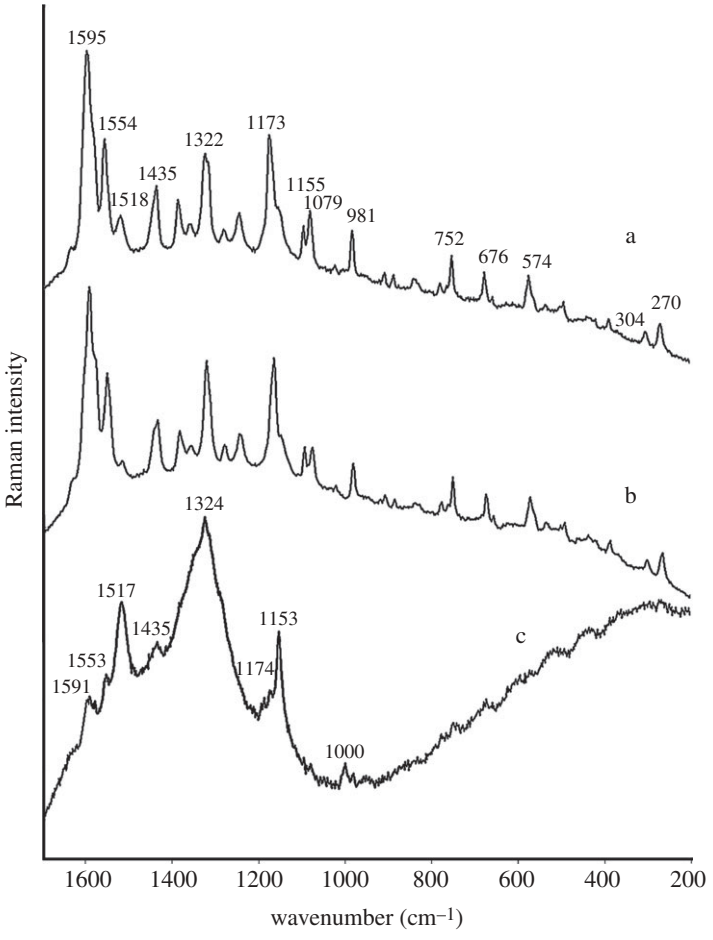

Figure 6. Another sample from the Yungay area (YUN-02) has very similar spectral signatures of the biogenic accumulations in the interior colonization zone as well as dark-green stripes at the surface pointing to scytonemin, carotenoid and chlorophyll. Moreover, the pale green zones associated within the colonization zone have different spectral features with a relatively weaker signal of scytonemin.

carotenoids observed are β-carotene, zeaxanthine and lutein. Lutein is probably also the compound observed in the SG-03 sample, where the sharp ν(C=C) band is located at 1526 cm^{-1}. Chlorophyll was registered by the bands around 1325, 915 and 745 cm^{-1} in all three samples (other chlorophyll bands of weaker intensity can be observed in spectrum b, figure 7). Medium weak to very weak signatures at 1638, 1586, 1468, 1369, 1281, 1236, 1049 and 665 cm^{-1} can be observed in spectra c and d (more clearly evident after baseline correction—data not presented). Based on the analysis of pure standards, these features were assigned to phycobiliproteins, which are accessory light-harvesting pigments. Weak to very weak signals assignable to scytonemin were observed in spectra b–d in figure 7, which are from diffuse colonization zones from the three samples. A markedly different signal was obtained from the constrained grey band in the

