Identification of microbial pigments in evaporites using Raman spectroscopy: implications for astrobiology

Petr Vítek

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Identifikace mikrobiálních pigmentů v evaporitech pomocí Ramanovy spektroskopie: využití v astrobiologii

Ph.D. thesis

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Charles University in Prague, Faculty of Sciences

Thesis supervisor

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Identification of microbial pigments in evaporites using Raman spectroscopy: implications for astrobiology

Ph.D. thesis

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Aims of the thesis, suggestions for the reader

The doctoral thesis focuses on Raman spectroscopic identification of pigments as biomarkers in evaporitic rocks in an astrobiological context. The work is intended a) to provide a data, which could shed light to potential of Raman spectroscopic instrumentation to detect selected organic compounds (particularly pigments) in mineral matrix with relevance to future Raman spectroscopic in-situ measurements on Mars and b) to primarily investigate endoevaporitic microbial colonies from the extreme conditions on Earth, namely Atacama Desert, through Raman spectroscopic signatures of pigment compositions analysed directly in the host rock.

The thesis is divided into an „Introduction“, to provide an overview and scientific background of the work to the reader, followed by the part of the results that has not yet been published, a general discussion and conclusions and appendices.

Most of the results are presented as published papers and a submitted manuscript in the appendices and can be divided in two major spheres. First, methodical work was performed, where the possible use of Raman instrumentation (both bench-top and portable) was evaluated for identification β-carotene as a model carotenoid in artificially prepared mixtures with various evaporites under different experimental conditions. The results are presented as two published papers and one manuscript submitted for publication (Appendices I, II, III).

The second part deals with Raman spectroscopic identification of pigments associated with endolithic phototrophs in natural evaporitic crusts from Atacama Desert. The results are presented as one published paper (Appendix IV) and unpublished results which are the subject of Chapter 2.

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List of publications included in the thesis as appendices:

I:
Vítek, P., Osterrothová, K., Jehlička, J. (2009). Beta-carotene—A possible biomarker in the Martian evaporitic environment: Raman micro-spectroscopic study. *Planetary and Space Science* **57**, 454-459.

II:
Vítek, P., Jehlička, J., Edwards, H. G. M., Osterrothová, K. (2009). Identification of β-carotene in an evaporitic matrix — evaluation of Raman spectroscopic analysis for astrobiological research on Mars. *Analytical and Bioanalytical Chemistry* **393**, 1967-1975.

III:
Vítek, P., Edwards, H. G. M., Jehlička, J., Cox, R. Evaluation of portable Raman instrumentation for identification of β-carotene and mellitic acid in two-component mixtures with halite. (Submitted to *Spectrochimica Acta A*).

IV:
Vítek, P., Edwards, H. G. M., Jehlička, J., Ascaso, C., De los Ríos, A., Valea, S., Villar, S. E. J., Davila, A. F., Wierzchos, J. (2010). Microbial colonization of halite from the hyper-arid Atacama Desert studied by Raman spectroscopy. *Philosophical Transactions of the Royal Society A* **368**, 3205-3221.

Summary

Raman spectroscopy is a powerful tool for identification both inorganic and organic compounds including microbial biomolecules. Together with the fact, that it is considered to be the important nondestructive instrument for use on Mars within future robotic missions, it is necessary to assess its capabilities in scenarios relevant for both Martian and terrestrial conditions. In this work, the potential of Raman spectrometry was tested – including both bench-top laboratory systems as well as portable counterparts – to detect traces of life within evaporitic matrices through biomolecular identification. Due to their chemical and physical nature resulting in optical properties, pigments are important organic compounds in Raman spectroscopic analysis using visible excitation. Hence in this work we have focused on the Raman spectroscopic identification of pigments as biomarkers with relevance for investigation of life in both extreme terrestrial and potentially extraterrestrial environments.

Results of methodical work are presented in Appendices I to III, dealing particularly with β-carotene as a model carotenoid pigment. The concentration limits of this biomarker in three different evaporitic matrices (halite, gypsum and epsomite) have been determined for artificially prepared powdered mixtures alone and mixtures analysed through a single crystal of gypsum or epsomite. We detected β-carotene content even as low as 1 mg kg^{-1} in an evaporitic matrix using Raman microspectrometry equipped with 785 nm excitation wavelength (non-resonant mode) which is a universal source for biomolecular identification. Comparison of these results with resonance Raman spectroscopy using a 514.5 nm laser for excitation showed that in this case resonance enhancement of the Raman signal can improve the limits of detection by about one order of magnitude. The analysis performed through the single sulphate crystals resulted in decrease of the Raman signal; however it was still possible to register at least one carotenoid band at concentrations of 1 - 10 mg kg^{-1} of the β-carotene, depending on the excitation wavelength used.

Results obtained using a portable (hand-held) Raman instrumentation equipped with a diode laser at 758 nm showed that Raman macro analysis can be favourable for finely ground mixtures. This miniaturized instrument yielded even slightly better results when analysing a β-carotene/halite mixture than a bench-top Raman microspectrometer using the same excitation.

Real geobiological systems from Atacama Desert which is one of the driest place on Earth and is considered a close analogue to the extremely arid conditions on the surface of Mars were studied as well. Results of Raman spectroscopic analyses of natural endoevaporitic colonies from Ca-sulphate crusts in Atacama Desert (dominated by gypsum, presented in Chapter 2) exhibit systematic variations in carotenoid composition along with the presence/absence of a phycobiliprotein signal. A phycobiliprotein Raman signal is indicative of cyanobacteria and was detected typically in deeper parts of the Ca-sulphate crust where a relatively low amount of light is available. This was accompanied by two clearly distinguished ν(C=C) carotenoid bands at approx. 1516 and 1498 cm^{-1}, pointing to carotenoids of different conjugation, probably including β-carotene and the long polyene chained (13-15 conjugated double bonds) carotenoid. On the other hand, the Raman signal of algal colonies from near the rock surface exhibited a ν(C=C) carotenoid band at approx.

1525 cm^{-1} interpreted as feature of lutein or similar xantophyll compound. These spectra lack a phicobiliprotein signal. Streamline Raman mapping of the algal colonies exhibited a great potential of such type of analysis for study of endolithic communities in their original habitats. Moreover, analytical aspects of using 785 nm and 514.5 nm excitation for analysis of carotenoids are discussed.

Appendix IV describes the Raman spectroscopic identification of pigments from endolithic cyanobacterial colonies in natural halite crusts from Atacama Desert. Spectral signatures revealed the presence of UV-protective biomolecule scytonemin as well as chlorophyll, carotenoids and phycobiliproteins. The spectral features of these biomolecules differed depending on the particular microhabitat. Substantial differences in the scytonemin Raman signal have been observed and suggested to correspond to variable biosynthesis of scytonemin according to the amount of light available inside the halite crust as well as other possible parameters.

It was proved that β-carotene – a typical carotenoid – can be detected in very low content in evaporitic matrix using Raman spectroscopy. The method showed to be also a valuable tool for examination of microbial colonies in their original rock habitats on the basis of pigment composition which allowed us to insight into the phototrophic microbial life in evaporites from Atacama Desert.

Shrnutí

Ramanova spektrometrie je důležitou nedestruktivní analytickou metodou pro identifikaci jak anorganických tak organických látek, včetně některých mikrobiálních biomolekul. Pigmenty, jak vyplývá z jejich fyzikálně-chemické podstaty, jsou důležitými látkami z pohledu spektrální analýzy, včetně Ramanovy spektrometrie. Miniaturní Ramanovský spektrometr bude součástí analytického vybavení sond plánovaných pro astrobiologický výzkum Marsu. Tato doktorská práce je proto zaměřena na vyhodnocení potenciálu Ramanovy spektrometrie – a to jak laboratorních tak přenosných přístrojů – pro detekci pigmentů, jako stop života v evaporitech pro účely výzkumu života jak v extrémních prostředích na Zemi, tak v podmínkách mimo naší planetu – konkrétně na Marsu.

V přílohách I-III je pojednáváno o výsledcích metodické práce zabývající se β-karotenem jako modelovým pigmentem. Byly zkoumány nejnižší možné detekovatelné koncemtrace tohoto biomarkeru ve třech různých evaporitických matricích – v halitu, sádrovci a epsomitu. Připravené směsi o různých koncentracích β-karotenu byly analyzovány jak přímo, tak přes krystal sádrovce či epsomitu pro simulaci analýzy β-karotenu uzavřeného v minerální matrici. Pomocí laseru o vlnové délce 785 nm jsme identifikovali koncentrace β-karotenu nízké až 1 mg kg^{-1}. Srovnání s výsledky rezonanční Ramanovy analýzy s použitím excitace 514,5 nm ukazuje, že tímto způsobem lze detekovat koncentrace β-karotenu ještě zhruba o jeden řád nižší. Analýza přes síranový krystal vedla k poklesu signálu, nicméně stale bylo možno identifikovat 1-10 mg kg^{-1} β-karotenu, v závislosti na použité vlnové délce laseru.

Výsledky získané pomocí přenosného Ramanova spektrometru s diodovým laserem o vlnové délce 785 nm ukazují, že pro analýzu jemnozrnných směsí může být tato instrumentace dokonce výhodnější a to zejména díky většímu průměru stopy laseru.

Navazující část práce se zabývá studiem endolitických mikroorganismů žijících v přírodních evaporitech v poušti Atacama v Chile, kde panují nejsušší podmínky na Zemi a je to tedy prostředí, které může částečně sloužit jako pozemský analog k extrémně aridním podmínkám na Marsu. V kapitole 2 jsou shrnuty dosud nepublikované výsledky měření, zabývající se Ramanovskou analýzou endoevaporitických kolonií žijících v krustách Ca-síranú (s převažujícím sádrovcem) v hyperaridním jádru pouště Atacama. Byl zjištěn systematicky se měnící Ramanovský signál karotenoidů který dobře koresponduje s polohou jednotlivých kolonií v rámci evaporitické krusty. Rozdíl ve složení karotenoidů, který byl pozorován spolu s přítomností/absencí phicobiliproteinů je důsledek kolonizace různými typy mikroorganismů - eukaryotickými řasami (většinou blíže k povrchu krusty) či sinicemi (typicky při spodní straně krusty). Ramanovský signál pigmentů lze dobře využít pro mapování výskytu jednotlivých kolonií v jejich původním mikrohabitatu pomocí streamline Ramanovské analýzy. Diskutovány jsou analytické aspekty použití excitačních délek 785 a 514.5 nm pro analýzu karotenoidů.

Článek prezentovaný jako příloha IV se věnuje Ramanovské analýze mikrobiálních společenstev (sinic) v přírodních vzorcích halitu, také z hyper-aridních oblastí pouště Atacama. Byly zjištěny různé typy pigmentů které mají UV-protektivní funkci (především scytonemin) či jsou součástí fotosyntetického aparátu. Ramanovský signál se liší v závislosti na konkrétním mikrohabitatu. Především velký rozdíl v přítomnosti spektrálních znaků

scytoneminu u jednotlivých kolonií se jeví jako velice zajímavý poznatek a může odrážet adaptaci na odlišné světelné podmínky panující v rámci jednotlivých mikrohabitatů stejně jako další faktory, které jsou diskutovány.

Ramanova spektrometrie se ukázala být vhodnou metodou pro identifikaci β-karotenu jako typického karotenoidu v nízkých koncentracích v evaporitických materiálech. Metoda je zároveň dobrým nástrojem pro studium mikroorganismů v jejich původním prostředí na základě nedestruktivní analýzy jejich pigmentů, která nám umožnila hlubší poznání autofototrofních mikroorganismů žijících v evaporitech v hyperaridních oblastech pouště Atacama.

1. Introduction

Pigments and their alteration products are the important biomarkers in sedimentary record. They originate mainly from a photosystem of higher plants, prokaryotes as well as algae. In addition to the light-harvesting function of pigments, some pigments act as photo-protective molecules, particularly under stressed conditions with high UV-flux. Brines and evaporites – minerals formed by precipitation from aqueous solutions can provide suitable habitats for organisms adapted to high salt concentrations. Microorganisms from all domains of life – bacteria, cyanobacteria, archaea as well as eukaryotic algae – harbour these environments and are often strongly pigmented in order to harvest light as an energy source or to protect against damaging radiation. This thesis is focused on Raman spectroscopic identification of microbial pigments, which we can find in extremophilic microorganisms living in such a high-salt environment. Since sulphate as well as chloride salts have been recovered on Mars, these types of rock are also assumed to be one of the potential habitats for past or even present life on the planet. At the very least, evaporites on Mars could play an important role in preservation of molecular remnants. Thus, the results of the work have implications for planetary exploration focusing on astrobiology, as well as for the primary study of adaptations of microbial life in extreme environments on the Earth.

The central theme of astrobiology is to search for life in the Universe. Such a scientific task is challenging and requires an interdisciplinary approach. It encompasses research related to extrasolar planets as well as the bodies in our Solar System. Planet Mars is one of the most promising objects in our Solar System in the search for traces of life beyond the Earth. Although it is necessary to be open-minded when suggesting possible extraterrestrial life forms, an important approach is based on study of the environment and its inhabitants, with which we are familiar from habitats on Earth. From this point of view, extreme localities and extremophilic organisms are especially important objects for astrobiological research. The term "extreme" sounds anthropocentric, as some of these organisms are well adapted to these conditions, which are their normal environment; nevertheless, they represent life at the limits from our point of view. Therefore, such environments and the organisms living in them provide us with an opportunity to learn more about the limiting conditions for the known metabolic processes and diverse adaptation strategies that they have evolved to survive challenging environmental stress. These extreme environments on Earth can also represent sites analogous in several parameters to Martian conditions, such as the McMurdo Dry Valleys in Antarctica or Atacama Desert and other locations (called Mars analogues).

The Raman spectroscopy is a vibrational spectroscopic technique based on inelastic scattering of light from an analyte first demonstrated by Raman and Krishnan (1928). The technique allows the identification of biomolecules in a nondestructive way, without any pretreatment, directly in the rock sample. Moreover, the Raman-microscopic technique allows us to focus on a single cell or accumulation of microbial cells in rock pore spaces. This approach is very important for terrestrial measurements, as it provides an opportunity to

examine the survival strategies of microorganisms in their original microhabitats based on the spectral record.

Raman spectroscopy is considered as a tool to identify both organic and inorganic compounds on Mars as a part of the scientific payload of the probes planned within future missions to the planet. The method will provide a nondestructive analysis prior to the measurements using more complex destructive methods with better sensitivity but very limited number of analyses available.

However, the recent plan of the ExoMars mission, which encompasses a Raman instrument as part of the scientific payload, includes the performance of Raman analyses on powdered Martian rock samples (without a microscope). Therefore, an Earth-based evaluation of Raman spectrometry to detect organic components, such as pigments in powdered mixtures, can also form an important basis for future planetary exploration. Nevertheless, the application of Raman spectroscopy in the micro-mode is crucial for the examination of microbial life strategies in extreme terrestrial environments in situ, without losing information about the spatial distribution of the colonies.

1.1 Conditions for life on Mars and the astrobiological context of the thesis

This chapter is concerned with the Martian environment in terms of habitability for life as we know it. One of the crucial preconditions for life is the presence of liquid water as a solvent in biochemical processes. Theories about possible nonaqueous biochemistry have also been proposed – dealing with hydrogen peroxide-based biochemistry (Houtkooper and Schulze-Makuch, 2007). Though such a completely different biochemistry can not be ruled out, our suggestions about the possible habitable environment of Mars will focus on known life forms, for which availability of liquid water – at least transient – is a crucial parameter defining habitability.

1.1.1 The availability of liquid water on Mars

On the current Mars surface, water ice is unambiguously present in the north polar cap (Kiefer et al. 1976). Moreover, polygonal terrain, resembling similar features in permafrost areas on Earth, has been reported in younger regions of Mars (Seibert and Kargel, 2001, Mellon et al., 2008). The presence of subsurface ice forming permafrost in polar regions was also indicated by a hydrogen signal detected by gamma-ray spectrometry onboard Mars Odyssey (Boynton et al., 2002). In 25th May 2008, the Phoenix Lander approached the northern arctic region of Mars and took close-up images of the polygonal terrain as well as water ice itself (Smith et al., 2009).

Existence of a liquid phase is defined by pressure/temperature conditions (see the water phase diagram, figure 1). The P/T conditions on recent Mars are very variable (see Jakosky and Phillips, 2001, Leovy, 2001). The temperature varies from about -140°C to +25°C depending on latitude, altitude as well as diurnal and annual cycle. The average value of the atmospheric pressure on Mars is 6 mbar (0.006 atm); however, this value can also vary quite extensively. Typically, ice sublimates directly to water vapor and condenses from vapor to the solid phase under recent Martian surface conditions. However, the temperature/pressure conditions on which the presence of liquid water depends most probably varied in time during the Martian geological history.

In contrast to the current climatic conditions, numerous landforms revealed within last decades on Martian surface provide evidence for extensive liquid water activity in the past (see the review by Baker, 2001). In 1972, the Mariner 9 spacecraft observed channels and valleys from the orbit of Mars for the first time. These features on the planet's surface were interpreted to originate in flowing water during the warmer periods in Martian history (Masursky, 1973). In the late 1970s, Viking orbiters took images of these structures with improved resolution, which long provided the basis for knowledge about water activity on Mars (Carr, 1996). A closer view of the structures related to water flow was provided by the MOC (Mars Orbiter Camera) onboard the Mars Global Surveyor spacecraft and, more recently, by the HiRISE (High Resolution Imaging Science Experiment) camera as a part of the payload on the Mars Reconnaissance Orbiter (Weitz et al., 2008, Lanza et al., 2010).

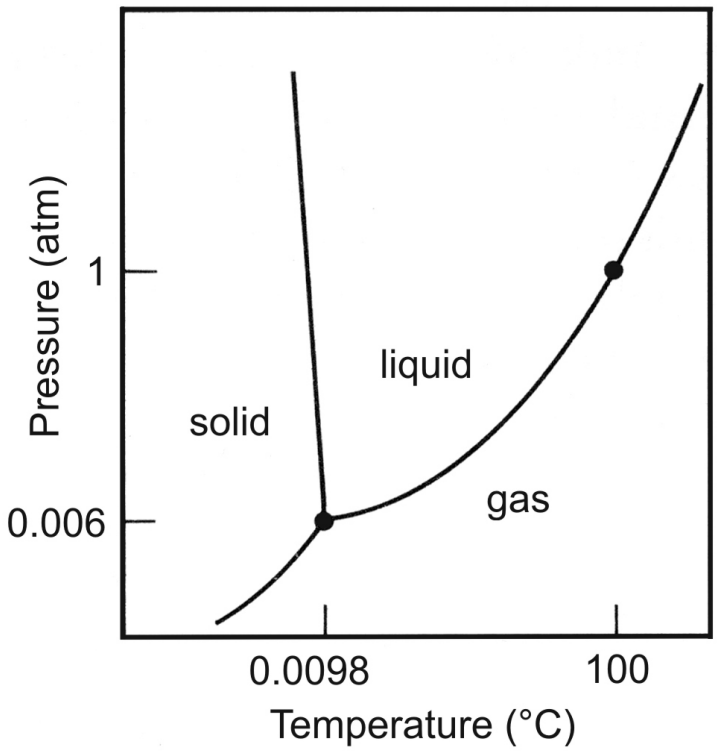

Figure 1: Simplified phase diagram of water.

The geological age of a particular terrain on Mars can be inferred from the densities of impact craters, which allows us to stratigraphically divide Mars into three main epochs. The Noachian period is the oldest and comprises heavily cratered regions formed prior to about 3.5 billion years ago, followed by the Hesperian epoch (3.5-1.8 billion years ago), represented by intermediate cratered areas, and the youngest Amazonian epoch, with less-cratered regions. It is generally supposed that, in the early stage of the planetary evolution (Noachian), the Martian atmosphere was significantly denser than it is today and that the climatic conditions were warmer and wetter. Most of the networks attributed to liquid water activity occur within the Noachian terrain (Carr and Clow, 1981) in the southern highlands. The hypothesis is strengthened by the systematic observation of a reduced number of impact craters smaller than 15-20 km in diameter which, together with degraded larger craters which is considered to be a result of water-related erosion processes which occurred at least prior to 3.7 billion years ago (Chapman and Jones, 1977, Carr, 1996). However, as many as 25-35 % of the valleys may represent Hesperian or Amazonioan age (Scott et al., 1995) pointing to younger periods of liquid water activity. Some gullies have been interpreted as being a result of recent water seepage and surface runoffs (Carr, 1996, Malin and Edgett, 2000). Braided channels and alluvial-like fans found in geologically recent craters were observed by HiRISE (McEwen et al., 2007). A syndepositional process - impact induced melting and subsequent “rainfall” have been proposed by the author as one of the possible mechanisms of formation of these fluvial features (see McEwen et al., 2007). The mechanism was formerly presented by Segura et al. (2002) as a possible process of transient liquid water formation during heavy bombardment in

the Noachian period, thus potentially corresponding to an alternative scenario to the constantly warm and wet climate hypothesis suggested for this epoch. In addition, evidence for standing water bodies in the form of impact crater lakes in the past can also be found on Mars, with ages mostly corresponding to the upper Hesperian and lower Amazonian period (Cabrol and Grin, 1999, 2001).

There are areas on Mars where the local P/T parameters allow at least the transient presence of liquid water even under current climatic conditions – these are the Amazonis, Arabia, and Elysium plains and the Argyre and Hellas impact basins (Haberle et al., 2001). Recently, Phoenix Mars Lander observation practically confirmed the suggestion that this corresponds to wet soil and segregated water ice, indicating condensation from liquid rather than directly from water vapour (Smith et al., 2009). The parameter that most probably affects the transient presence of liquid water is the obliquity (Laskar et al., 2004). During high obliquity periods, a warmer and wetter climate occurs, which could result in liquid water activity in the recent past (Haberle et al., 2003, Smith et al., 2009).

An important indicator for aqueous processes is the Martian mineralogy. Crystalline haematite has been discovered in the Meridiani Planum and other areas on Mars using the Thermal Emission Spectrometer (TES) instrument on the Mars Global Surveyor (MGS) spacecraft (Christensen et al., 2000, 2001). The presence of such haematite-rich surface deposits has been confirmed by the Opportunity Rover, which performed in-situ measurements on Meridiani Planum (Christensen et al., 2004). Microscopic images revealed spherules about up to 5 mm in diameter. Mössbauer, APXS (Alpha-particle X-ray spectrometry), Mini-TES (Thermal Emission Spectrometer) and pancam – all the analyses of these spherules confirmed their haematite nature. Pancam observations of the cross-section of an abraded spherule point to the presence of haematite throughout the volume, not just as a coating (Squyres et al., 2004). The spherules are interpreted as concretions formed in aqueous environment during early burial diagenesis. One of the possible (thermodynamically plausible) reactions involved in the haematite formation is dissolution of jarosite (Squyres et al., 2004).

Spectroscopic features obtained by the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) on the Mars Reconnaissance Orbiter (see Murchie et al., 2007) have demonstrated the presence of hydrated silicate minerals on the Martian surface (Mustard et al., 2008) which is consistent with the earlier observations obtained from the OMEGA imaging spectrometer onboard the Mars Express spacecraft (Poulet et al., 2005, Bibring et al., 2006). Recently, the presence of $CaCO_3$ has been revealed in soil on the Phoenix landing site by a Thermal and Evolved-Gas Analyzer (TEGA) instrument (Boynton et al., 2009); the carbonate was probably precipitated from liquid water.

Additionally, secondary porosity observed within outcrops in Meridiani Planum by the Opportunity Rover has been interpreted as corresponding to diagenetic features, suggesting liquid water activity (McLennan et al., 2005). Crystal shaped molds and sheet-like vugs have been described by the authors. These features are significantly larger than the size of framework sand grains and are interpreted as being a result of dissolution of relatively soluble evaporitic minerals (Squyres et al., 2004, McLennan et al., 2005). The evaporitic deposits discovered on Mars are summarized separately in the next chapter.

Recently, evidence for the presence of perchlorate ($Mg(ClO_4)_2$ or $Ca(ClO_4)_2$) in concentrations 0.4 to 0.6 wt % (ClO_4) in Martian soil from the polygon-patterned northern plains of the Vastitas Borealis was observed by the Phoenix Mars Lander (Hecht et al., 2009). It is assumed to have originated relatively recently. The presence of perchlorates supports the possibility of the existence of low-temperature brines in recent Martian environments, possibly related to contemporary liquid-mediated processes, such as gully formation. Under recent Martian conditions, perchlorate does not readily oxidize organics; however its presence may indicate an oxidant-forming chemistry in the Martian atmosphere or on its surface (Hecht et al., 2009).

Finally, all the findings described here represent strong evidence for liquid water processes involved in the formation of the Martian surface in the past and possibly in recent times, which is extremely important from the astrobiological point of view.

1.1.2 Evaporites on Mars

Deposits of evaporitic minerals have been widely observed to date within the Martian surface by both orbit-based measurements (Gendrin et al., 2005, Bibring, 2006, Bishop et al., 2008, Roach et al., 2007, Christensen et al., 2008, Wiseman et al., 2008, 2009, Osterloo et al., 2008) and by in-situ analyses by robotic missions (see the references below).

Sulphate minerals have been identified in outcrops as well as soils on Mars, analysed within the Mars Exploration Rover (MER) missions Spirit and Opportunity in two distinct areas on Mars – Gusev crater and Meridiani Planum, respectively (see Squyres et al, 2004, Haskin et al., 2005). A fine-grained whitish/yellowish material was observed during the Spirit's traverse within Gusev crater as a result of excavation of subsurface material by Rover's wheels (figure 2). High sulphur contents were revealed (up to 35 wt % SO_3) by an alpha particle x-ray spectrometer (APXS), as was enrichment in Cl, Cr and Br. Moreover, strong correlation between Mg (attributed to $MgSO_4$) and S was found which, together with enrichment in Br (typically occurs as highly soluble salts) in this several-centimetre-thick subsurface layer, suggests possible deposition of salts by groundwater (Haskin et al., 2005, Gellert et al., 2006, Wang et al., 2006). The APXS/Mossbauer data from excavated soil (dust) were interpreted as indicating the presence of primarily Mg-sulphates, with minor amounts of Ca- and also perhaps Fe-sulphates (Wang et al., 2006), whereas the Mössbauer spectrometry of other excavated layers suggested that the soils has high contents of Fe^{3+} bearing sulphates (Gellert et al., 2006, Ming et al., 2006, Morris et al., 2006). The features of bound water were observed by Mini-TES in these soils, pointing to the presence of hydrated minerals (Bandfield and Smith, 2003). The discovery of a subsurface layer enriched in salts provides strong evidence for the role of water in area of Gusev crater.

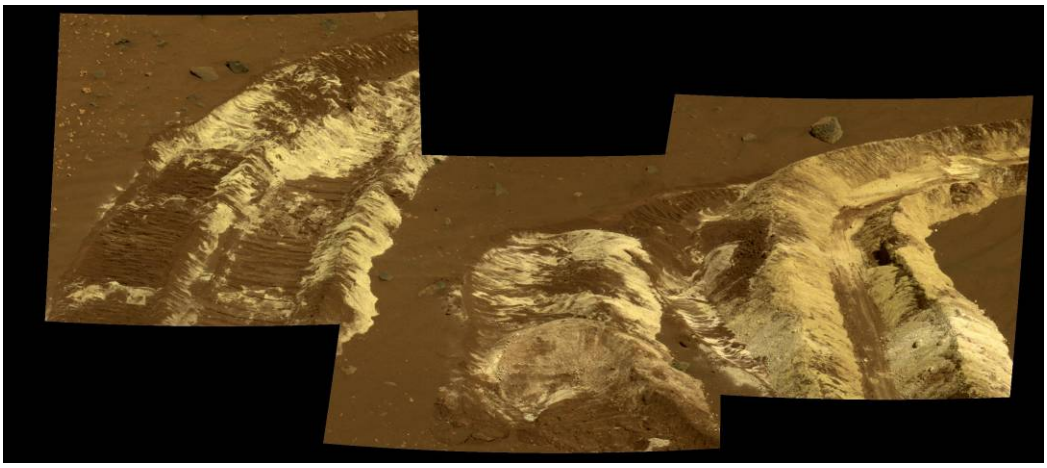

Figure 2: Bright soil (dust) excavated by rover wheels from shallow subsurface in Gusev crater (Courtesy NASA/JPL-Caltech/Cornell).

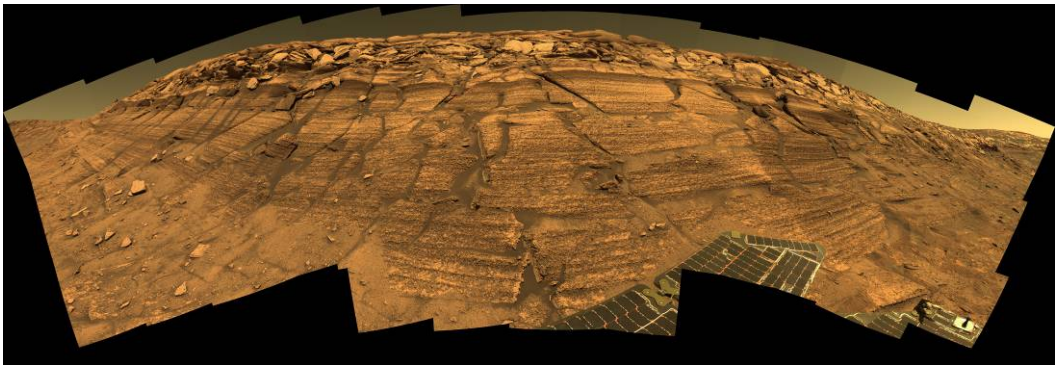

Figure 3: Mosaic of the „Burns cliff“ forming the inner wall of the Endurance crater rim at Opportunity landing site, Meridiani Planum, Mars (Courtesy NASA/JPL/Cornell).

The Opportunity Rover enabled study of outcrops exposed on the rims of impact craters along the Meridiani Planum. Genetically related strata observed at four different places (Eagle, Fram and Endurance impact craters and a regional fracturing called Anatolia) have been informally named the Burns Formation (see figure 3), after Roger Burns, who predicted (based on Viking chemistry data) ferric sulphates, including jarosite as stable phases that should occur on the Martian surface (Burns, 1987). The stratigraphic units of the Burns Formation reflect both subaqueous and eolian transport and deposition acting periodically on the Meridiani Planum in the late Noachian to early Hesperian epochs (Arvidson et al., 2003). These genetically related sediments were observed all over the landing site of the Rover at Meridiani planum (more than 8 km), with a thickness of at least 10 m. However, orbital data even point to probably a much larger extent and greater thickness of the Burns Formation (Christensen and Ruff, 2004). Mg, Fe and Ca sulphates, including jarosite, occur within the studied outcrops at different localities studied by Opportunity (Christensen et al., 2004, Rieder

et al., 2004, Clark et al., 2005, Grotzinger et al., 2005). Similarly as with bright soils, the outcrops exhibited high sulphur contents (6.8-11.5). Abraded outcrop rocks in Endurance crater exhibited a correlation of the Mg and S contents, suggesting that $MgSO_4$ is a major component here. If all the SO_3 is bound to Mg, Ca and ferric sulphates (as suggested by Mössbauer data, Klingelhöfer et al., 2004), the outcrop contains about 40 wt % sulphates. Interestingly, the Br content varied by more than an order of magnitude, depending on the position within the outcrop, although no systematic variations, either vertical or horizontal, were recorded (for a detailed description of the chemistry and mineralogy of outcrops at Meridiani Planum, see Squyres et al., 2004, Clark et al., 2005, Squyres and Knoll, 2005, Brückner et al., 2008).

The chemical composition of evaporitic minerals is derived from the fluid (brine) chemistry, from which the minerals precipitate. This is controlled by the chemical components of the initial lithologies from which the brines were enriched and the pH and Eh conditions. Basaltic lithology predominates on Mars which together with high SO_4^{2-} concentrations in water reservoirs due to volcanic activity resulted in acidic weathering of olivines and other minerals in basaltic rocks as the key process of forming brines on Mars. The fluid resulting from acidic weathering of Martian basalts are assumed to be enriched in Fe, Mg, SiO_2(aq), SO_4, and less in Ca (Tosca et al., 2005). In contrast, typical ocean water on Earth contains predominantly Na, Cl, Mg and SO_4 ions (Taylor and McLennan, 1985). Hence, contrary to common Earth scenarios, sulphates largely dominate over chloride salts in Meridiani Planum outcrops which correspond to the S-rich Martian lithosphere with a high SO_3/Cl ratio in volatiles (Clark and Baird, 1979, Clark et al., 2005).

The cation abundance identified by the APXS instrument onboard the Opportunity Rover points to Mg-sulphates as the dominant sulphate component, with subordinate amounts of Ca-sulphate and jarosite (Clark et al., 2005). The presence of jarosite at Meridiani Planum has been unambiguously confirmed by Opportunity's Mössbauer spectrometer (Klingelhöfer et al., 2004). Jarosite provides important environmental information, as it precipitates only under acidic conditions. Hence, it strengthens the hypothesis (Clark and Baird, 1979, Burns, 1987) that sulphuric acid had a strong influence on basalt weathering and sediment deposition on Meridiani Planum (Squyres and Knoll, 2005). Moreover, water with pH above 4-5 could not have occurred since jarosite formation (Madden et al. 2004). From geochemical modelling of the evaporation processes at Meridiani Planum, Tosca et al. (2005) suggested H_3O-jarosite as a source for releasing Fe^{3+} into solution. Transformation to goethite is thermodynamically favoured in the low Fe^{3+}, SO_4^{2-} aqueous solution with pH ~2-2.5. However, relative proportions of these two phases at equilibrium depends on other factors, such as porosity, the initial abundance of H_3O-jarosite and the presence of other Fe and SO_4^{2-} bearing phases before the diagenetic process (Tosca et al., 2005).

Beside the sulphate-rich sediments, the orbital mapping by the Thermal Emission Imaging system (THEMIS) onboard the Mars Odyssey also indicated the presence of chloride-bearing deposits on the southern highlands of Mars (Osterloo et al., 2008). Moreover, SNC meteorites (named after three representative members of the group - Shergotty, Nakhla and Chassigny) originating from Mars were found to contain halite (Gooding, 1992).

These evaporitic deposits are the result of aqueous conditions and are one of the indicators pointing to the presence of liquid water on Mars. Although the acid environment

predicted for formation of sedimentary rocks at Meridiani Planum might have complicated prebiotic chemistry, terrestrial experience suggests that environmental conditions inferred from Meridiani outcrop rocks could allow microbial adaptation at least for some periods of time when liquid water was unambiguously present (Knoll et al., 2005). Hence, brines and evaporites could serve as a potential habitat in the past and, if no extant life is present within these deposits, traces of extinct life in the form of microfossils or biomarkers could be still preserved.

1.1.3 Challenges for life on Mars

Assuming there is a promising habitat for microbial life and there was liquid water on Mars at least in the past or that it still occurs in several areas today (at least periodically), some other challenges for potential life still remain. These consists particularly in the harsh radiation reaching the surface of the planet. The lack of a magnetic field allows solar energetic particles (SEP) from the Sun as well as galactic cosmic radiation (GCR) from the surrounding cosmic space to reach the Martian surface or shallow subsurface.

UV flux on the planet‘s surface is an important parameter in terms of potential life (Rontó et al., 2002). On Mars, the incident solar irradiation on the atmosphere is 43% of that on the Earth’s atmosphere. Significant amounts of O_3 in Earth’s atmosphere result in a UV cutoff near 290 nm. The recent thin Martian atmosphere (average atm. pressure ~6 mbar) contains approx. 95% CO_2 and ozone corresponding to about 2% of that on the Earth. Thus, there is little protection, and the CO_2 absorbs all the irradiation below 190 nm (Kuhn and Atreya, 1979). The dose of UV irradiation above 190 nm on the surface of Mars creates hazardous, mutagenic or even lethal conditions for organisms known on Earth (Jager, 1985). Harsh UV irradiation can damage DNA and the other biomolecules directly or indirectly through the formation of reactive oxygen radicals (Yen et al. 2000). High UV flux on Martian surface and reactive oxidizing surface can be responsible for lack of organics there (at the ppb level), even those of abiotic origin, as recorded by pyrolysis-gas chromatography-mass spectrometry (pyr-GC-MS) onboard the Viking Lander (Biemann, 1977). However, in history of the planet, when the atmosphere was denser - more favourable conditions for evolution of life occured, comparable to those on early Earth.

Several strategies for life to survive in environments exposed to harsh radiation are known from recent Earth conditions and can also be applied to the Martian environment. Microorganisms use repairing processes to cope with the destructive irradiation. Simultaneously, they often use various strategies to screen against the UV irradiation. These are shielding by the non-translucent mineral layer or living inside the rocks including the evaporites, which can act as UV shielding agents and simultaneously allow the photosynthetically active irradiation (PAR) to reach the microbial layer, which can be extremely important for survival and growth of the phototrophs. Additionally, living underneath a layer of dead organisms within the microbial mat is another survival strategy observed in modern terrestrial systems (Garcia-Pichel, 1994).

As shown by Mancinelli and Klovstad (2000), spores of *Bacillus subtilis* can 100% survive exposure to a UV dose when screened by a 1 mm thick layer of Martian regolith simulant, while unprotected spores were 100% destroyed. Moeller et al. (2005) showed the role of pigmentation of *Bacillus* sp. endospores, including carotenoids, in protection against UV irradiation. Possible survivability of cyanobacteria of the genus *Chroococcidiopsis* covered by 1 mm thick mineral layer under simulated Martian irradiation was demonstrated by Cockell et al. (2005). The positive role of shielding by a mineral matrix has also been confirmed by Rettberg et al. 2002 and 2004 during exposure experiments performed in space (PERSEUS mission on MIR and the BIOPAN facility onboard the FOTON satellite, respectively). Moreover Fendrihan et al. (2009a) showed that the survivability of *Halococcus dombrowskii* towards UV exposure is significantly higher when it is embedded in halite crystals (the dose for 37% survivability was about 400x higher than for unprotected cells).

Taking account these facts, any life forms or even dead organic remnants are most probably not present on the surface of Mars. On the other hand, the Martian subsurface still may be a habitat for microbial life (Kanavarioti and Mancinelli, 1990). Chemolithotrophic microorganisms are amongst the likely candidates for potential life forms there. However, presence of photosynthetic microorganisms protected by layer of translucent minerals like evaporites may also be a possible survival strategy, favourable at least in the earlier stage of Martian history. In addition, besides protecting living microorganisms, evaporitic rocks can play an important role in preserving microorganisms and molecular remnants through geological history, as shown in Chapter 1.2.1.

1.2 Overview of microbial life in an evaporitic environment

According to Warren (2006), evaporites are defined as salt rocks that were originally precipitated from a saturated surface or subsurface brine by solar evaporation, comprising a wide range of chemically precipitated salts including alkaline earth carbonates (for comprehensive information related to evaporites see Warren, 2006). Evaporites are typically formed by sequential precipitation (from less soluble to more soluble) during evaporation of concentrated solutions. The first minerals precipitating from the evaporating sea water are Ca-carbonates (calcite, aragonite), followed by gypsum at salt concentrations above 140 – 150 g l^{-1}, halite, which precipitates when the salt concentration exceeds 300 g l^{-1} and highly soluble salts like sylvite (KCl).