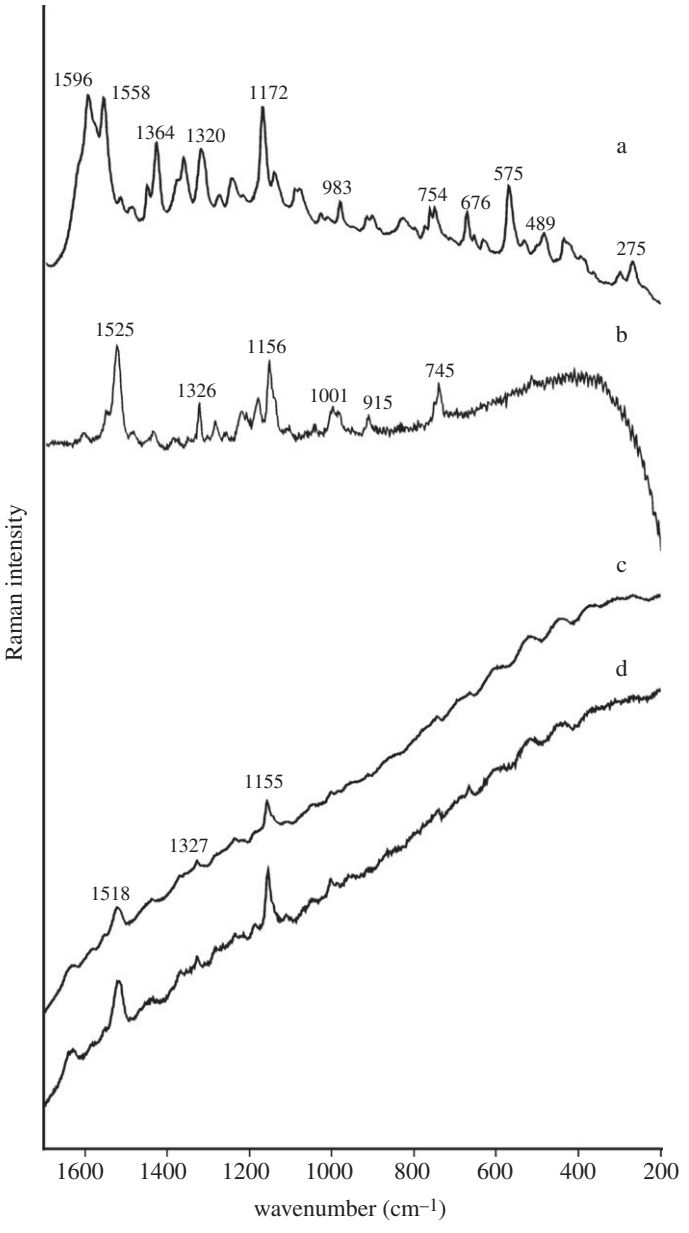

Figure 7. Spectral signatures as obtained from the colonization zones of the three samples from Salar Grande. a, Grey band from the SG-03 sample with a strong scytonemin signal; b, Raman signal from the diffuse colonization zones in sample SG-03 with signatures of carotenoids, chlorophyll and weak scytonemin; c,d, Raman signal from the diffuse colonization zones in samples SG-01 and SG-02 with features of carotenoids, chlorophyll, weak bands owing to phycobiliproteins and very weak signatures of scytonemin.

SG-03 sample (figure 7, spectrum a), where an intense signal of scytonemin was registered by the corroborative bands at 1596, 1558, 1321 and 1172 cm^{-1} (and other bands of medium weak intensity).

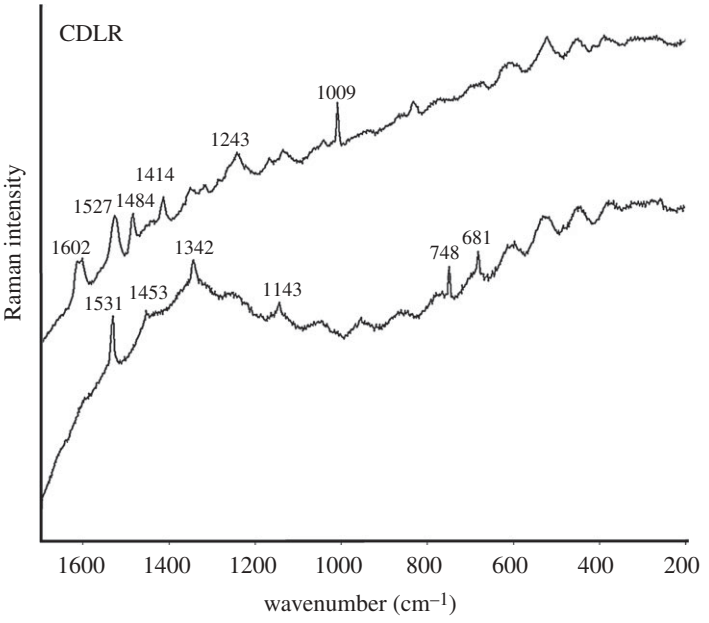

Figure 8. The Raman spectrum of the widespread microbial accumulations in halite from the Iquique area revealed the probable presence of mycosporine-like amino acids (upper) and signatures of porphyrin molecules (lower).