Organisms living in an environment with high salt concentrations have to cope with osmotic balance to grow and live in very low water activity (a_w 0.75 at NaCl saturation, see Grant, 2004). Two ways of adaptation to life in high salinity can be found between halophilic microorganisms (Oren, 1999, 2000a). The first consists in the accumulation of inorganic salts in the cell, mainly KCl, to maintain osmotic balance with surrounding environment. The second option consists in the accumulation of organic solutes in the cytoplasm to provide osmotic balance. Accumulation of KCl in the cytoplasm requires much less energy than the synthesis of organic solutes (1 ATP equivalent required for the accumulation of 1.5-2 molecules of KCl, see Oren, 1999) and is thus favourable for microorganisms of metabolic types producing small amounts of energy, such as fermentative bacteria in the *Haloanaerobiales* family (Oren, 1999, 2000a). On the other hand, high concentrations of KCl inside the cell require adaptation of the intracellular enzymatic system (Oren, 1999, Lanyi, 1974, Dennis and Shimmin, 1997). Microorganisms that use compatible organic solutes do not need special enzymatic adaptation; however, the biosynthesis of these organic molecules requires much more energy.

Typical compatible solutes used by halophilic microorganisms are glycerol (found only in Eukarya, like the green alga *Dunaliella*), glycine, betaine, ectoine, glucosylglycerol, disaccharides sucrose and trehalose (for more, see Galinski, 1993, 1995, Oren, 1999, 2000a). The precise amount of equivalent ATP molecules needed for the synthesis of the organic solutes varies between 30 ATP and 109 ATP, depending on the particular solute used and the metabolic types (for more details see Oren, 1999, 2000a).

Brines and salt lakes are often inhabited by halophilic organisms, even at a salt concentration of halite saturation. It is evident from the salt concentration limits for particular metabolic types presented by Oren (1999, 2000a) that just a few metabolic types occur at halite saturation – namely oxygenic photosynthesis, anoxygenic photosynthesis, aerobic respiration, denitrification (based on laboratory studies on pure cultures) and aerobic methane oxidation (based on study of natural communities).

The archaea *Halobacterium salinarum* is a typical representative of these extremely halophilic microorganisms. This archaea is pink-red in colour mainly due to a C-50 carotenoid pigment α-bacterioruberin (and its derivatives) (Kelly et al., 1970), see figure 4. Other pigments responsible for colouration of photo-active haloarchaea are rhodopsins. Bacteriorhodopsin is a lipid – protein complex that forms patches in cell membranes. Although

halophilic archaea with purple membranes are mostly heterotrophs, bacteriorhodopsin pigment which act as a light-driven proton pump enables facultative autotrophic activity under low oxygen conditions. The light energy harvested by the bacteriorhodopsin is converted to the proton gradient and almost all of this energy is consumed to maintain osmotic balance via pumping K^+ across the cell membrane.

Figure 4: Saltern evaporation pond in Eilat coloured pink-red due to haloarchaeal bacterioruberin pigment and other carotenoids.

The green alga *Dunaliella* is a typical primary producer in hypersaline environments (see Oren, 2005), including the saltern evaporation ponds as well as natural systems like Great Salt Lake, Utah, USA (Brock, 1975) or cold Antarctic saline lakes (Tominaga and Fukui, 1981), for example. Certain *Dunaliella* strains produce extremely high amounts of β-carotene (up to 12%, as a response to a particular dose of irradiation). Cyanobacteria, both unicellular and filamentous are representants of prokaryotic phototrophs found within hypersaline systems through the world (Golubic, 1980, Javor, 1989, Oren, 2000b). More recently extremely

halophilic bacteria have been also recognized within saltern brines approaching NaCl saturation. The rod-shaped bacteria previously described as "*Candidatus* Salinibacter" (Antón et al., 1999, 2000) are now known as *Salinibacter ruber* (Antón et al., 2002). The bright red colour due to the carotenoid pigment of *Salinibacter* is also thought to contribute in part to the colouration of saltern crystallizer ponds (Oren et al., 2004). The carotenoid pigments are produced as protective biomolecules under conditions where high dose of irradiation – harmful for organisms – is present (see Chapter 1.3.1).

Microbial life was also reported from environments, where highly saline conditions occur together with other stress factors. Cold saline environments, including Antarctic saline lakes, have been reported to be habitable for microorganisms (see Hand and Burton, 1981, Tominaga and Fukui, 1981, Wright and Burton, 1981, Green and Lyons, 2009), as have arctic areas, where halophilic bacteria as well as archaea were characterized in sediments of cold saline sulphate-rich springs in a Canadian arctic region (Perreault et al., 2007). Interestingly, microbial communities of saline lakes from western Australia were reported (Benison and Beitler Bowen, 2006, Mormile et al., 2009). Some of these lakes are examples of an acid saline lake ecosystems with pH <4 which represent an important Mars analogue site.

1.2.1 Evaporites as a refuge for microorganisms

Even precipitated halite can provide a refuge for microbes in the inclusions within the crystal and can possess conditions for long-term preservation. Crystalline halite coloured by embedded *Halobacterium salinarum* NRC-1 is depicted in figure 5.

Figure 5: Halobacterium salinarum (pink colour) embedded in halite grown in laboratory conditions.

Viable microorganisms have been isolated from ancient halite. The first isolates were described in Permian salt deposits in the 1960s by Reiser and Tasch (1960) and Dombrowski (1963). Dombrowskii's results have been confirmed by Bibo et al. (1983) under highly controlled conditions to avoid contamination. They isolated extreme halophiles from Zechstein salt cores of Permian age, described as Gram-positive cocci and rod-shaped sporeformers (see the minireview by McGenity et al., 2000). More recently, Vreeland et al. (1998) isolated viable halophiles utilising cellulose from a Permian (250 MA old) Salado formation (USA). Viable haloarchaea including new species have been reported by Radax et al. (2001) and Stan-Lotter et al. (2004) in the Zachstein deposits dated late-Permian. The frequent isolate *Halococcus solofodinae* (so far isolated only from rock salt) was isolated by the authors from distinct salt deposits of the same age from England, Germany and Austria (Radax et al., 2001, Stan-Lotter et al., 2004). Interestingly, spore-forming bacteria (*Bacillus*) have been extracted and reactivated from inclusions in halite crystals from Permian salt deposits in New Mexico (Vreeland et al., 2000). Although the claimed age of 250 Ma has been questioned (Hazen and Roedder, 2001, Hebsgaard, 2005), the real possibility that dormant or even active microbes or their remnants may be preserved in ancient halite rocks is very distinct and has important implications for astrobiology.

Different, more recent scenarios of life adaptations to highly saline conditions can be found in hot desert areas throughout the world. Ancient prokaryotes have been observed microscopically by Schubert et al. (2009) in ancient halite inclusions from a core drilled 90 m deep in up to 100 ky old evaporite deposits in Death Valley. The presence of eukaryotic *Dunaliella* cells within these fluid inclusions in halite was recently confirmed; they still contained intact pigment molecules such as chlorophyll and carotenoids (Schubert et al., 2010a). Within the same core, halophilic archaea have been cultured from a section at a depth 13 – 17.8 m (22,000 – 34,000 years old) containing perennial saline lake deposits. Another 876 crystals from different depths yielded no living archaea, hence microbial survival in inclusions has been found to be rare (Schubert et al., 2010b). The occurrence of *Dunaliella* cells was systematically associated with prokaryotes. As *Dunaliella* produces glycerol intracellularly to maintain osmotic balance (see review by Oren, 2005), the authors (Schubert et al. 2010a) hypothesized that glycerol that leaked from the cell in halite inclusion served as a nutrient source for associated heterotrophic prokaryotes. The hypothesis was strengthened by confirmation that two halophilic Archaea from the same core in Death Valley (34,000 years old) were successfully cultured in medium containing glycerol as the sole carbon source (Schubert et al., 2010a).

Interestingly, crystals of carotenoids (most probably β-carotene) originating from *Dunaliella* cells were discovered in these halite inclusions. β-carotene is sensitive to light, oxygen and temperature and easily degrades under their influence. However, it did not show any evidence of degradation in inclusions in up to 34,000 year-old halite crystals studied by Schubert et al. (2010a), which was interpreted as being a result of the low oxygen environment of a fluid inclusion and dark conditions as no sunlight reached the buried halite deposits. The results are an important example of how pigments can be preserved in halite inclusions for thousands of years, both incorporated within the biomass (cells of *Dunaliella*) as well as in the form of pure crystalline β-carotene released from the cell.

Figure 6: The halite crust in the hyper-arid core of Atacama Desert – Yungay area (photo by J. Wierzchos).

Salt-crusted pans and playas (locally known as salars) occur in depressions in the semi-arid to hyper-arid regions of the central Andes (see the figure 6). These are formed by massive halite crusts (deepest parts of the crust in Salar Grande, Chile are more than 160 m thick) (see Ericksen, 1993, Warren, 2006). Location between two mountain ranges prevents the moisture from the coast from approaching the central regions of Atacama Desert resulting in extreme dryness in this area. Wierzchos et al. (2006) have shown that halite can provide a suitable habitat for cyanobacteria (dominated by *Chroococcidiopsis* morphospecies) in the hyper-arid core of Atacama Desert, which is one of the driest areas on Earth with mean annual rainfall <2 mm yr^{-1} (see McKay et al., 2003). Endolithic (endoevaporitic) colonies have been observed in pore spaces within the halite crust by a variety of microscopic techniques – namely light microscopy, confocal laser scanning microscopy, low temperature scanning electron microscopy and transmission electron microscopy. Soils in this hyper arid region contain almost no bacteria, as documented by DNA amplification as well as examination of culturable bacteria (Navarro-González et al., 2003). Even hypolithic (living below the rocks) organisms are rare and exist in small isolated patches in this region (Warren-Rhodes et al., 2006) – so why is there abundant life in halite? The reason lies in great part in halite diliquescence – the hygroscopic properties of halite enable water to condense within the pores during periods when the relative humidity exceeds 70 – 75% – consequently, water is available for photosynthesis at this relatively low humidity levels together with photosynthetically active radiation (PAR) at the same point (Wierzchos et al., 2006, Davila et al., 2008). Raman spectroscopic analysis was performed on these halite-inhabiting cyanobacterial colonies (Vítek et al., 2010). Variable

pigment composition has been identified within the studied colonies, particularly the scytonemin signal varied greatly, which is suggested to be a microbial response mainly to the local light conditions in a particular microhabitat, defined by mineralogy (presence of mineral impurities) and morphology of the crust. Other factors are discussed as well – the results are part of this thesis (see Appendix IV).

As halite can provide an important habitat for microbial life in the most extreme areas on Earth and the survival of halophiles within crystalline halite over geological times seems very likely, the presence of chloride-bearing deposits on Mars makes the search for halophilic biota and their organic remnants on Mars plausible.

Sulphate deposits can harbour various life forms as well. Microorganisms inhabiting gypsum ($CaSO_4.2H_2O$) have been observed through the world including wet (brine bottom or within the capillary fringe) as well as extremely dry environments. Gypsum is a suitable substrate colonized by endolithic microbial communities which enable the photosynthetically active radiation to penetrate through the matrix and simultaneously protects against harmful UV irradiation.

Gypsum crusts growing in the bottom of saltern evaporation ponds have been intensively studied (see for example Rothschild et al., 1994, Oren et al., 1995, 2009, Vogel et al., 2009). Such a type of gypsum crust covered by a shallow brine pond is depicted in figure 7, as recorded during the field trip in April 2010 (illustrative photos, the work is not a part of this thesis). The gypsum crust covered by a brine of salt concentration between ~190 and 240 g l^{-1} contain stratified pigmented layers of phototrophs (see Oren et al., 1995, 2009). The uppermost part of the crust is inhabited by unicellular cyanobacteria, below which a green layer of filamentous cyanobacteria occurs, followed typically by purple anoxygenic phototrophs (see Oren et al., 2009). Pigment composition of these layered crusts was studied by Oren et al. (1995) – see Chapter 1.3.

Another important hyper-saline environment consists in the sabkha regions. Sabkha is an Arabic word for salt flat; these areas are typically formed along arid coastlines, with the water table just beneath the surface. The typical mineralogy of sabkha consists of gypsum, anhydrite and carbonates, accompanied by halite precipitates. Microbial signatures in gypsum from the sabkha regions have been reported, including microscopic (Barbieri et al., 2006) as well as Raman spectroscopic observations (Edwards et al., 2006). Moreover, intertidal and supratidal sabkha areas were described by Vogel et al. (2009), where the groundwater (brine) exists near the surface, allowing biofilms of phototrophs to grow. Orange, green and purple layered colonization of the gypsum crust has been found there, with associated subsurface brine of salinity 240 g l^{-1} or more. Biocolonized gypsum has also been reported from a desert environment (see Dong et al., 2007, for example). Endolithic communities dominated by green algae and cyanobacteria were described by Stivaletta and Barbieri (2009) in spring mound gypsum in Tunisia. The evaporite precipitation on top of the spring mounds allowed the rapid sealing of the remnants of endolithic microorganisms and preserved them from oxidation processes (Stivaleta and Barbieri, 2009). Endoevaporitic communities in gypsum and carbonates have been observed in Death Valley, California by Douglas (2004), together with significant biologically induced mineral modification. Microbialites (organosedimentary formations mediated by microorganisms) of unusual mineral lamination – Mn-

hydroxide/calcite/gypsum have been described in the same area (Douglas et al., 2008). Recently Wierzchos et al. (in press) reported on the microbial colonization of Ca-sulphate crusts in the hyper-arid core of Atacama Desert. Epilithic lichens, algae, cyanobacteria as well as fungal hyphae and heterotrophic bacteria have been observed by the authors. Raman spectroscopic study of cyanobacterial/algal pigments in these Ca-sulphate samples is part of this thesis (see Chapter 2).

Figure 7: The gypsum crust growing at the bottom of the saltern evaporation pond in Eilat (Israel) – the uppermost layer is inhabited by orange-brown coloured unicellular cyanobacteria, followed by a green layer dominated by filamentous cyanobacteria and purple layer of anoxygenic phototrophs.

As mentioned in Chapter 1.1.2, Mg-sulphates are abundant sulphates on Mars and their relative abundance on Mars is probably much greater than on Earth. An example of how halophiles can be entrapped in crystalline Mg-sulphates has been published by Foster et al. (2010), who performed an IR-reflectance spectroscopy study on natural hylersaline systems (Basque Lakes, BC, Canada). The authors claimed an ability to detect spectral signatures of biomass greater than or equal to concentrations of 0.78 mg/g within a salt matrix.

The environments described above represent extreme environments on Earth and can be important analogues for Martian scenarios. Although these modern terrestrial habitats are more or less wetter and warmer than present-day Mars where photosynthetic life is unlikely, the evaporite deposits on modern Mars are thought to have arisen during a warmer, wetter periods in its past, when halophilic phototrophs could have occured. The possibility of preservation of halophilic organisms throughout geological times strengthens probability of the hypothesis about microbial life in evaporitic environment as a model system for search of life on Mars proposed by Rothschild (1990).

1.3 Microbial pigments in an evaporitic environment

Organic pigments are compounds that can not be synthesized abiotically in nature. Thus, their eventual presence and identification in extraterrestrial sediments would unambiguously point to biological activity. Pigments are biomolecules that absorb light in a particular wavelength range. Microorganisms synthesize pigments to a) yield energy from absorbed light, b) screen against harmful irradiation by pigments absorbing at lower wavelength ranges (UV- near VIS), c) prevent damage of cellular components caused by reactive oxygen species and d) for membrane stabilization. The basic informations about light-harvesting pigments in next paragraphs have been taken from Medigan et al. (2008).

All known photosynthetic organisms use some form of chlorophyll for oxygenic photosynthesis or bacteriochrorophyll for anoxygenic photosynthesis. The chlorophyll molecule is formed by a porphyrin (tetrapyrole) ring with Mg^{2+} ion in the middle and an alcohol (phytol) side chain (figure 8). Various types of chlorophyll, differ in side functional groups, resulting in different absorption maxima. The most widespread is chlorophyll *a* with absorption maximum in the red region at 680 nm and in the blue region at 430 nm. It is used by higher plants as well as cyanobacteria. The absorption maxima of bacteriochlorophyll *a*, which differs from chlorophyll in having different side groups in two of the pyrole rings, is shifted to above 800 nm. The exact position of the absorption peak varies with bonding to the various proteins in the particular species (Medigan et al., 2008).

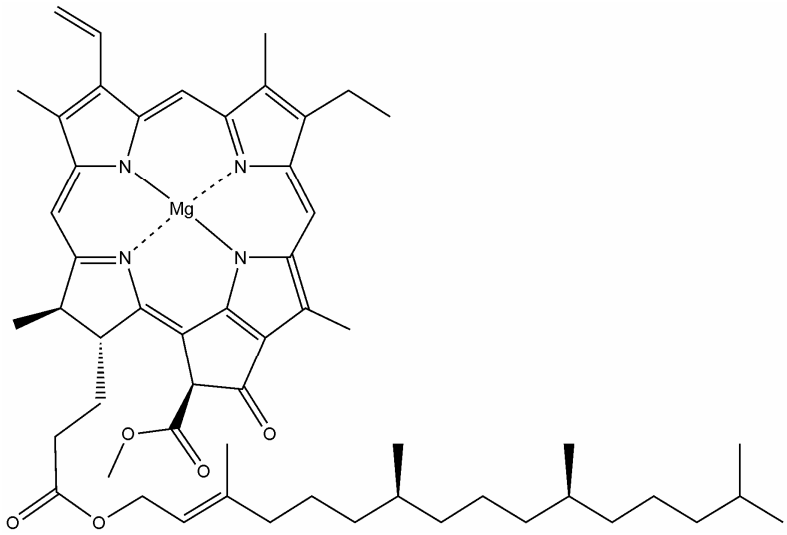

Figure 8: Molecular structure of chlorophyll *a*.

Phycobiliproteins are accessory pigments that enable coverage of a broader spectrum of visible sunlight (and thus more efficient photosynthesis). Their molecular structure is formed by an open tetrapyrole chain bonded to proteins (figure 9). These blue or reddish pigments can be found in cyanobacteria (cyan = blue, the name of the family is derived from blue pigmentation caused by phycobilins) and red algae. Three types of phycobiliproteins can

be found in the photosynthetic apparatus of these organisms – allophycocyanin, phycocyanin (blue) and phycoerythrin (reddish). They form aggregates called phycobilisomes, formed by allophycocyanin attached to the photosynthetic membrane surrounded by phycocyanin or phycoerythrin, or both depending on the particular organism. The energy is transported from the phycocyanin and phycoerythrin, absorbing low wavelengths (around 620 and 550 nm, respectively, high energy) to allophycocyanin (around 650 nm, lower energy), from which the energy is transferred to chlorophyll absorbing in the red region of the visible spectrum (680 nm, lower energy) (Medigan et al., 2008).

Phicobiliproteins increase the efficiency of photosynthesis, which allows the cyanobacteria to grow at low light intensities. The amount of phycobiliprotein content in cyanobacteria increases as the light intensity decreases (Medigan et al., 2008). These facts are extremely important when we consider endolithic communities from extreme habitats on Earth. Villar et. al (2006) observed the light-dependent biosynthesis of phycocyanin in cyanobacteria inhabiting volcanic rocks in Svalbard (Norway) using Raman spectroscopy. As these pigments are indicative for cyanobacteria and its biosynthesis is light-dependent, Raman spectroscopic probing of colonies directly in their microhabitats can be useful for examination of the life strategies of these phototrophs in-situ.

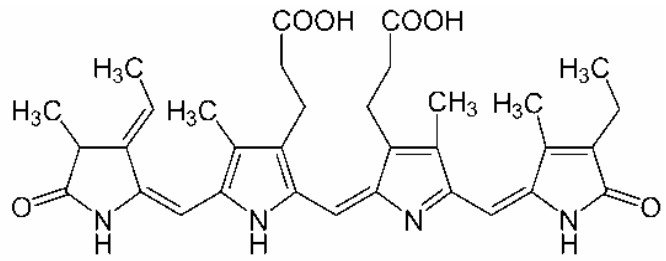

Figure 9: Open-chain tetrapyrole structure of phycocyanin.

Carotenoids are also accessory pigments involved in photosynthesis, photoprotection and membrane stabilization. A characteristic feature of carotenoid structures lies in the long conjugated double-bond system composed of isoprenoic units. These compounds are hydrophobic and are closely linked to chlorophyll or bacteriochlorophyll. As part of photosynthetic apparatus, carotenoids play an important role in harvesting energy and transfer this energy to the chlorophyll. Carotenoids absorb mainly in the blue region of visible irradiation; the accumulated energy can be transferred to the reaction centre and then can be used in ATP synthesis (but carotenoids do not act directly in ATP synthesis) (Medigan et al., 2008). Another important function of carotenoids is photoprotective and is described in the next chapter.

Pigment composition of layered community of cyanobacteria and purple bacteria living in gypsum crust on the bottom of the hypersaline solar saltern pond in Eilat, Israel was studied in detail by Oren et al. (1995). Chlorophyll, bacteriochlorophyll and variety of carotenoids were identified using high-performance liquid chromatography (HPLC) with detection based on the absorption spectra. Composition of pigments varied

depending on particular layer. Molar pigment ratios of myxoxantophyll/chlorophyll *a*, echinenone/chlorophyll *a* and β-carotene/chlorophyll *a* decreased with depth. Spectral light measurements showed that the extreme dose of irradiation often approaching the upper orange-brown layer of unicellular cyanobacteria can not be completely utilized in photochemical reactions. The high relative carotenoid content observed within the upper orange-brown layer is consistent with the need for photoprotection against photoinhibition and damage of photosynthetic apparatus (see next chapter). Another well-known example of increased carotenoid biosynthesis under stressed conditions is green alga *Dunnaliella salina* which accumulates β-carotene at concentrations up to 12 wt % or *Haematococcus pluvialis* – a freshwater green alga accumulating asthaxantin in concentrations up to 2 wt % (dry mass). Therefore, these organisms are harvested for commercial production of these specific carotenoids (see Dufossé, 2009).

1.3.1 Photoprotective pigments

As already mentioned, microorganisms use a variety of strategies to cope with damaging UV radiation or visible radiation exceeding the capacity of photosynthetic aparatus. They can benefit from the passive UV screening by inorganic compounds - living underneath the mineral layer providing specific UV absorption (iron compounds, sulphur, halite) or non-translucent rock/sediment layer (Cockell and Knowland, 1999). An important adaptation strategy to cope with harsh UV radiation consists in production of screening organic compounds that absorb in the UV region, thus providing passive protection against UV irradiation (see the database of photoprotective compounds in cyanobacteria, phytoplankton and macroalgae by Gröniger et al., 2000). In addition, mechanisms to cope with reactive oxygen species and UV damaged cell compounds, especially DNA, exist as indirect adaptations to a high UV dose. For a comprehensive review of the UV-screening compounds, see Cockell and Knowland (1999).

Generally, the UV screening capability (absorption in UV region) of organic compounds is most often related to the π-electron system. Such an electron configuration is primarily found in linear chained molecular structures with conjugated double bonds (see molecular structure of two carotenoids – β-carotene and bacterioruberin depicted in figure 10) and many aromatic and cyclic molecules with electron resonance (Cockell and Knowland, 1999).

The primary role of carotenoids is not well known (Cockell and Knowland, 1999). First, some short-chained carotenoids can potentially act directly as UV-screening pigments but are not favoured by organisms, whereas ubiquitous carotenoids with nine or more conjugated double bonds absorb more in the visible region (Cockell and Knowland, 1999), particularly in the range corresponding to blue irradiation (440 – 520 nm). These longer-chained carotenoids are able to cope with toxic reactive oxygen species which can be formed during photosynthesis or due to UV-irradiation and would destroy the biomolecules forming the photosynthetic apparatus. Carotenoids act as protective agents because of their ability to prevent the formation of singlet oxygen ($^1O_2$), by rapidly quenching chlorophyll triplet states and rapid direct scavenge of singlet oxygen if any is formed (Anderson and Robertson, 1960, Krinsky, 1979,

Siefermann-Harms, 1987, Telfer et al., 2008). Shahmohammadi et al. (1998) showed role of bacterioruberin in protection of *Halobacterium salinarum* against UV radiation and oxidative DNA-damaging agents, such as ionizing radiation and hydrogen peroxide. The observation is supposed to be in great part a result of bacterioruberin radical scavenging ability which was found to be greater than that of β-carotene due to highly conjugated polyene structure (Saito et al., 1997). Moreover, Fong et al. (2001) pointed to another role of bacterioruberin in psychrophilic bacteria, which is the cell membrane rigidification under low temperatures.

Carotenoids are vulnerable towards oxidation and photo- decomposition during senescence (see Rontani, 2001). Figure 11 depicts the time-dependent effect of UV-rich irradiation on the β-carotene recovery determined using the HPLC technique (Vítek et al., unpublished results). However, they can nonetheless be preserved under the anoxic conditions of the sedimentary record as shown on the example of carotenoid crystals recovered from the 34,000 year-old halite inclusions from Death Valley (Schubert et al., 2010b). The oldest intact carotenoids were identified in sediments of Miocene age whereas diagenetically altered carotenoids are reported from much older sediments and petroleum (see review by Sinninghe Damsté and Koopmans, 1997 and references therein). Diagenetic products of extremely old prokaryotic carotenoids have been identified by Brocks et al. (2005), who discovered a hydrogenated form of carotenoids – β-carotane, lycopane and okenane in sediments dating to mid-Proterozoic. See Chapter 1.4.1 for the Raman spectroscopy of carotenoids and hydrogenated carotenoids.

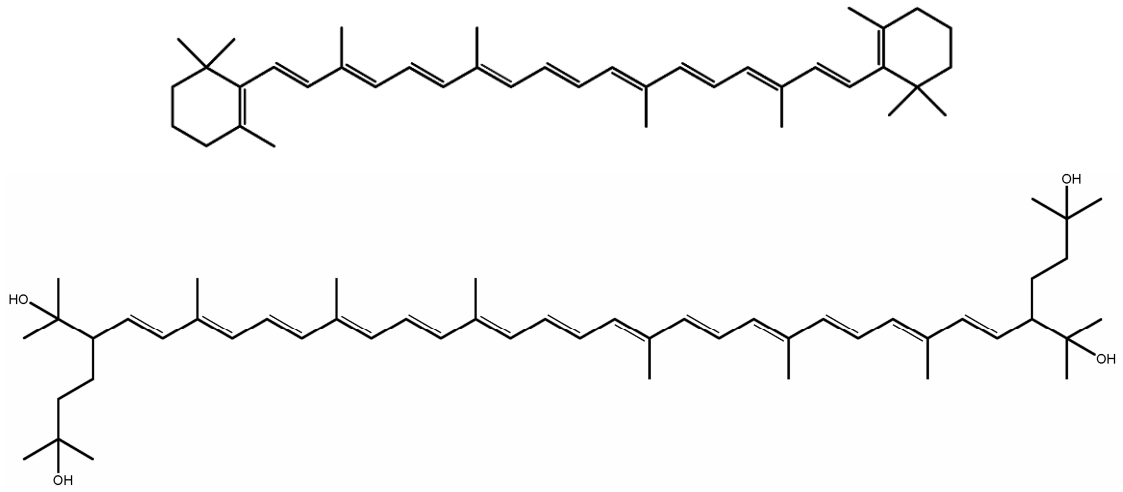

Figure 10: Molecular structure of two carotenoid pigments β-carotene (upper) and bacterioruberin (lower).

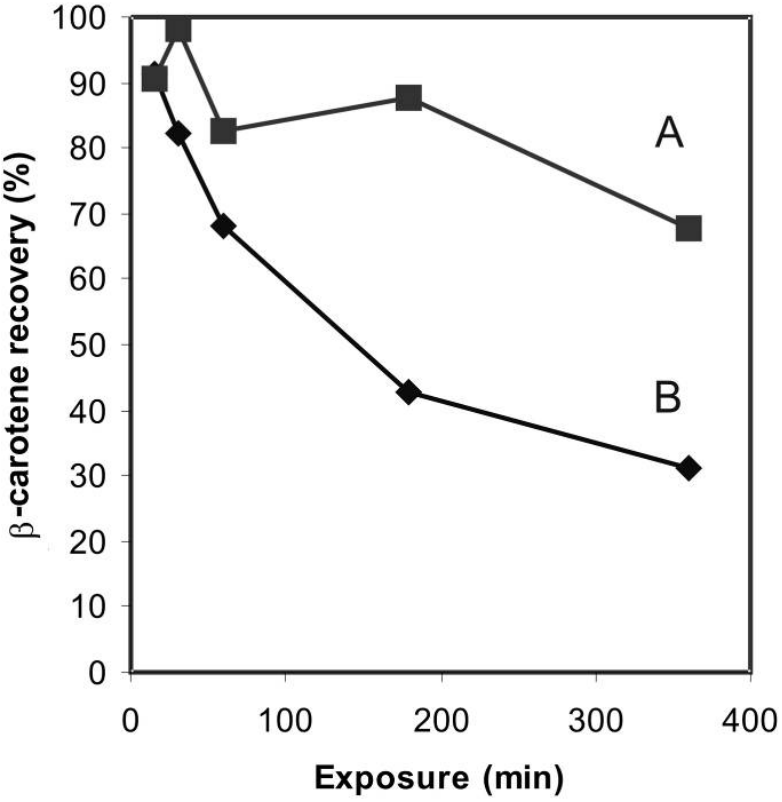

Figure 11: Degradation of β-carotene at temperature 278 K in the air: (A) in the dark, (B) exposed to UV-rich irradiation calibrated to give ~40 W m^{-2} in the range 250-400 nm, which is about 42% of the irradiation in the range 250-850 nm.

Scytonemin is a very effective UV-screening compound. This is a yellow-brown pigment produced as part of the extracellular sheath of certain cyanobacteria (Garcia-Pichel and Castenholz, 1991, Garcia-Pichel et al., 1992, Sinha et al., 1998) and is chemically more stable compared to carotenoids and chlorophylls. The structure of scytonemin is based on indolic and phenolic subunits (figure 12). The absorption maxima of purified scytonemin are located at 386, 252, 278 and 300 nm (Proteau et al., 1993, Sinha et al., 1998). In nature, scytonemin can be found mainly in the green oxidized form; however, it can be reduced to red forms in reducing layers (Proteau et al., 1993). It was demonstrated that scytonemin production by cyanobacteria is induced by UV-A radiation under laboratory conditions (Garcia-Pichel and Castenholz, 1991) and that this process can be affected by other forms of environmental stress, such as temperature, salinity and desiccation (Dillon et al., 2002). The scytonemin level was found to be positively correlated with the light flux under natural conditions in *Rivulari* sp., whereas negative correlation was found in *Scytonema* sp. (Pentecost, 1993). This observation is explained by the author as a response to different water availability in the two studied sites and differing cell division rates, which affect the scytonemin production, and concluded that the data are in agreement with the laboratory observations (Pentecost, 1993). Our results of Raman spectroscopic analysis of cyanobacteria inhabiting halite crusts in Atacama Desert confirm the hypothesis about the UV-induced biosynthesis of scytonemin under natural conditions,

although other parameters which may have affected these observations are also discussed. Measurements performed directly on colonies in the rock samples showed a strong scytonemin signal in communities living near the crust surfaces in contrast to the scytonemin-poor signal from colonies inhabiting the parts of the halite crust where lower dose of UV irradiation is expected (see the Appendix IV).

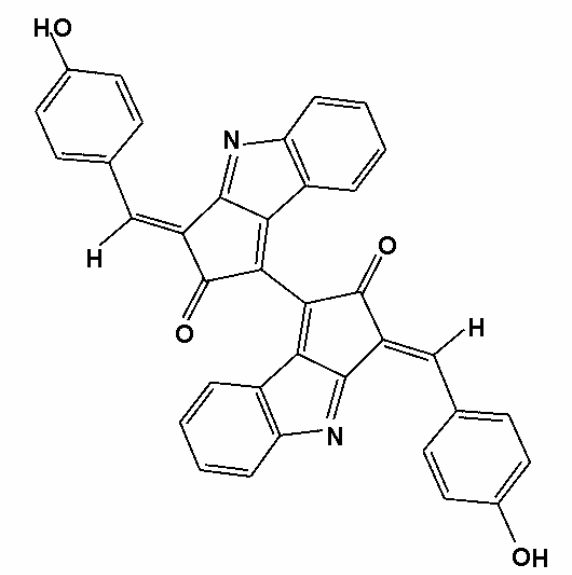

Figure 12: Dimeric molecular structure of scytonemin.

Mycosporine-like amino acids (MAAs) are UV-screening compounds that are widespread across the taxonomic range (Cockell and Knowland, 1999). These are water-soluble biomolecules containing cyclohexanone or an imino cyclohexane ring and are biosynthesized by a wide range of microorganisms including prokaryotic cyanobacteria, as well as eukaryotic microalgae, yeasts, and fungi (Oren and Gunde-Cimerman, 2007). Garcia-Pichel and Castenholz (1993) proved that MAA production of cyanobacteria *Gloeocapsa* sp. is induced by increased UV radiation under laboratory conditions. This effect corresponds to observations in natural systems with varying amount of UV incident on the organisms (see Cockell and Knowland, 1999 – Chapter VI and the references therein). However, Oren (1997) pointed out another physiological function of MAAs in cyanobacteria inhabiting saline environments, i.e. osmoregulation. MAAs were identified by Oren et al. (1995) in the orange-brown cyanobacterial layer (an analogous sample is depicted in figure 4) of the uppermost parts of the gypsum crust covering the bottom of crystallizer ponds in Eilat, Izrael. An absorption maximum observed in extracts at 332 nm and shoulder between 360 and 365 nm point to two different mycosporine-like compounds (Oren et al., 1995). The compounds were identified as mycosporine-2-glycine (Kedar et al., 2002) and 2-(E)-3-(E)-2,3-dihydroxyprop-1-enylimino-mycosporine-alanine (Volkmann et al., 2006).

1.4 Raman spectroscopy as a tool for identification of microbial biomolecules

It has been shown that Raman spectroscopy can be a powerful tool for analysis of biomolecules related to extant and fossil microbes (Marshall et al., 2006), as well as microbial communities inhabiting rocks from extreme environments like Antarctic habitats (Russell et al., 1998, Wynn-Williams and Edwards, 2000a,b, Edwards et al., 2005a, Villar et al., 2005a), volcanic rocks and travertines on Svalbard (Villar et al. 2006, 2007) and halophiles from a hot desert (Edwards et al. 2005b, 2006). Pigments are generally the prominent biomolecules dominating Raman spectra excited in the visible region from a variety of living microorganisms or unaltered microbial remnants. These include both light-harvesting and protective pigments. DNA and proteins can be rather identified using deep UV excitation (e.g., 244 nm) based on the resonance Raman effect (Manoharan et al., 1990, Chadha et al., 1993, Storrie-Lombardi, 2001, Wu et al., 2001, Jarvis and Goodacre, 2004, Tarcea et al., 2007). Interestingly, Storrie-Lombardi et al. (2001) reported on ultraviolet resonance Raman spectral detection limit of ~60 ppb for identification of Gram negative bacteria *Shewanella oneidensis* in Mars soil analog. Fendrihan et al. (2009b) showed that the signal of an amino acid compound (namely phenylalanine) can be observed in the Raman spectra of *Halococcus dombrowskii* when excited by a 1064 nm laser (FT-Raman). Among other biomolecules which were identified by Raman spectroscopy of natural microbial colonies from Antarctic habitats are osmoregulative solutes trehalose and erythritol (Winn-Williams and Edwards 2000a,b) or various organic substances of epilithic lichens, studied using FT-Raman spectroscopy (1064 nm excitation) (see Holder et al., 2000, Edwards et al., 2003a,b).

Several excitation wavelengths have been used for selective excitation of the resonance Raman spectra of chlorophylls (Lutz, 1972) and to study chlorophyll associations in chloroplasts (Lutz and Breton, 1973). Ten excitation wavelengths ranging from 441.6 nm to 514.5 nm were used and allowed determination of the relative enhancement of chlorophyll *a* (at wavelengths below 450 nm), chlorophyll *b* (at 450-475 nm) and carotenoids (using the excitation sources above 475 nm). The Raman spectra of chlorophyll *d* and bacteriochlorophyll *a* have been published by Zheng-Li et al. (2002) and Ceccarelli et al. (2000), respectively. Detailed assignments of resonance Raman spectra (using the 363.8 and 488 nm lines of an argon laser) of phycocyanin, allophycocyanin and phycocyanobilin chromophore isolated from cyanobacterium *Synechococcus* 6301 have been reported by Szalontai et al. (1994).

The Raman spectra of the UV protective pigment scytonemin were investigated by Edwards et al. (2000) using FT-Raman spectroscopy; the characteristic bands occur at 1590 (ν(CCH) aromatic ring quadrant stretch), 1549 (ν(CCH) *p*-disubstituted aromatic ring), 1323 (ν(C=N) indole ring) and 1172 cm^{-1} (ν(C=C–C=C) system (*trans*)). More recently, Varnali et al. (2009, 2010) reported the *ab initio* calculations for scytonemin and its derivates with relevance for Raman spectroscopic characterization. Application of surface-enhanced Raman spectroscopy (SERS) to detect scytonemin and carotenoids of endolithic cyanobacteria at nanomolar concentrations was published by Wilson et al. (2007). Scytonemin signal strongly dominated the spectra obtained from the pale grey-to-green colonization zones in halite crust from Atacama Desert (Vítek et al., 2010, Appendix IV).

The Raman spectrum assigned to mycosporine-like amino acid compound was reported by Edwards et al. (2007) for a jarosite matrix originating from Rio Tinto, Spain. One of the Raman spectral signatures obtained from microbial colonization of halite in Atacama Desert (CDLR sample, see Appendix IV) has been also tentatively assigned to mycosporine-like amino acid; further work needs to be done to confirm this suggestion. Comprehensive Raman assignment of particular MAAs remains unresolved.

The Raman spectroscopy of carotenoids is described separately in the next chapter because of its importance related to this thesis.

The choice of a particular excitation laser wavelength is crucial for Raman spectroscopic analysis (Villar et al., 2005b, 2006, Villar and Edwards, 2006). The compromise wavelength for identification of both organics and minerals is 785 nm. A wavelength of 514.5 nm or near is ideal for enhancement of the carotenoid signal due to the resonance Raman effect (described more in detail in next chapter) and is useful for analysis of minerals (Marshall et al., 2010). Combination of both wavelengths - 785 nm excitation to identify a broad range of organics and 514.5 nm excitation for resonance Raman enhancement of carotenoids can be important to obtain more information about the microbial communities studied (Villar et al., 2006). Some materials fluoresce at a particular excitation wavelength, complicating the Raman spectroscopic analysis. In fluorescence interference, a small Raman peak is superimposed on a high background or is totally overlapped. The solution is to choose a laser wavelength where the fluorescence is minimized or fully eliminated from the Raman spectrum. In general, the fluorescence decreases at longer wavelengths where electronic transitions become prohibited. However, even for 785 nm laser excitation, many materials can be compromised with varying levels of fluorescence emission. A frequent solution consists in multiplex detection based on Fourier Transform (FT) Raman spectroscopy coupled with 1064 nm laser excitation laser sources. FT based Raman instruments have excellent frequency precision, but tend to have lower signal-to-noise ratios than their multichannel counterparts. Recently, portable dispersive systems using a 1064 nm laser have also become available (Carron and Cox, 2010).