(*d*) *Iquique area sample*

A very different spectral record was obtained from the colonized halite crusts collected near Iquique, where probably a different life story occurs. The colonization pattern in the halite sample from the Iquique area (CDLR) is widespread and diffuse and has a pale green tone, as can be seen in figure 1*e*. The surface of the halite crust is coated by minerals forming a brown cover, while the interior of the crust is white with little clay inclusions. Only very dispersed and small clumps of organics can be recognized at the microscopic scale within the colonization zone. The Raman spectra of these accumulations (figure 8) revealed signatures at 1484, 1414 and 1350 cm^{-1}, which was tentatively attributed to mycosporine-like amino acids. The bands at 1612 and 1602 cm^{-1} can be assigned to a six-member ring possibly related to mycosporine-like amino acids as well. The bands in the lower spectra in figure 8 at 1531, 1452, 1342, 1143, 748 and 681 cm^{-1} are interpreted as porphyrin of unknown origin.

4. Discussion

(*a*) *Detection of organic biomarkers with Raman spectroscopy*

Based on the results of previous work by Wierzchos *et al.* (2006), a cyanobacteria morphospecies is responsible for primary production within the halite crusts. These organisms use solar radiation as an energy source via photosynthesis, taking advantage of the translucent properties of halite for PAR. Simultaneously, these micro-organisms have to be protected against harsh UV radiation. As Raman

microspectrometry does not allow us to quantitatively analyse the chemical composition of studied spots based simply on relative intensities, we have to be careful in further interpretations. Therefore, suggestions regarding scytonemin abundance are based on very strong spectral differences that have been detected repeatedly in multiple spots.

Scytonemin is well known as a UV radiation screening pigment characteristic of cyanobacteria (Proteau *et al.* 1993; Garcia-Pichel 1998; Dillon & Castenholz 1999; Edwards *et al.* 2000), and its Raman spectroscopic detection in these samples is consistent with previous observations (Edwards *et al.* 2005*a*,*b*) and shows that these micro-organisms have evolved adaptations to cope with the extreme conditions in the Atacama Desert. The key parameter controlling the biosynthesis of scytonemin by cyanobacteria is the amount of UVA irradiance (Garcia-Pichel & Castenholz 1991). Together with UV radiation stress, other stress factors can affect synthesis of this screening pigment (Dillon *et al.* 2002). Production of UV-protective pigments is energy consuming and an important part of their survival strategy. In samples from the Yungay region, halite crusts typically contain relatively high amounts of clay minerals in the interior, which often reduce the area for colonization to only a thin layer close to the crust surface. In this case, the Raman detection of abundant scytonemin associated with these microbial communities seems to be a response to the relatively high UV dose approaching the colonization zone owing to its proximity to the surface of the crust. This is a result of limited space for colonization and survival strategy against harmful UV radiation. Climate data from this region also show long periods, often more than three weeks, of extreme dryness with RH no higher than 40 per cent by night, during which little or no primary productivity is expected. During these periods, UV-shielding pigments probably play an important role in protecting the cells. The dark layers visible on the surface of the halite crusts are perhaps mainly composed of dead cells and their sheaths. These traces of cells are probably exposed on the surface of halite owing to repeated salt dissolution and re-precipitation events. Therefore, we interpret our observations of syctonemin, carotenoid and chlorophyll associated with this surface layer as organic remnants of past colonizing cells. These remnants are considered relatively young as no Raman spectroscopic evidence for their degradation was observed.