Dickensheets et al. (2000) tested a miniature Raman system equipped with 852 nm excitation for identification of biomolecules from the Antarctic lichen Acarospora as an analogue for future Martian exploration. However, comprehensive testing of portable Raman instrumentation for identification of natural biomolecules in field conditions with relevance for terrestrial as well as planetary applications remain an important task for future investigation.

The potential of Raman spectroscopy for astrobiological purposes has been reviewed by Villar and Edwards (2006) and more recently by Marshall et al. (2010), focusing on the interpretation of the Raman spectroscopy of kerogenous microfossils and differences from identification of unaltered biomolecular remnants.

1.4.1 Raman spectroscopy of carotenoids

In fact, carotenoids are exceptional pigments in Raman spectroscpic analysis of microbial communities because they a) have an extremely strong Raman signal even in a non-resonant mode and b) are widespread in microbial communities and are present in all phototrophic microorganisms. The strong colouration of carotenoids (from yellow to black) is due to the high electric dipole transition moment of the $\pi-\pi^*$ electronic transition resulting in the absorption in visible range of electromagnetic spectrum. The colours are dependent on the number of the double bonds in the polyene chain – the more conjugated C=C double bonds cause the darker colouration (Withnall et al., 2003). The Raman signal of carotenoids can be significantly enhanced by using the proper excitation wavelength which coincides with the absorption band of an allowed $\pi-\pi^*$ electronic transition, resulting in the resonance Raman effect (Gill et al., 1970, Merlin, 1985, Withnall et al., 2003, Marshall et al., 2007).

Due to the resonance Raman effect, generally the most favourable excitation wavelength for the identification of carotenoids lies around 500 nm (e.g. 488 nm, 514.5 nm) (Withnall et al., 2003, Marshall et al., 2007). However, for identification of a broader range of organic molecules, 785 nm is an ideal compromise excitation wavelength (Villar et al., 2005b, Villar and Edwards, 2006) with still relatively high sensitivity towards carotenoids, as is evident from the works that form part of this thesis (Vítek et al., 2009a,b, Vítek et al., submitted; Appendices I-III).

Resonance Raman spectroscopy of carotenoids was reported by Gill et al. (1970) and Merlin (1985). More recently, Withnall et al. (2003) pointed to application of resonance Raman Spectroscopy of carotenoids in natural products like sea shells, fruits and vegetables. Carotenoids have two strong Raman bands due to in-phase ν_1(C=C) and ν_2(C–C) stretching vibrations of the polyene chain (Gill et al., 1970, Merlin, 1985). See the Raman spectrum of β-carotene – a typical carotenoid – which is characterized by the ν_1 band located at 1515 cm^{-1} and ν_2 band at 1157 cm^{-1}. A feature of medium intensity occurs at 1008 cm^{-1}, corresponding to the in-plane rocking modes of the CH_3 groups attached to the polyene chain. The wavenumber positions of both ν_1 and ν_2 bands are dependent on the length of the polyene chain (number of conjugated double bonds). The shift in the band position is much more pronounced in the case of the ν_1 band. The longer polyene chain causes a shift in the ν_1 band to lower wavenumber positions and vice versa. Compare the spectra of β-carotene - 11 conjugated double bonds and bacterioruberin - 13 conjugated double bonds (figure 13), where a shift in the ν_1 band from 1515 cm^{-1} (β-carotene) to 1506 cm^{-1} (bacterioruberin) can be observed. The systematic dependence of the ν_1 band on the conjugation is depicted in figure 14 (adopted after Withnall et al., 2003[*]).

[*] Figure 14 reprinted from Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Vol. 59, Withnall, R., Chowdhry, B. Z., Silver, J., Edwards, H. G. M., de Oliveira, L. F. C., Raman spectra of carotenoids in natural products, Pages No. 2207–2212, Copyright (2003), with permission from Elsevier.

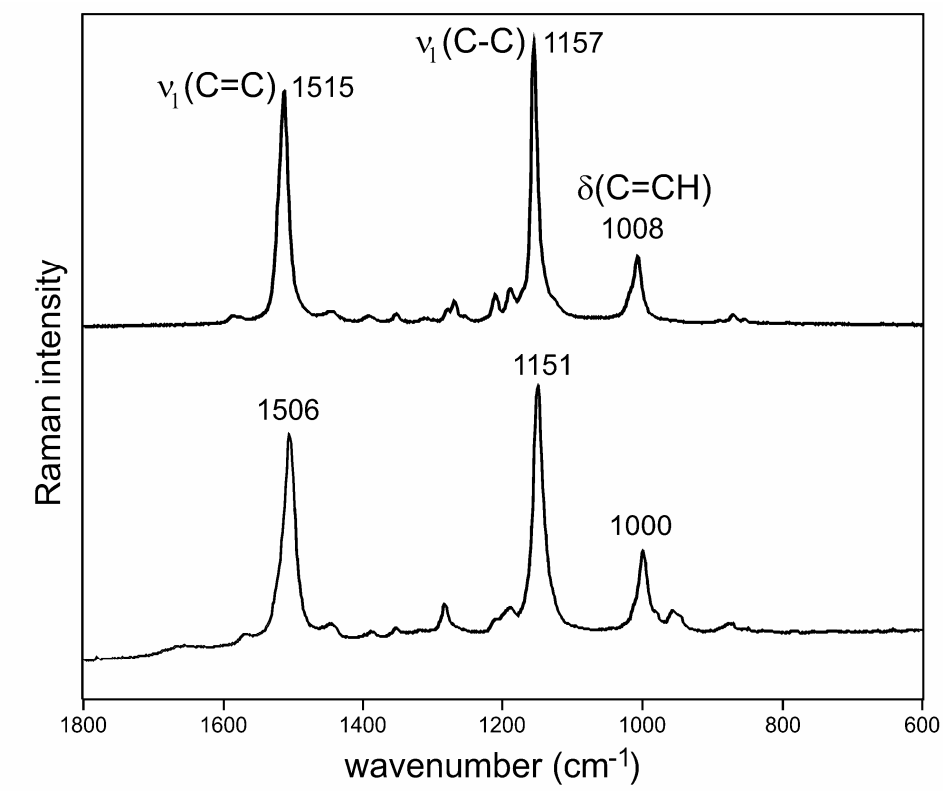

Figure 13: Raman spectra of syntethic pure β-carotene (upper) and bacterioruberin obtained by analysis of laboratory-grown colonies of *Halobacterium salinarum* using 785 nm laser for excitation.

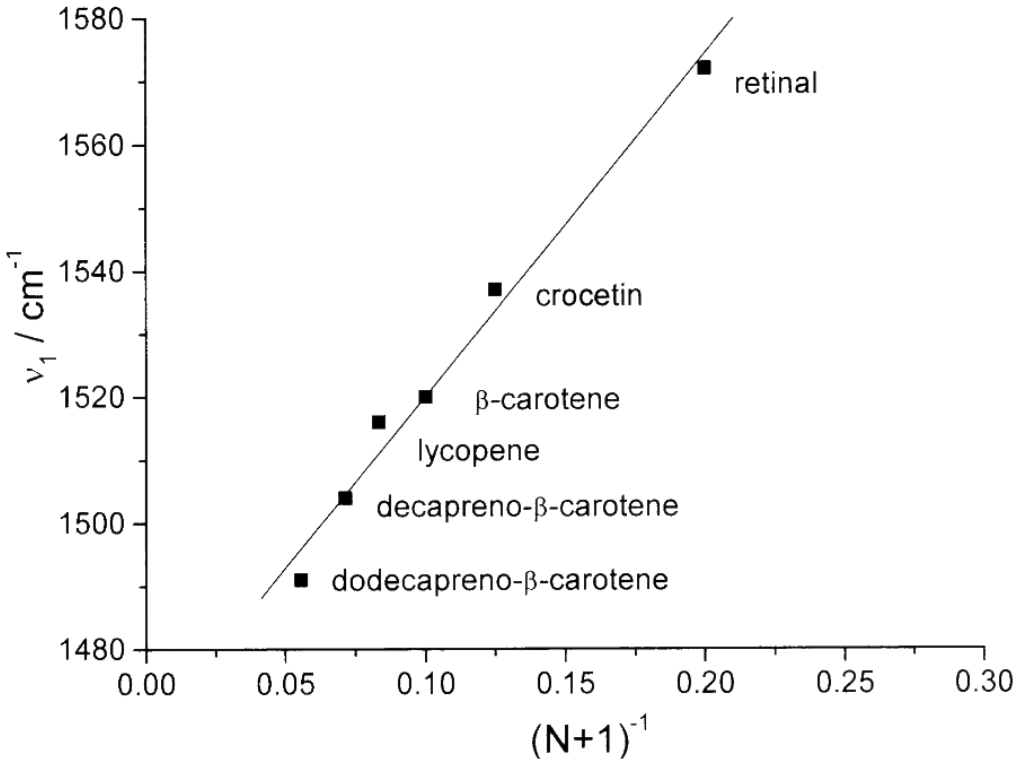

Figure 14: Carotenoids – ν_1 band dependence on the conjugation of the polyene chain (after Withnall et al., 2003)

Binding of carotenoids to proteins in antenna complexes within photosystem leads to specific changes in the ν_4 region (940 – 980 cm^{-1}) which is active only when carotenoid molecules are distorted from their planar configuration and can be indicative for particular xantophylls (see Ruban et al., 2001, Andreeva and Velitchkova, 2004, Gruszecki et al., 2009). As described by de Oliveira et al. (2010), carotenoid bonding within the biomass which affects the main polyene chain can cause significant shift of the ν_1 band position due to change in electronic delocalization. This means, that unambiguous identification of particular carotenoids in complex organic material on the basis of Raman spectra alone may not be possible. Nevertheless, although careful interpretation is necessary, information about the carotenoid band positions can be still useful for investigation of rock-inhabiting phototrophic organisms as described in Chapter 2. Other factors affecting the band shifts in carotenoid Raman spectra are substitution related to molecular termination which results in very small wavenumber change, isomerism and molecular conformation in solid and liquid state, respectively (see Liaaen-Jensen, 1997, Withnall et al., 2003, Barnard and de Waal, 2006, and discussion in de Oliveira et al., 2010). Moreover, the ν_1 band position of carotenoids depends on the laser wavelength used for excitation (see Ruban et al., 2001, Andreeva and Velitchkova, 2005).

The resonance Raman spectra of extremely halophilic archaea – *Halobactrium salinarum* was published by Marshall et al. (2007). The archaea is pigmented due to the presence of bacterioruberin. Another halobacterial pigment bacteriorhodopsin is an light harvesting pigment (proton pump) that is synthesized under stressed conditions and contains a shorter polyene chain with the ν(C=C) band position at 1536 cm^{-1} (Lewis et al., 1974, Marshall et al., 2007).

Recently, Marshall and Marshall (2010) described the spectra of hydrogenated carotenoids – β-carotane and lycopane. These can be the stable diagenetic products of the original carotenoids as the saturation of double bonds in the chain protects the molecule against damage by reactive radical. The "perhydro" carotenoids derivatives as observed by Marshall and Marshall (2010) have more complicated spectra. An intense band at 1455 cm^{-1} is the dominant mode assigned to the $\delta(CH_2)$ scissoring mode of methylene. The spectra contain 8 bands between 1390-1000 cm^{-1}, which are assigned to the ν(C-C) stretching modes from the main hydrocarbon chain, and 6 bands between 1000-800 cm^{-1} that were assigned to a combination of δ(C=CH) methyl in-plane rocking and δ(C-H) out-of-plane bending modes (Marshall and Marshall, 2010).

2. Results of Raman spectroscopic study of microbial colonization of Ca-sulphate crust from Atacama Desert: Methodical aspects

As described in Chapter 1.2.1, gypsum can provide a suitable habitat for microorganisms in extreme environments including arid and hyperarid regions on Earth and is one of the sulphate minerals suggested to be present on Mars. Recently Wierzchos et al. (in press) described colonization of Ca-sulphate crusts from the hyper-arid core of Atacama Desert. The authors used various microscopic methods for identification and determination of the spatial distribution of colonists of these gypsum specimens and discovered the presence of cyanobacteria, algae, heterotrophic bacteria, fungi and epilithic lichens in the samples.

Samples of the same Ca-sulphate crust were subject of Raman spectroscopic study. In this chapter, the tentative results are presented and the important methodical aspects are discussed resulting from employment of the two different excitation wavelelengths (785 and 514.5 nm) in point analysis and application of streamline mapping using 785 nm laser.

2.1 Materials and methods

Five samples of Ca-crust collected in April 2008 and February 2010 in Atacama Desert have been analyzed. Samples originate from area called Jacek Hills located within the Central Depression in the hyperarid core of Atacama Desert, separated by Coastal Cordillera from the Pacific coast (about 45 km inland). The Ca-sulphate forms small crusts about 10 cm in diameter which exhibit clear epilithic and/or endolithic colonization patterns. Crust thickness varies between 0.5 – 5 cm.

Colonization zones comprising endolithic phototrophs were examined using point analysis on Renishaw *InVia* Reflex Raman microspectrometer using 785 diode laser and 514,5 nm line of argon laser for excitation. A standard 50x objective and long working distance objective (50x) were used. The spectral laser power was typically set to 3-15 mW using 785 nm excitation and 0,2 – 2 mW using 514,5 nm laser. Typically, 15 s scans were accumulated 1-20 times. Point measurements were replicated on several spots within studied colonization zones. Analyses were performed particularly on the transected Ca-sulphate crust.

Streamline Raman analysis was tested for mapping of the algal layers through the pigment composition on the transections of Ca-sulphate crust. The *InVia* Renishaw microscope equipped with 785 nm laser was used for this purpose. Objective of 20x magnification was applied providing the compromise between spatial resolution and sufficient field-of-depth. The laser intensity was kept at 100%. The data obtained by a streamline Raman analysis was processed using Wire 3.2 software.

2.2 Raman signal of pigments excited by a 785 nm laser

Raman spectra obtained from endolithic phototrophic communities inhabiting Ca-sulphate from Atacama Desert are presented in figure 15. Three different carotenoids, chlorophyll and phycobiliproteins have been identified in the spectra. The Raman spectra systematically revealed 2 different types of Raman signal obtained on green coloured phototrophs. These differences are reflected especially by the ν_1 band position (C=C stretching vibrations) pointing to variable contents of carotenoids with different conjugation and presence/absence of phycobiliprotein signal. Spectral record of algal colonies (based on study of Wierzchos et al., in press) inhabiting typically pore spaces just beneath the surface and living within the epilithic lichen thallus as phycobiont revealed carotenoid ν_1 band position at 1525 cm^{-1} which is assignable to lutein or similar xantophyll compound.

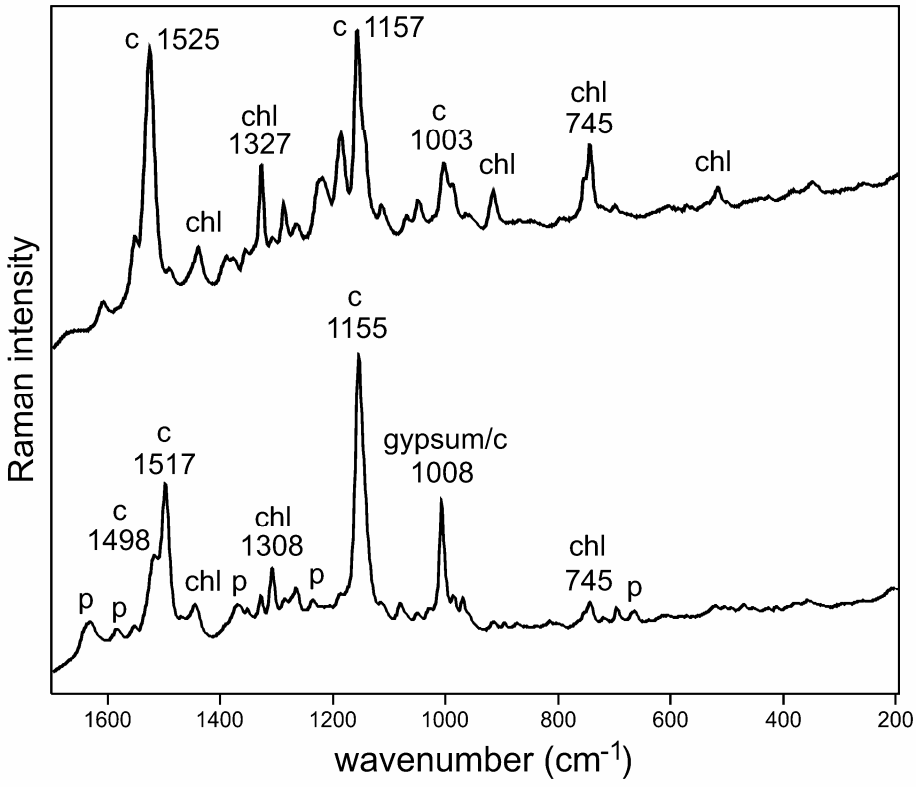

Figure15: Raman spectra of two different colonization zones – near the crust surface (upper spectrum) and colonization observed deeper in the crust matrix, typically near the soil contact (lower spectrum). The analyses were performed using 785 nm excitation.
(c = carotenoid, chl = chlorophyll, p = phycobiliprotein)

Different spectra were typically obtained from colonies living deeper in the sulphate matrix and near the base of the crust, which was originally in contact with the soil. Two carotenoid ν_1 bands have been identified at 1517 and 1498 cm^{-1} (see the carotenoid ν_1 region in figure 16). The strong differences observed between the bands at 1525 and 1498 cm^{-1} are due to different carotenoid polyene chain lengths (as described in Chapter 1.4.1). Hence, the band at 1498 cm^{-1} is interpreted as a signature for presence of carotenoid with 13 or more conjugated double bonds, such as decapreno-β-carotene. Weaker shifts of ν_1 band position can be caused by different functional groups in the structure, different bonding in the cell or isomerisation. The band at 1517 cm^{-1} can be due to β-carotene. Strict assignment of the observed spectral features to particular carotenoid compound can not be done as the ν_1 band position can be identical for several carotenoids and is dependent on carotenoid bonding as well (Oliveira et al., 2010). The differences in carotenoid composition are consistent with the signal of phycobiliproteins, identified by the bands at 1631, 1583, 1370, 1283, 1235, 874, 815, 665 cm^{-1} observed in the spectra together with the 1517 and 1498 cm^{-1} carotenoid bands. Phycobiliproteins are indicative of cyanobacteria which are hence thought to be responsible for this spectral record (see figures 15, 16).

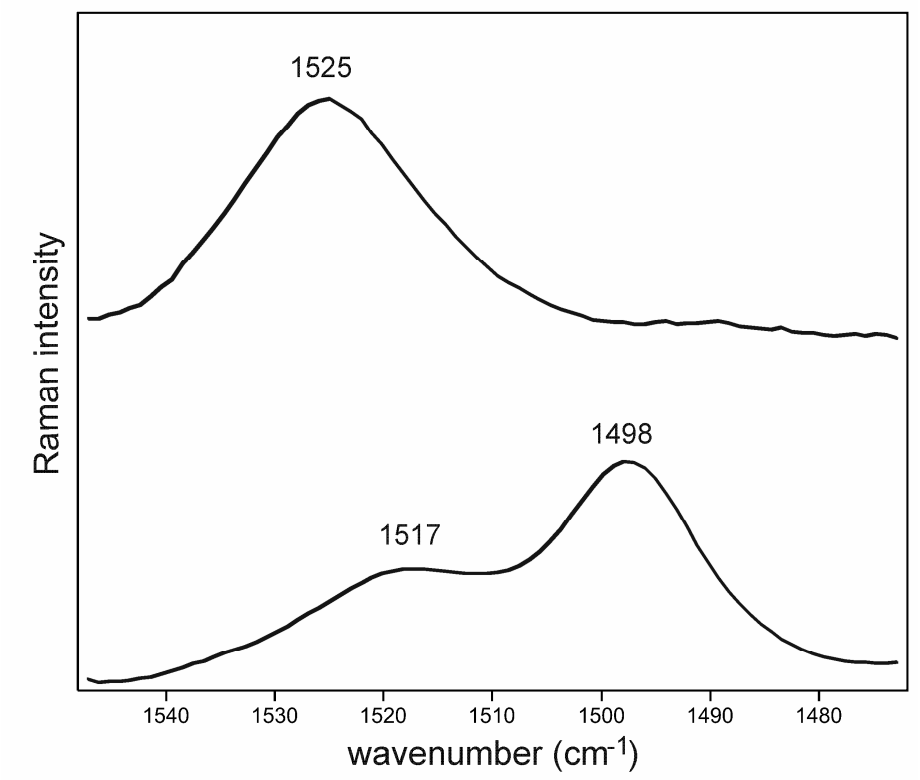

Figure 16: Closer view on the difference in carotenoid ν_1 band position observed within the two different colonization zones.

2.3 Comparison of 785 nm and 514.5 nm excitation

It is well known that the 785 nm excitation wavelength is a universal source for analysis of various biomolecules as well as minerals (see Villar et al., 2005b). On the other hand, as mentioned above, the carotenoid signal can be significantly enhanced due to the already described resonance Raman effect by using 514.5 nm excitation or a close-by lower wavelength. This can be an important point if only small carotenoid content with only a weak Raman signal is analysed. This was proven by analysis of some natural samples (Villar et al., 2005b), as well as artificially prepared mixtures (β-carotene/evaporitic mineral) with defined content of the components (Vítek et al., 2009b).

High-quality spectra were obtained using both excitation sources for analysis of endolithic colonization of Ca-sulphate crusts from Atacama Desert. A stronger carotenoid signal was recorded by the 514.5 nm laser as expected, together with very weak bands due to phycobiliproteins at 1636, 1581 and 1284 cm^{-1}, but no other organic bands were detected using this excitation, in contrast to measurements using the 785 nm laser as described above.

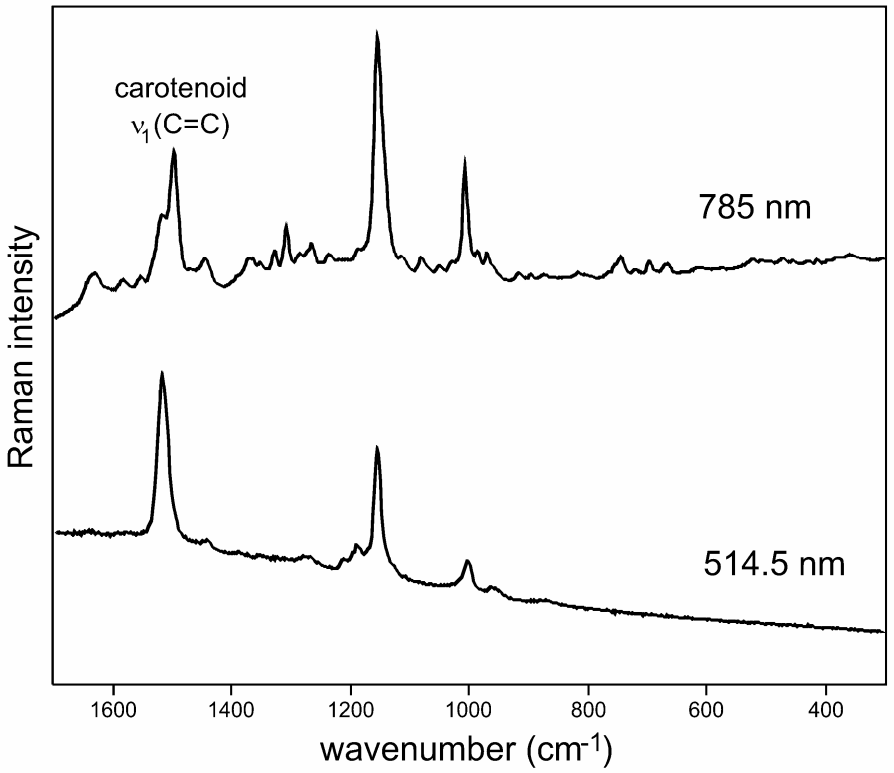

Figure 17: Comparison of the Raman spectra from the same colonization layer (deeper part of the crust) as obtained by the two different excitation wavelength – 785 and 514,5 nm. Lack of chlorophyll spectra and very weak features of phycobiliproteins is evident in the spectra obtained using 514,5 nm laser. Moreover the resonance Raman effect using 514,5 nm laser resulted in selective enhancement of the ν_1 carotenoid band at 1517 cm^{-1} precluding the identification of the band at 1498 cm^{-1} corresponding to long-chained carotenoid.

However, another important effect was observed. Use of these two different excitation wavelengths showed a distinct carotenoid spectral record in analysis of the identical colonization zone (see figure 17). At least two carotenoids have been recognized from the spectra excited by the 785 nm laser as two ν_1 bands can be clearly observed (contribution of more carotenoids can not be ruled out). On the other hand, just one ν_1 band can be clearly distinguished when the Raman spectra using 514.5 nm are recorded. This is mostly ascribed to selective resonant enhancement of a particular carotenoid compound (the ν_1 band at 1516-1518 cm^{-1}) using 514.5 nm excitation, while the signal of the other carotenoid component (longer chained carotenoid) is eclipsed. This effect has important implications for carotenoid analysis in natural systems of unknown carotenoid composition. In addition, a slight shift of the carotenoid ν_1 band as a function of excitation wavelength has been detected and also contributed to the observed band positions.

Systematic resonance Raman analysis using several different wavelengths within resonance region of chlorophylls (440-514,5 nm) and carotenoids (470 - 515 nm) can yield relatively precise information about pigment composition, including different carotenoids in the biological system (Lutz 1972, Lutz and Breton, 1973). However, from this study it can be concluded that, for multi-component pigment identification in one single measurement of microbial communities in-situ in a rock habitat, the 785 nm excitation is strongly favoured a) due to the possibility of identifying other pigments in addition to carotenoids and b) because of the more precise separation of ν_1 bands of different carotenoids. On the other hand, in cases where low carotenoid content can be limiting factor the resonance Raman analysis using excitation near 500 nm is recommended.

2.4 Streamline Raman mapping

A streamline Raman mapping of the green algal zone beneath the surface allowed to observe spatial distribution of particular pigments, namely carotenoid and chlorophyll (as already detected by the point analysis). The analysis provides signal from both organic and inorganic compounds, hence allows mapping of the endolithic communities and their surrounding habitat in one measurement. Subsequent data processing allows imaging based on chemical composition reflected in Raman spectra. Figure 18 represents Raman map based on signal intensity in selected wavenumber range relative to baseline. The signal of carotenoid (red) is represented by the ν_1(C=C) band centered at approx. 1525 cm^{-1} reflecting the distribution of algae in mineral matrix. The occurence of dehydration of gypsum matrix can be observed in the same image represented by the anhydrite signal (light green contrary to dark green colour of gypsum matrix) mapped through the intensity of Raman signal relative to baseline in the $\nu_1(SO_4)$ region.

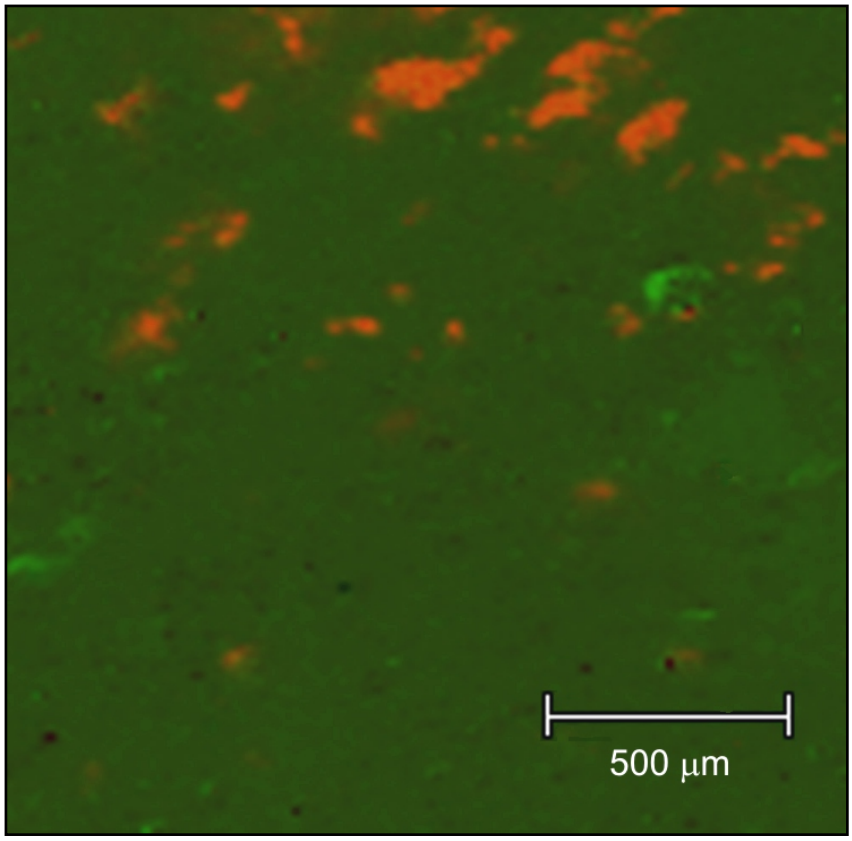

Figure 18: Raman map based on streamline Raman analysis showing an example of distribution of carotenoid (red) and anhydrite (light green) within the gypsum crust (dark green). The crust surface is situated about 1 mm above the top margin.

According to particular advantages of 785 and 514.5 nm excitation wavelengths, both can be extremely valuable for streamline Raman mapping of endolithic communities – 785 nm for imaging of broader range of biomolecules and better spectral separation of different carotenoids and 514.5 nm for its increased sensitivity towards carotenoids for search of any sign of life in the sample.

Streamline Raman analysis proved to be extremely powerful for mapping of spatial distribution of microbial colonies through the pigment composition directly in their microhabitat. Both organic and inorganic compounds are analysed at the same time, thus the method can be recommended as a valuable tool for future studies of bio-mineral interactions.

3. General discussion and conclusions

3.1 Suggestions resulting from the methodical approach on identification of β-carotene in powdered mixtures with evaporites by Raman spectroscopy

Several important points have been demonstrated in these studies:

- relatively high sensitivity towards β–carotene even in non-resonance Raman analysis using 785 nm excitation, permitting recording of a signal even at a concentration level of 1 mg kg^{-1} (Appendix I).

- comparison of these results with resonance Raman measurements (employing 514.5 nm excitation) showed enhancement of Raman signal of β-carotene in the resonance mode, permitting detection of ν_1 band of β-carotene at concentrations of up to one order of magnitude lower (0,1 mg kg^{-1} in sulphate matrix) (Appendix II).

- measurements of the same two-component mixtures through an approx. 2 mm thick sulphate crystal resulted in reduction of the signal; the lowest concentration when any signal of β-carotene was identified was 1 – 10 mg kg^{-1}, depending on the excitation wavelength.

- a halite matrix prevent the occurrence of band overlapping as seen for a sulphate matrix as halite itself does not have a Raman-active mode. However, the spectral background resulting from halite when excited in the visible region can compromise the measurement at low concentration levels (e.g., 0.1 mg kg^{-1}) where no signal of β-carotene was detected in halite in contrast to a sulphate matrix.

- portable Raman instrumentation in the macro-mode can yield comparable or even better results than bench-top Raman micro-spectrometry in analysis of β-carotene at low concentrations in powdered mixtures using the same excitation wavelength. We interpret this observation as being a function of the laser spot-size. As the powdered mixtures are not homogenous on a micro-scale, macro-mode analysis averaging the signal over a larger area is favoured in this case (Appendix III).

In general, sensitivity towards low concentrations of the analyte is not a strong point of ordinary Raman spectroscopy in non-resonant mode, as demonstrated for example by Osterrothová and Jehlička (2009) who analysed usnic acid using the same methods as described in Appendices I-III. This was also demonstrated in Appendix III where mellitic acid was analysed by portable Raman instruments. The lowest content of these organics detected in evaporitic matrix varies between 1 – 10 g kg^{-1} using 785 nm excitation. This is in great contrast to the limiting concentrations of β-carotene obtained here (Appendices I - III), or results reported by Alajtal et al. (2010) who analysed polycyclic aromatic hydrocarbons (PAHs) in

low concentrations as well. Together with the fact that carotenoids are ubiquitously present as cellular components of extremophiles across the taxonomic domains found in extreme environments on Earth, the sensitivity of Raman spectroscopy towards carotenoids could be potentially important for search for traces of life in extreme habitats on Earth and beyond.

It has been described in the previous text that evaporitic environment can harbour phototrophic microorganisms in areas which are supposed as dry limit for life on Earth (Atacama Desert) and could have potentially served as habitat for photoautotrophic life on Mars at least in periods when water activity was greater than today. As mentioned by Warren-Rhodes et al. (2006), the potential microbial life or its remnants on Mars (if present) will probably be widely dispersed and difficult to detect. The lowest content of β-carotene identified by both bench-top and portable Raman instrumentation is low enough to allow detection of carotenoid in many of naturally occurring systems known on Earth. However, as pointed in Appendix III - the routine usage of portable Raman instrumentation under field conditions for identification of natural biomolecules in geological samples has not yet been established and remains a goal for future experiments. Several problems may occur that can potentially hinder the analysis. The first is that a fluorescing agent could cause fluorescence which overlaps the Raman signal even in a carotenoid-rich matrix. An example of such an agent could be a slimy extracellular polysaccharide matrix produced in huge amounts by some cyanobacterial communities in hypersaline environment, which probably hinders the identification of any organics using the 785 nm excitation. In this case, the use of some other excitation source can, in principle, resolve the problem (Jan Jehlička and Petr Vítek, personal observation).

Due to relative instability of carotenoids, the two conditions are considered for their potential presence on Mars – a) occurrence of relative recent microbial life, or b) preservation in dark in anoxic conditions, for example within mineral inclusions. According to my experience, positioning of aggregates of microbial colonies at the micro-scale which is necessary for direct analysis without any pretreatment of the sample can be difficult and sometimes time-consuming in some cases of widespread diffuse colonization or analysis of organic material embedded in inclusions even when the whole matrix is clearly coloured due to microbial pigmentation. The systematic analysis in micro-mode is important for terrestrial scenarios (see next chapter), however this approach would potentially be a difficult or almost impossible in the case where remote control of instrumentation deployed on another planetary body is necessary. Current plans for the ExoMars mission (now scheduled to launch in 2018) deal with the Raman spectroscopic analysis of powdered Martian samples drilled from the subsurface (no direct Raman analysis of rock is intended on this mission). Raman spectrometer will operate with the 532 nm excitation source with laser spot size about 50 μm (Vago, 2009). Analysis of homogenized mixture of potential organic compounds and mineral matrix can be an advantage for such situation. However, the question arises whether potential organics embedded in mineral matrix will be released to be eventually detected by Raman spectrometer and whether the grinding will be efficient enough to sufficiently homogenize the mixture. It is a task for future research to answer these questions.

3.2 Raman spectroscopy of natural endoevaporitic colonies

Microbial colonies in extreme dry parts of Atacama Desert have been studied for the first time by Raman micro-spectrometry (Chapter 2 and Appendix IV). Analysis in a micro-mode was crucial here for focusing on particular aggregates of cyanobacteria in their microhabitats.

An excitation wavelength of 785 nm (non-resonance mode) proved to have some advantages in carotenoid analysis of the studied biocolonized Ca-sulphate samples compared to resonance Raman measurements using 514.5 nm for excitation. It is already well known that a broader range of cell components (pigments) can be observed using the 785 nm laser (Villar et al., 2005b). Although a stronger signal of carotenoids can be obtained in the resonance mode, selective enhancement of one carotenoid compound can preclude unambiguous identification of other bands of different carotenoids in the system using one excitation wavelength. Hence 785 nm excitation provided us with more valuable data in some cases, where long-chained carotenoid (~13-15 conjugated double bonds) with a ν_1 band at approx. 1498 cm^{-1} observed using this wavelength was missing using the 514.5 nm laser.

Following the initial work of Wierzchos et al. (2006), who discovered the endoevaporitic cyanobacterial colonization of halite crusts in different parts of Atacama Desert, our Raman spectroscopic investigation revealed different pigment composition in a particular microhabitat pointing to adaptation strategies evolved by the cyanobacteria living there. Colonies occupying the halite crust relatively near the surface typically produced scytonemin, whereas more diffuse colonization observed in deeper parts of the crust typically exhibited no or only a very weak signal of this photoprotective molecule. Significant variability of the scytonemin signal was interpreted as being a result of differences in biosynthesis of this important UV-screening compound mainly as a response to variable UV-exposure – depending on the light properties of a particular microhabitat. According to the work of Dillon et al. (2002), the effect of other environmental parameters, such as high temperatures or salinity, together with the UV-A dose in production of scytonemin can not be ruled out.

Phycobiliproteins were identified within some of the colonies inhabiting Ca-sulphate and halite crusts in Atacama Desert. These light-harvesting pigments are synthesised by cyanobacteria as a response to the amount of photosyntetically active radiation – lower energy from the sunlight typically leads to an increase in the biosynthesis of the phycobiliproteins so that more energy is gained by the photosystem (Medigan et al., 2008). This phenomenon was already observed by Villar et al. (2006) using Raman spectroscopy. Moreover, in the analysed Ca-sulphate samples from Atacama Desert, the Raman signal of phycobiliproteins could be used as an indicative biomolecule for cyanobacteria and was systematically coupled with the specific carotenoid composition compared to microbial colonization originating from the aggregates of eukaryotic algae. Interestingly, the carotenoid composition of the cyanobacterial colonies inhabiting halite (diffuse colonization patterns lacking in scytonemin) and Ca-sulphate clearly differ based on the carotenoid ν_1 band positions observed.

Raman instrumentation coupled with an optical microscope allows a single cell or only a small number of micro-organisms in aggregates to be unambiguously analysed. As the biomolecular composition in particular microbial strains is due to gene expression, some

limited taxonomical interpretations of the Raman spectra can be derived from the Raman spectroscopic data, particularly in systems with known diversity. The taxonomically-dependent carotenoid Raman signal excited by the 514.5 nm line of an argon laser was depicted by Petry et al. (2003), who described the Raman spectroscopic features of β-carotene from *Rhodotula rubra* and sarcinaxanthin from *Micrococcus luteus*.