On the other hand, in the Salar Grande and Iquique area, the halite crust reveals different endolithic colonization patterns. This may be mainly a response to the different texture and structure of the interior of the crusts rather than the geographical location. Contrary to the halite samples from Yungay, these halite crusts are composed almost entirely of pure and white halite where microbial colonization is distributed in a diffuse manner inside the rock. Often, microbial colonization is also found close to the bottom part of the crusts, where micro-organisms probably use light reflected from the ground. Moreover, slightly more habitable conditions for microbial life (higher mean annual relative humidity values) could also induce diffuse colonization of the rock interior. In this interior position, the light radiation could be significantly attenuated by the high amount of halite crystals, and, for this reason, the production of scytonemin by microbes could be diminished. Moreover, samples from Salar Grande and Iquique are typically covered by a layer of dark-brown clay and sand particles. This, together with the presence of impurities above the colonization zones, such as clays and other non-transparent minerals, probably acts as protection against incident

UV radiation, and, therefore, the energy-consuming production of scytonemin is not necessary for micro-organisms in such a case. This fact could contribute to the very low scytonemin signal detected in some parts of these samples (lower three spectra in figure 7). However, when the micro-organism colonies reach the underneath position, a strong signal of the scytonemin pigment is detected, as reflected in spectrum a in figure 7. According to the work of Dillon *et al.* (2002), environmental parameters such as high temperatures or salinity together with UVA dose can also increase the normal production of scytonemin. This phenomenon could affect in part the scytonemin synthesis by cyanobacteria in studied areas; however, this is not fundamental. Work is in progress to confirm this hypothesis as a possible explanation for the spectral differences described above.

Microscopic visualization of endolithic aggregates from the Yungay area revealed that cyanobacteria colonies are surrounded by a layer of heterotrophic bacteria (fig. 2*b* in Wierzchos *et al.* 2006). Heterotrophic bacteria layers adjoining the phototrophic micro-organisms are much thicker and more abundant in the case of endolithic aggregates from Salar Grande (J. Wierzchos 2009, personal communication). This fact can distinctly affect the results of Raman micro-analysis, revealing lower Raman signals characteristic of cyanobacteria morphospecies pigments owing to poorer excitation of these compounds that lie below the heterotrophic bacteria layer and out of laser-induced beam excitation range.

Spectral features obtained from the colonization in the sample originating from the Iquique area are distinctly different from the other samples. One explanation for these differences could indicate the different survival strategy of biota in this locality. Another explanation might point to possible degradation processes of organic matter within the sample in the field and/or during the sample storage time. However, the very probable explanation of Raman spectral differences between the CDLR sample and samples from other localities is that it might be the consequence of biodiversity differences in phototrophic and heterotrophic species. Proposed mycosporine-like amino acids are other UV-screening compounds, being used by many prokaryotic and eukaryotic organisms, especially in a saline environment (see minireview by Oren & Gunde-Cimerman 2007). Conclusive interpretation of the results from this sample remains open.

The results presented here show that local environmental conditions, fabric of halite crusts and their mineralogy may induce a different biota strategy in defence against UV radiation, which can become a relevant factor in the synthesis of UVA screening pigments such as scytonemin. In part, similar conclusions were achieved by Dillon *et al.* (2002) in controlled laboratory studies. Furthermore, our results show that endoevaporitic micro-organisms in the Atacama Desert probably synthesize these types of pigments in abundance under drier conditions, and that these pigments become exposed on the surface of the halite, providing a source of biomarkers readily detectable by Raman spectroscopic and imaging techniques.

(*b*) *Implications for the search for biomarkers on Mars*

The observed tendency of micro-organisms to colonize the interior of rocks in the extreme environments that are considered to be Mars analogues suggests that a similar type of colonization ought to be expected on Mars if life ever arose on

the planet. The core of the Atacama Desert has endured hyper-arid conditions for more than 15 Ma, and therefore evaporitic deposits such as the halite salt flats have been stable on the surface long enough to enable colonization by micro-organisms. The fact that these types of minerals provide better access to liquid water (the limiting factor for life in these extreme environments) suggests that this may be a common strategy among micro-organisms inhabiting extreme hyper-arid environments on Earth and possibly on Mars. The same type of minerals has indeed been detected on the surface of Mars, either as discrete deposits or as components of the soil. Osterloo *et al.* (2008) recently identified and mapped widespread deposits with a chloride salt component in the Terra Syrenum region and across the entire planet at latitudes between −10° and −50°. Individually, most chloride-bearing deposits are small in area (less than 25 km^2), and commonly occur in topographic lows relative to the surrounding terrain. These deposits have been interpreted as the result of brines in an evaporitic environment (Osterloo *et al.* 2008), and their significance resides in their strong similarities to evaporitic deposits found in the Atacama Desert.