Nevertheless, in natural systems, where the unknown community living within rock substrate in extreme environments is investigated, spectral differences obtained from the colonies can be attributed to various reasons apart from the species diversity. Environmental stress and the subsequent biochemical response of the particular organism, as well as alteration of organic remnants, are two other very important parameters potentially reflected in the spectra. These parameters can be mapped nondestructively using Raman micro-spectrometry. However, it may be difficult to discriminate between these individual factors affecting the Raman spectra of microbial colonies in their natural habitats. Precise taxonomic information about the analysed microbial colony is of interest for more detailed interpretation of the Raman spectroscopic data. Phylogenetic examination by culture-independent methods, namely based on ribosomal RNA studies is an important and precise method for identification of microbial diversity in natural habitats as shown, for instance, by Navarro-González et al. (2003) for Atacama Desert soils. However, for examination of spatial relationship and adaptation strategies of the studied colonies, other methods, allowing in-situ study of microbial communities are necessary (Wierzchos et al., in press). An optical microscope coupled to a Raman spectrometer is crucial for positioning and focusing particular cell aggregates for in-situ analysis within the microhabitat, but it may not be sufficient in some cases for precise identification of the organism. Therefore, additional information provided by other techniques (complementary to Raman) may be necessary in the future to facilitate Raman analysis of endolithic communities. Such information could be provided by other microscopic techniques such as scanning electron microscopy (SEM) based methods, fluorescence microscopy or atomic force microscopy (AFM) used together with Raman, thus allowing the assessment of close relationships between different colonies along with their spatial arrangement. A great potential lies in combination of Raman with the other analytical methods for study of geo-biological systems like this.

Despite the limitations of Raman spectroscopy alone described above, it was shown here (Chapter 2 and Appendix IV), that Raman micro-spectroscopic analysis of endoevaporitic colonies in-situ can help us to study life adaptation strategies on the basis of pigment composition. Moreover, streamline Raman mapping proved to be a very promising method for study and imaging of rock-inhabiting pigmented microorganisms in their original microhabitat. Importantly, the method allows mapping of both organic and inorganic compounds in the same time, thus potentially allowing the direct observation of microbial colonies and surrounding mineralogy defining the microhabitat and in future may be used for study of bio-mineral interaction like microbially induced mineral weathering. Hence, the application of the streamline Raman mapping can contribute to future progress in Earth-based research of geo-biological systems.

In conclusion, the Raman microspectrometry proved to be an efficient tool for detection of β-carotene – a typical carotenoid - in very low concentrations in model mixtures with evaporites in resonance Raman mode (0,1 – 1 mg kg^{-1} level) and very good results were obtained in non-resonance mode using more universal 785 nm excitation as well (e.g., lowest content detected was 1-10 mg kg^{-1}). Macro-mode analysis using large laser spot-size (~100 μm) is considered as a favourable for analysis of finely ground mixtures studied here as concluded from the results obtained by the portable (hand-held) Raman instrumentation using 785 nm laser for excitation. Together with detection of carotenoids, scytonemin as well as other pigments in natural evaporitic systems from Atacama Desert, these results point to successful application of Raman spectroscopy for nondestructive identification of pigments as traces of life in evaporites – both terrestrially and potentially in future within missions aimed to search for life on another planetary body.

References

Alajtal, A. I., Edwards, H. G. M., Scowen, I J. (2010). Raman spectroscopic analysis of minerals and organic molecules of relevance to astrobiology. *Analytical and Bioanalytical Chemistry* **397**, 215-221.

Anderson, I. C., Robertson, D. S. (1960). Role of carotenoids in protecting chlorophyll from photodestruction. *Plant Physiology* **35**, 531-534.

Andreeva, A., Velitchkova, M. (2005). Resonance Raman spectroscopy of carotenoids in Photosystem I particles. *Biophysical Chemistry* **114**, 129-135.

Antón, J., Llobet-Brossa, E., Rodríguez-Valera, F., Amann, R. (1999). Fluorescence in situ hybridization analysis of the prokaryotic community inhabiting crystallizer ponds. *Environmental Microbiology* **1**, 517-523.

Antón, J., Roselló-Mora, R., Rodríguez-Valera, F., Amann, R. (2000). Extremely halophilic Bacteria in crystallizer ponds from solar salterns. *Applied and Environmental Microbiology* **66**, 3052-3057.

Antón, J., Oren, A., Benlloch, S., Rodríguez-Valera, F., Amann, R., Roselló-Mora, R. (2002). *Salinibacter ruber* gen. nov., sp. nov., a new species of extremely halophilic Bacteria from saltern crystallizer ponds. *International Journal of Systematic and Evolutionary Microbiology* **52**, 485-491.

Arvidson, R. E., Seelos, F. P., Deal, K. S., Koeppen, D. C., Snider, N. O., Kieniewicz, J. M., Hynek, B. M., Mellon, M. T., Garvin, J. B. (2003). Mantled and exhumed terrains in Terra Meridiani, Mars. *Journal of Geophysical Research – Planets* **108**, E8073-E8077.

Baker, V. R. (2001). Water and the martian landscape. Nature **412**, 228-236.

Barbieri, R., Stivaletta, N., Marinangeli, L., Ori, G. G. (2006). Microbial signatures in sabkha evaporite deposits of Chott el Gharsa (Tunisia) and their astrobiological implications. *Planetary and Space Science* **54**, 726-736.

Bandfield, J. L., Smith, M. D. (2003). Multiple emission angle surface-atmosphere separations of Thermal Emission Spectrometer data. *Icarus* **161**, 47-65.

Benison, K. C., Beitler Bowen, B. (2006). Acid saline lake ecosystems give clues about past environments and the search for life on Mars. *Icarus* **183**, 225-229.

Bernard, W., de Waal, D. (2006) Raman investigation of pigmentary molecules in the molluscan biogenic matrix. *Journal of Raman Spectroscopy* **37**, 342-352.

Bibo, F.-J., Söngen, R., Fresenius, R. E. (1983). Vermehrungsfähige Mikroorganismen in Steinsalz aus primären Lagerstatten. *Kali und Steinsalz* **8**, 367-373.

Bibring, J. P., Langevin, Y., Mustard, J. F., Poulet, F., Arvidson, R., Gendrin, A., Gondet, B., Mangold, N., Pinet, P., Forget, F. (2006). Global mineralogical and aqueous mars history derived from OMEGA/Mars express data. *Science* **312**, 400-404.

Biemann, K., Oro, J., Toulmin III, P., Orgel, L. E., Nier, A. O., Anderson, D. M., Simmonds, P. G., Flory, D., Diaz, A. V., Rushneck, D. R., Biller, J. E., La Fleur, A. K. (1977). The search for organic substances and inorganic volatile compounds in the surface of Mars. *Journal of Geophysical Research* **82**, 4641-4658.

Bishop, J. L., Lane, M. D., Darby Dyar, M., Parente, M., Roach, L. H., Murchie, S. L., Mustard, J. F. (2008). Sulfates on Mars: how recent discoveries from CRISM, OMEGA and the MERs are changing our view of the planet. *Geochimica et Cosmochimica Acta* **72**, A86.

Boynton, W. V., Feldman, W. C., Squyres, S. W., Prettyman, T. H., Brückner, J., Evans, L. G., Reedy, R. C., Starr, R., Arnold, J. R., Drake, D. M., Englert, P. A. J., Metzger, A. E., Mitrofanov, I., Trombka, J. I., d'Uston, C., Wänke, H., Gasnault, O., Hamara, D. K., Janes, D. M., Marcialis, R. L., Maurice, S., Mikheeva, I., Taylor, G. J., Tokar, R., Shinohara, C. (2002). Distribution of hydrogen in the near surface of Mars: Evidence for subsurface ice deposits. *Science* **297**, 81-85.

Boynton, W. V., Ming, D. W., Kounaves, S. P., Young, S. M. M., Arvidson, R. E., Hecht, M. H., Hoffman, J., Niles, P. B., Hamara, D. K., Quinn, R. C., Smith, P. H., Sutter, B., Catling, D. C., Morris, R. V. (2009). Evidence for Calcium Carbonate at the Mars Phoenix Landing Site. *Science* **325**, 61-64.

Brock, T. D. (1975). Salinity and the ecology of *Dunaliella* from the Great Salt Lake. *Journal of General Microbiology* **89**, 285-292.

Brocks, J. J., Love, G. D., Summons, R. E., Knoll, A. H., Logan, G. A., Bowden, S. A. (2005). Biomarker evidence for green and purple sulphur bacteria in stratified Paleoproterozoic sea. *Nature* **437**, 866-870.

Brückner, J., Dreibus, G., Gellert, R., Squyres, S. W., Wänke, H., Yen, A., Zipfel, J. (2008). Mars Exploration Rovers: chemical composition by the APXS. In: The Martian Surface, Composition, Mineralogy and Physical Properties (Bell III, J. F. Ed.), Cambridge University Press, pp. 58.

Burns, R. G. (1987). Ferric sulphates on Mars. *Journal of Geophysical research – Planets* **92**, E570-E574.

Cabrol, N. A., Grin, E. A. (1999). Distribution, classification, and ages of Martian impact crater lakes. Icarus **142**, 160-172.

Cabrol, N. A., Grin, E. A. (2001). The evolution of lacustrine environments on Mars: is Mars only hydrologically dormant? *Icarus* **149**, 291-328.

Carr, M. H., Clow, G. D. (1981). Martian channels and valleys: their characteristics, distribution, and age. *Icarus* **48**, 91-117.

Carr, M. H. (1996). Water on Mars. Oxford University Press, New York, 248 p.

Carron, K., Cox, R., (2010). Qualitative Analysis and the Answer Box: A Perspective on Portable Raman Spectroscopy. *Analytical Chemistry* **82**, 3419-3425.

Ceccarelli, M., Lutz, M., Marchi, M. (2000). A density functional normal mode calculation of a bacteriochlorophyll *a* derivative. *Journal of American Chemical Society* **122**, 3532-3533.

Chadha, S., Manoharan, R., Moënne-Loccoz, P., Nelson, W. H., Peticolas, W. L., Sperry, J. F. (1993) Comparison of the UV resonance Raman-spectra of bacteria, bacterial-cell walls, and ribosomes excited in the deep UV. *Applied Spectroscopy* **47**, 38-43.

Chapman, C. R., Jones, K. L. (1977). Cratering and obliteration history of Mars. *Annual Reviews in Earth and Planetary Sciences* **5**, 515-540.

Christensen, P. R., Bandfield, J. L., Clark, R. N., Edgett, K. S., Hamilton, V. E., Hoefen, T., Kieffer, H. H., Kuzmin, R. O., Lane, M. D., Malin, M. C., Morris, R. V., Pearl, J. C., Pearson, R., Roush, T. L., Ruff, S. W., Smith, M. D. (2000). Detection of crystalline hematite mineralization on Mars by the Thermal Emission Spectrometer: Evidence for near-surface water. *Journal of Geophysical Research - Planets* **105**, 9623-9642.

Christensen, P. R., Morris, R. V., Lane, M. D., Bandfield, J. L., Malin, M. C. (2001). Global mapping of Martian hematite mineral deposits: Remnants of water-driven processes on early Mars. *Journal of Geophysical Research - Planets* **106**, 23873-23885.

Christensen, P. R., Wyatt, M. B., Glotch, T. D., Rogers, A. D., Anwar, S., Arvidson, R. E., Bandfield, J. L., Blaney, D. L., Budney, C., Calvin, W. M., Fallacaro, A., Fergason, R. L., Gorelick, N., Graff, T. D., Hamilton, V. E., Hayes, A. G., Johnson, J. R., Knudson, A. T., McSween, H. Y. Jr., Mehall, G. L., Mehall, L. K., Moersch, J. E., Morris, R. V., Smith, M. D., Squyres, S. W., Ruff, S. W., Wolff, M. J. (2004). Mineralogy at Meridiani Planum from the Mini-TES Experiment on the Opportunity Rover. *Science* **306**, 1733-1739.

Christensen, P. R., Ruff, S. W. (2004). Formation of hematite-bearing unit in Meridiani Planum: Evidence for deposition in standing water. *Journal of Geophysical Research – Planets* **109**, E08003.

Christensen, P. R., Bandfield, J. L., Rogers, A. D., Glotch, T. D., Hamilton, V. E., Ruff, S. W., Wyatt, M. B. (2008). Global mineralogy mapped from the Mars Global Surveyor Thermal Emission Spesctrometer. In: The Martian Surface, Composition, Mineralogy and Physical Properties (Bell III, J. F. Ed.), Cambridge University Press, pp. 195.

Clark, B. C., Baird, A. K. (1979). Is the Martian lithosphere sulfur rich? *Journal of Geophysical Research* **84**, 8395-8402.

Clark, B. C., Morris, R. V., McLennan, S. M., Gellert, R., Jolliff, B., Knoll, A. H., Squyres, S. W., Lowenstein, T. K., Ming, D. W., Tosca, N. J., Yen, A., Christensen, P. R., Gorevan, S., Brückner, J., Calvin, W., Dreibus, G., Farrand, W., Klingelhoefer, G., Waenke, H., Zipfel, J., Bell III, J. F., Grotzinger, J., McSween, H. Y., Rieder, R. (2005). Chemistry and mineralogy of outcrops at Meridiani Planum. *Earth and Planetary Science Letters* **240**, 73-94.

Cockell, C. S., Knowland, J. (1999). Ultraviolet radiation screening compounds. *Biological Reviews* **74**, 311-345.

Cockell, C. S., Schrueger, A. C. Billi, D., Friedmann, E. I., Panitz, C. (2005). Efects of a simulated martian UV flux on the cyanobacterium, *Chroococcidiopsis* sp 029. *Astrobiology* **5**, 127-140.

Davila, A. F., Gomez-Silva, B., de los Rios, A., Ascaso, C., Olivares, H., McKay, C. P., Wierzchos, J. (2008) Facilitation of endolithic microbial survival in the hyperarid core of the Atacama Desert by mineral deliquesence. *Journal of Geophysical Research – Biogeosciences* **113**, G01028.

de Oliveira, V. E., Castro, H. S., Edwards, H. G. M., de Oliveira, L. F. C. (2010). Carotenes and carotenoids in natural biological samples: a Raman spectroscopic analysis. *Journal of Raman Spectroscopy* **41**, 642-650.

Dennis, P. P., Shimmin, L. C. (1997). Evolutionary divergence and salinity-mediated selection in halophilic archaea. *Microbiology and Molecular Biology Reviews* **61**, 90-104.

Dickensheets, D. L., Wynn-Williams, D. D., Edwards, H. G. M., Schoen, C., Crowder, C., Newton, E. M. (2000). A novel miniature confocal microscope/Raman spectrometer system for biomolecular analysis on future Mars missions after Antarctic trials. *Journal of Raman Spectroscopy* **31**, 633-635.

Dillon, J. G., Tatsumi, C. M., Tandingan, P. G., Castenholz, R. W. (2002). Effect of environmental factors on the synthesis of scytonemin, a UV-screening pigment, in a cyanobacterium (*Chroococcidiopsis* sp.). *Archives of Microbiology* **177**, 322–331.

Dombrowski, H. (1963). Bacteria from Oaleozoic salt deposits. *Annals of the New York Academy of Sciences* **108**, 453-460.

Dong, H. L., Rech, J. A., Jiang, H. C., Sun, H., Buck, B. J. (2007). Endolithic cyanobacteria in soil gypsum: Occurrences in Atacama (Chile), Mojave (United States), and Al-Jafr Basin (Jordan) deserts. *Journal of Geophysical Research – Biogeosciences* **112**, G02030.

Douglas, S. (2004). Microbial biosignatures in evaporite deposits: Evidence from Death Valley, California. *Planetary and Space Science* **52**, 223-227.

Douglas, S., Abbey, W., Mielke, R., Conrad, P., Kanik, I. (2008). Textural and mineralogical biosignatures in unusual microbialite from Death Valley, California. *Icarus* **193**, 620-636.

Dufossé, L. (2009). Microbial and Microalgal Carotenoids as Colourants and Supplements. In: Carotenoids (Eds. Britton, G., Liaanen-Jensen, S., Pfander, H.), Birkhäuser, Basel, vol. 5, pp 83-98.

Edwards, H. G. M., Garcia-Pichel, F., Newton, E. M., Wynn-Williams, D. D. (2000). Vibrational Raman spectroscopic study of scytonemin, the UV-protective cyanobacterial pigment. *Spectrochimica Acta A.* **56**, 193-200.

Edwards, H. G. M., Newton, E. M., Wynn-Williams, D. D. (2003a). Molecular structural studies of lichen substances II: atranorin, gyrophoric acid, fumarprotocetraric acid, rhizocarpic acid, calycin, pulvinic dilactone and usnic acid. *Journal of Molecular Structure* **651**, 27-37.

Edwards, H. G. M., Newton, E. M., Wynn-Williams, D. D., Lewis-Smith, R. I. (2003b). Non-destructive analysis of pigments and other organic compounds in lichens using Fourier-transform Raman spectroscopy: a study of Antarctic epilithic lichens. *Spectrochimica Acta A* **59**, 2301-2309.

Edwards, H. G. M., Moody, C. D., Villar, S. E. J., Wynn-Williams, D. D. (2005a). Raman spectroscopic detection of key biomarkers of cyanobacteria and lichen symbiosis in extreme Antarctic habitats: evaluation for Mars Lander missions. *Icarus* **174**, 560-571.

Edwards, H. G. M., Villar S. E. J., Parnell, J., Cockell, Ch. S., Lee P. (2005b). Raman spectroscopic analysis of cyanobacterial gypsum halotrophs and relevance for sulfate deposits on Mars. *Analyst* **130**, 917-923.

Edwards, H. G. M., Mohsin, M. A., Sadooni, F. N., Hassan, N. F. N., Munshi, T. (2006). Life in the sabkha: Raman spectroscopy of halotrophic extremophiles of relevance to planetary exploration. *Analytical and Bioanalytical Chemistry* **385,** 46-56.

Edwards, H. G. M., Vandenabeele, P., Villar, S. E. J., Carter, E. A., Rull Perez, F., Hargreaves, M. D. (2007). The Rio Tinto Mars Analogue site: An extremophilic Raman spectroscopic study. *Spectrochimica Acta A* **68**, 1133-1137.

Ericksen, G. E. (1993). Upper Tertiary and Quaternary continental saline deposits in the central Andean region. *Geological Association of Canada Special Paper* **40**, 89-102.

Fendrihan, S., Bérces, A., Lammer, H., Musso, M., Rontó, G., Polacsek, T. K., Holzinger, A., Kolb, C., Stan-Lotter, H. (2009a). Investigating the Effects of Simulated Martian Ultraviolet Radiation on *Halococcus dombrovskii* and Other Extremely Halophilic Archaebacteria. *Astrobiology* **9**, 104-112.

Fendrihan, S., Musso, M., Stan-Lotter, H. (2009b). Raman spectroscopy as a potential method for the detection of extremely halophilic archaea embedded in halite in terrestrial and possibly extraterrestrial samples. *Journal of Raman Spectroscopy* **40**, 1996-2003.

Fong, N. J. C., Burgess, M. L., Barrow, K. D., Glenn, D. R. (2001). Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. *Applied Microbiology and Biotechnology* **56**, 750-756.

Foster, I. S., King, P. I., Hyde, B. C., Southam, G. (2010). Characterization of halophiles in natural MgSO4 salts and laboratory enrichment samples: Astrobiological implications for Mars. *Planetary and Space Science* **58**, 599-615.

Galinsky, E. A. (1993). Compatible solutes of halophilic eubacteria – molecular principles, water-solute interaction, stress protection. *Experientia* **49**, 487-496.

Galinsky, E. A. (1995). Osmoadaptation in Bacteria. In: *Advances in Microbial Physiology* (Ed: Poole, R. K.), Academic Press limited, San Diego, vol. 37, 273-328.

Garcia-Pichel, F., Castenholz, R. W. (1991). Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *Journal of Phycology* **27**, 395-409.

Garcia-Pichel, F., Sherry, N. D., Castenholz, R. W. (1992). Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. *Photochemistry and Photobiology* **56**, 17-23.