Apart from its capability to absorb and retain moisture, halite is also efficient at protecting and shielding micro-organisms and organic compounds for long periods of time. For example, intact and abundant cellulose fibres have been recovered from subsurface halite deposits 250 Ma old (Griffith *et al.* 2008). Spore-forming bacteria have been extracted and reactivated from inclusions in the same halite crystals (Vreeland *et al.* 2000; Satterfield *et al.* 2005). Although these results have been questioned (Hazen & Roedder 2001; Hebsgaard *et al.* 2005), the possibility that dormant microbes or their remnants may be preserved in ancient halite rocks is very distinct and has important implications for astrobiology. In contrast to the surrounding soil, halite crusts from the Atacama Desert concentrate large amounts of organic compounds as a result of biological activity. These organics may be better protected from non-biological oxidation within the halite crusts, and could therefore represent important targets in the search for biogenic signatures. Recently Bada *et al.* (2008) reported that not only organic compounds such as amino acids, but also their chirality would be preserved for billions of years on Mars if trapped within halite crystals, owing to the cold temperatures on the surface of the planet; the same authors, as well as others, concluded that closed basin lacustrine and dry desert valley regions with evaporite-rich deposits would be suitable environments in the search for preserved biosignatures on Mars (Rothschild 1990; Mancinelli *et al.* 2004; Bada *et al.* 2008).

In the work of Vítek *et al.* (2009*a*,*b*), it was proved that, when analysing pure β-carotene in a fine ground mixture with halite, a very low concentration of this biomarker, which is an excellent Raman scatterer, can be detected by Raman micro-spectrometry (e.g. 0.1–1 mg kg^{-1} by the 514.5 nm laser and 1–10 mg kg^{-1} using the 785 nm laser for excitation) with a randomly targeted laser beam. Using mobile Raman instrumentation (785 nm) equipped with a much larger laser spot diameter (without a microscope) can provide similar or even better results for such artificially prepared mixtures than the bench instrument equipped with the same excitation wavelength (P. Vítek, J. Jehlička & H. G. M. Edwards 2009, unpublished data). On the other hand, for *in situ* analysis of natural rock samples without any treatments, employing a Raman microscope to target a particular microbial aggregate seems very important. Positioning of the aggregates of microbial colonies at the micro-scale can be

difficult and time consuming in some cases of widespread diffuse colonization, even appearing as a clear greenish zone at the macroscopic scale. In the salt crystals, microbial communities predominantly accumulate in fluid inclusions, as shown recently by Schubert *et al.* (2009) on the natural samples from Death Valley or by Fendrihan *et al.* (2009) in the case of laboratory-made halite crystals with embedded halophilic archaea. Laser spot size and focusing of the laser beam are also important for such types of colonization, different from the colonization zones studied here. Therefore, if biomarkers are present within Martian rocks, the laser spot size and positioning/focusing method of the future Raman instrumentation used for Mars exploration will be crucial for successful detection of such traces of life on that planet. Indeed, for these reasons, the application of Raman spectroscopy studies for characterization of halite rocks naturally colonized by endoevaporitic micro-organisms in the Atacama Desert is very important in Mars astrobiology.

5. Conclusions

— Endolithic colonies from the halite crust of the Atacama Desert have been identified non-destructively by Raman microspectrometry.
— Signals of various pigments and UV-protective compounds were registered—namely scytonemin, carotenoids, chlorophyll, as well as spectral features attributed to the mycosporine-like amino acids.
— We have detected scytonemin—a UV-protective pigment in samples from the Yungay area as well as from Salar Grande. The spectral features of colonization zones from the particular microhabitat differed; we hypothesize that these differences are the possible result of distinct ways of microbial adaptation to the harsh conditions depending mainly on local mineralogical composition and morphology of the halite crust forming the microhabitat as well as the different biodiversity of halite colonizers.
— The results presented here support the suggestion that evaporitic, chloride-bearing rocks revealed on Mars may represent a suitable habitat for life—in the past or even present—enabling photosynthetic activity and adaptation to a harsh UV and hyper-arid environment.
— Raman microspectrometry appears to be a highly efficient tool in the detection and characterization of biomarkers—traces of present and/or past microbial life are hidden in the evaporitic rocks.

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