Garcia-Pichel, F., Wingard, C. E., Castenholz, R. W. (1993). Evidence regarding the UV sunscreen role of mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. *Applied and Environmental Microbiology* **59**, 170-176.

Garcia-Pichel, F. (1994). A model for internal self-shading in planctonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnology and Oceanography* **39**, 1704-1717.

Gellert, R. Rieder, R., Brückner, J., Clark, B. C., Dreibus, G., Klingelhöfer, G., Lugmair, G., Ming, D. W., Wänke, H., Yen, A., Zipfel, J., Squyres, S. W. (2006). Alpha particle X-ray spectrometer (APXS): results from Gusev crater and calibration report. *Journal of Geophysical Research* **111**, E02S05.

Gendrin, A., Mangold, N., Bibing, J.-P., Langevin, Y., Gondet, B., Poulet, F., Bonello, G., Quantin, C., Mustard, J., Arvidson, R., LeMouélic, S. (2005). Sulfates in Martian layered terrains: the OMEGA/Mars Express view. *Science* **307**, 1587-1591.

Gill, D., Kilponen, R. G., Rimai, L. (1970). Resonance Raman scattering of laser radiation by vibrational modes of carotenoid pigment molecules in intact plant tissues. *Nature* **227**, 743.

Golubic, S. (1980). Halophily and halotolerance in cyanophytes. *Origins of life and Evolution of Biospheres* **10**, 169-183.

Gooding, J. L. (1992). Soil mineralogy and chemistry on Mars: possible clues from salts and clays in SNC meteorites. *Icarus* **99**, 28-41.

Grant, W. D. (2004). Life at low water activity. *Philosophical Transactions of the Royal Society of London B* **359**, 1249-1266.

Green, W. J., Lyons, W. B. (2009). The Saline Lakes of the McMurdo Dry Valleys, Antarctica. *Aquatic Geochemistry* **15,** 321-348.

Gröniger, A., Sinha, R. P., Klisch, M., Hader, D. P. (2000). Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – a database. *Journal of Photochemistry and Photobiology B – Biology* **58**, 115-122.

Grotzinger, J. P., Arvidson, R. E., Bell III, J. F., Calvin, W., Clark, B. C., Fike, D. A., Golombek, M., Greeley, R., Haldemann, A., Herkenhoff, K. E., Jolliff, B. L., Knoll, A. H., Malin, M., McLennan, S. M., Parker, T., Soderblom, L., Sohl-Dickstein, J. N., Squyres, S. W., Tosca, N. J., Watters, W. A. (2005). Stratigraphy and sedimentology of a dry to wet eplian depositional system, Burns formation, Meridiani Planum, Mars. *Earth and Planetary Science Letters* **240**, 11-72.

Gruszecki, W. I., Gospodarek, M., Grudziński, W., Mazur, R., Gieczewska, K., Garstka, M. (2009). *Journal of Physical Chemistry* B **113**, 2506-2512.

Haberle, R. M., McKay, C. P., Schaeffer, J., Cabrol, N. A., Grin, E. A., Zent, A. P., Quinn, R. (2001). On the possibility of liquid water on present-day Mars. *Journal of Geophysical Research – Planets* **106**, 23317-23326.

Haberle, R. A., Murphy, J. R., Schaeffer, J. (2003). Orbital change experiments with a Mars general circulation model. *Icarus* **161**, 66-89.

Hand, R. M., Burton, H. R. (1981). Microbial ecology of an Antarctic saline meromictic lake. *Hydrobiologia* **82**, 363-374.

Haskin, L.A., Wang, A., Jolliff, B. L., McSween, H. Y., Clark, B. C., Des Marais, D. J., McLennan, S. M., Tosca, N. J., Hurowitz, J. A., Farmer, J. D., Yen, A., Squyres, S. W., Arvidson, R. E., Klingelhöfer, G., Schröder, C., de Souza, P. A., Ming, D. W., Gellert, R., Zipfel, J., Brückner, J., Bell, J. F., Herkenhoff, K., Christensen, P. R., Ruff, S., Blaney, D., Gorevan, S., Cabrol, N. A., Crumpler, L., Grant, J., Soderblom, L. (2005). Water alteration of rocks and soils on Mars at the Spirit rover site in Gusev crater, *Nature* **436**, 66-69.

Hazen, R. M. & Roedder, E. (2001). How old are bacteria from the Permian age? *Nature* **411**, 155-155.

Hebsgaard, M. B., Phillips, M. J., Willerslev, E. (2005). Geologically ancient DNA: fact or artifact? *Trends in Microbiology* **13**, 121-220.

Hecht, M. H., Kounaves, S. P., Quinn, R. C., West, S. J., Young, S. M. M., Ming, D. W., Catling, D. C., Clark, B. C., Boynton, W. V., Hoffman, J., DeFlores, L. P., Gospodinova, K., Kapit, J., Smith, P. H. (2009). Detection of Perchlorate and the Soluble Chemistry of Martian Soil at the Phoenix Lander Site. *Science* **325**, 64-67.

Holder, J. M., Wynn-Williams, D. D., Rull Perez, F., Edwards, H. G. M. (2000). Raman spectroscopy of pigments and oxalates in situ within epilithic lichens: *Acarospora* from the Antarctic and Mediterranean. *New Phytologist* **145**, 271-280.

Houtkooper, J. M., Schulze-Makuch, D. (2007). A possible biogenic origin for hydrogen peroxide on Mars: the Viking results reinterpreted. *International Journal of Astrobiology* **6**, 147-152.

Jagger, J. (1985). Solar-UV Actions on Living Cells. Praeger, New York, 202 p.

Jakosky, B. M., Phillips, R. J. (2001). Mars‘ volatile and climate history. *Nature* **412**, 237-244.

Jarvis, R. M., Goodcare, R. (2004). Ultra-violet resonance Raman spectroscopy for the rapid discrimination of urinary tract infection bacteria. *FEMS Microbiology Letters* **232**, 127-132.

Javor, B. (1989). Hypersaline Environments, Microbiology and Biogeochemistry. Springer-Verlag, Berlin.

Kanavaroti, A., Mancinelli, R. (1990). Could organic matter have been preserved on Mars for 3.5 years? *Icarus* **84**, 196-202.

Kedar, L., Kashman, Y., Oren, A. (2002). Mycosporine-2-glycine is the major mycosporine-like amino acid in a unicellular cyanobacterium (*Euhalothece* sp.) isolated from a gypsum crust in a hypersaline saltern pond. *FEMS Microbiology Letters* **208**, 233-237.

Kelly, M., Norgard, S., Liaanen-Jensen, S. (1970) C50-carotenoids. 5. Carotenoids of *Halobacterium salinarum*, especially bacterioruberin. *Acta Chemica Scandinavica* **24**, 2169-2182.

Kiefer, H. H., Chase, S. C., Martin, T. Z., Minre, E. D., Don Palluconi, F. (1976). Martian north pole summer temperature: Dirty water ice. *Science* **194**, 1341-1344.

Klingelhöfer, G., Morris, R. V., Bernhardt, B., Schröder, C., Rodionov, D. S., de Souza, P. A., Yen, A., Gellert, R., Evlanov, E. N., Zubkov, B., Foh, J., Bonnes, U., Kankeleit, E., Gutlich, P., Ming, D. W., Renz, F., Wdowiak, T., Squyres, S. W., Arvidson, R. E. (2004). Jarosite and Hematite at Meridiani Planum from Opportunity’s Mössbauer Spectrometer. *Science* **306**, 1740-1745.

Knoll, A. H., Carr, M., Clark, B., Des Marais, D. J., Farmer, J. D., Fischer, W. W., Grotzinger, J. P., McLennan, S. M., Malin, M., Schröder, C., Squyres, S., Tosca, N. J., Wdowiak, T. (2005). An astrobiological perspective on Meridiani Planum. *Earth and Planetary Science Letters* **240**, 179-189.

Krinsky, N. I. (1979). Carotenoid protection against oxidation. *Pure and Applied Chemistry* **51**, 649-660.

Kuhn, W. R., Atreya, S. K. (1979). Solar radioation incident on the Martian surface. *Journal of Molecular Evolution* **14**, 57-64.

Lanyi, J. K. (1974). Salt-dependent properties of proteins from extremely halophilic bacteria. *Bacteriological Reviews* **38**, 272-290.

Lanza, N. L., Meyer, G. A., Okubo, C. H., Newsom, H. E., Wiens, R. C. (2010). Evidence for debris flow gully formation initiated by shallow subsurface water on Mars. *Icarus* **205**, 103-112.

Laskar, J., Correia, A. C. M., Gastineau, M., Joutel, F., Levrard, B., Robutel, P. (2004). Long term evolution and chaotic diffusion of the insolation quantities of Mars. *Icarus* **170**, 343-364.

Leovy, C. (2001). Weather and climate on Mars. *Nature* **412**, 245-249.

Lewis, A., Spoonhower, J., Bogomolni, R. A., Lozier, R. H., Stoeckenius, W. (1974). Tunable Laser Resonance Raman Spectroscopy of Bacteriorhodopsin. *Proceedings of the National Academy of Sciences* **71**, 4462-4466.

Liaaen-Jensen, S. (1997). Stereochemical aspects of carotenoids. *Pure and Applied Chemistry* **57**, 649-658.

Lutz, M. (1972). Raman resonance spectroscopy of plant pigments in solution and included in chloroplast lamellae. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences Serie B* **275**, p 497.

Lutz, M, Breton, J. (1973). Chlorophyll associations in the chloroplast: Resonance Raman spectroscopy. *Biochemical and Biophysical Research Communications* **53**, 413.

Madden, M. E. E., Bodnar, R. J., Rimstidt, J. D. (2004). Jarosite as an indicator of water-limited chemical weathering on Mars. *Nature* **431**, 821-823.

Malin, M. C., Edgett, K. S. (2000). Evidence for recent groundwater seepage and surface runoff on Mars. *Science* **288**, 2330-2335.

Mancinelli, R. L., Klovstad, M. (2000). Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. *Planetary and Space Science* **48**, 1093-1097.

Manoharan, R., Ghiamati, R. A., Dalterio, R. A., Britton, K. A., Nelson, W. H., Sperry, J. F. (1990). UV resonance Raman-spectra of bacteria, bacterial-spores, protoplasts and calcium dipicolinate. *Journal of Microbiological Methods* **11**, 1-15.

Marshall, C. P., Carter, E. A., Leuko, S., Javaux, E. J. (2006). Vibrational spectroscopy of extant and fossil microbes: Relevance for the astrobiological exploration of Mars. *Vibrational Spectroscopy* **41**, 182-189.

Marshall, C. P., Leuko, S., Coyle, C. M., Walter, M. R., Burns, B. P., Neilan, B. A. (2007). Carotenoid Analysis of Halophilic Archaea by Resonance Raman Spectroscopy. *Astrobiology* **7**, 631-643.

Marshall C. P., Marshall, A. O. (2010). The potential of Raman spectroscopy for the analysis of diagenetically transformed carotenoids. *Philosophical Transactions of the Royal Society A* **368**, 3137-3144.

Marshall, C. P., Edwards, H. G. M., Jehlička, J. (2010). Understanding the Application of Raman Spectroscopy to the Detection of Traces of Life. *Astrobiology* **10**, 229-243.

Masursky, H. (1973). An overview of geologic results form Mariner 9. *Journal of Geophysical Research* **78**, 4037-4047.

Medigan, M. T., Martinko, J. M., Dunlap, P. V., Clark, D. P. (2008). Brock biology of microorganisms. Benjamin Cummings, 12th edition, 1168 p.

Mellon, M. T., Arvidson, R. E., Marlow, J. J., Phillips, R. J., Asphaug, E. (2008). Periglacial landforms at the Phoenix landing site and the northern plains of Mars. *Journal of Geophysical Research* **113**, E00A23.

Merlin, J. C. (1985). Resonance Raman spectroscopy of carotenoids and carotenoid-containing systems. *Pure and Applied Chemistry* **5**7, 785–792

McEwen, A. S., Hansen, C. J., Delamere, W. A., Eliason, E. M., Herkenhoff, K. E., Keszthelyi, L., Gulick, V. C., Kirk, R. L., Mellon, M. T., Grant, J. A., Thomas, N., Weitz, C. M., Squyres, S. W., Bridges, N. T., Murchie, S. L., Seelos, F., Okubo, C. H., Millazo, M. P., Tornabene, L. L., Jaeger, W. L., Byrne, S., Russell, P. S., Griffes, J. L., Martínez-Alonso, S., Davatzes, A., Chuang, F. C., Thomson, B. J., Fishbaugh, K. E., Dundas, C. M., Kolb, K. J., Banks, M. E., Wray, J. J. (2007). A Closer Look at Water-Related Geologic Activity on Mars. *Science* **317**, 1706-1709.

McGenity, T. J., Gemmell, R. T., Grant, W. D., Stan-Lotter, H. (2000). Origins of halophilic microorganisms in ancient salt deposits. *Environmental Microbiology* **2**, 243-250.

McKay, C. P., Friedmann, E. I., Gomez-Silva, B., Caceres-Villanueva, L., Andersen, D. T., Landheim, R. (2003). Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: Four years of observations including the El Nino of 1997-1998. *Astrobiology* **3**, 393-406.

McLennan, S. M., Bell III, J. F., Calvin, W. M., Christensen, P. R., Clark, B. C., de Souza, P. A., Farmer, J., Farrand, W. H., Fike, D. A., Gellert, R., Ghosh, A., Glotch, T. D., Grotzinger, J. P., Hahn, B., Herkenhoff, K. E., Hurowitz, J. A., Johnson, J. R., Johnson, S. S., Jolliff, B., Klingelhöfer, G., Knoll, A. H., Learner, Z., Malin, M.

C., McSween, H. Y. Jr., Pocock, J., Ruff, S. W., Soderblom, L. A., Squyres, S. W., Tosca, N. J., Watters, W. A., Wyatt, M. B., Yen, A. (2005). Provenance and diagenesis of the evaporite-bearing Burns formation, Meridiani Planum, Mars. *Earth and Planetary Science Letters* **240**, 95-121.

Ming, D. W., Mittlefehlt, D. W., Morris, R. V., Golden, D. C., Gellert, R., Yen, A., Clark, B. C., Squyres, S. W., Farrand, W. H., Ruff, S. W., Arvidson, R. E., Klingelhöfer, G., McSween, H. Y., Rodionov, D. S., Schröder, C., de Souza, P. A., Wang, A. (2006). Geochemical and mineralogical indicators for aqueous processes from the Columbia Hills in Gusev crater, Mars. *Journal of Geophysical Research* **111**, E02S12.

Moeller, R., Horneck, G., Facius, R., Stackebrandt, E. (2005). Role of pigmentation in protecting *Bacillus* sp. endospores against environmental UV radiation. *FEMS Microbiology Ecology* **51**, 231-236.

Mormile, M. R., Hong, B. Y., Benison, K. C. (2009). Molecular Analysis of the Microbial Communities of Mars Analog Lakes in Western Australia. *Astrobiology* **9**, 919-930.

Morris, R. V., Klingelhöfer, G., Schröder, C., Rodionov, D. S., Yen, A., Ming, D. W., de Souza, P. A., Fleischer, I., Wdowiak, T., Gellert, R., Bernhardt, B., Evlanov, E. N., Zubkov, B., Foh, J., Bonnes, U., Kankeleit, E., Gutlich, P., Renz, F., Squyres, S. W., Arvidson, R. E. (2006). Mössbauer mineralogy of rock, soil and dust at Gusev crater, Mars: Spirit's journey through weakly altered olivine basalt on the plains and pervasively altered basalt in the Columbia Hills. *Journal of Geophysical Research* **111**, E02S13.

Murchie, S., Arvidson, R., Bedini, P., Beisser, K., Bibring, J.-P., Bishop, J., Boldt, J., Cavender, P., Choo, T., Clancy, R. T., Darlington, E. H., Des Marais, D., Espiritu, R., Fort, D., Green, R., Guinness, E., Hayes, J., Hash, C., Heffernan, K., Hemmler, K., Heyler, G., Humm, D., Hutcheson, J., Izenberg, N., Lee, R., Lees, J., Lohr, D., Malaret, E., Martin, T., McGovern, J. A., McGuire, P., Morris, R., Mustard, J., Pelkey, S., Rhodes, E., Robinson, M., Roush, T., Schaefer, E., Seagrave, G., Seelos, F., Silverglate, P., Slavney, S., Smith, M., Shyong, W. J., Strohbehn, K., Taylor, H., Thompson, P., Tossman, B., Wirzburger, M., Wolff, M. (2007). Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) on Mars Reconnaissance Orbiter (MRO). *Journal of Geophysical Research - Planets* **112**, E05S03.

Mustard, J. F., Murchie, S. L., Pelkey, S. M., Ehlmann, B. L., Milliken, R. E., Grant, J. A., Bibring, J.-P., Poulet, F., Bishop, J., Dobrea, E. N., Roach, L., Seelos, F., Arvidson, R. E., Wiseman, S., Green, R., Hash, C., Humm, D., Malaret, E., McGovern, J. A., Seelos, K., Clancy, T., Clark, R., Des Marais, D., Izenberg, N., Knudson, A., Langevin, Y., Martin, T., McGuire, P., Morris, R., Robinson, M., Roush, T., Smith, M., Swayze, G., Taylor, H., Titus, T., Wolff, M. (2008). Hydrated silicate minerals on Mars observed by the Mars Reconnaissance Orbiter CRISM instrument. *Nature* **454**, 305-309.

Navarro-González, R., Rainey, F. A., Molina, P., Bagaley, D. R., Hollen, B. J., de la Rosa, J., Small, A. M., Quinn, R. C., Grunthaner, F. J., Caceres, L., Gomez-Silva, B., McKay, C. P. (2003). Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life. *Science* **302**, 1018-1021.

Oren, A., Kühl, M., Karsten, U. (1995). An evaporitic microbial mat within a gypsum crust: zonation of phototrophs, photopigments, and light penetration. *Marine Ecology Progress series* **128**, 151-159.

Oren, A. (1997). Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. *Geomicrobiology Journal* **14**, 231-240.

Oren, A. (1999). Biogenetic aspects of halophilism. *Microbiology and Molecular Biology Reviews* **63**, 334-348.

Oren, A. (2000a). Life at high salt concentrations: possibilities and limitations. In: Journey to Diverse Microbial Worlds – Adaptations to Exotic Environments (Ed: Seckbach, J.), Book series: Cellular Origin and Life in Extreme Habitats, Kluwer Academic Publishers, Dordrecht, volume 2, pp 229.

Oren, A. (2000b). Salts and brines. In: Ecology of Cyanobacteria: Their Diversity in Time and Space (Eds: Whitton, B. A., Potts, M.), Kluwer Academic Publishers, Dordrecht, pp. 281.

Oren, A., Rodríguez-Valera, F., Antón, J., Benlloch, S., Roselló-Mora, R., Amann, R., Coleman, J., Russell, N. J. (2004). Red, Extremely Halophilic, but not Archaeal: The Physiology and Ecology of *Salinibacter ruber*, a Bacterium Isolated from Saltern Crystallizer Ponds. In: Halophilic Microorganisms (Ed. Ventosa, A.), Springer-Verlag, Heidelberg, pp 63.

Oren, A. (2005). A century of *Dunaliella* research: 1905-2005. In: Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya (Eds: Gunde-Cimerman, N., Oren, A., Plemenitas, A.), Book series: Cellular Origin and Life in Extreme Habitats, Springer, Dordrecht, vol. 9, pp 491.

Oren, A., Gunde-Cimerman, N. (2007). Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiology Letters* **269**, 1-10.

Oren, A., Sørensen, K. B., Canfield, D. E., Teske, A. P., Ionescu, D., Lipski, A., Altendorf, K. (2009). Microbial communities and processes within a hypersaline gypsum crust in a saltern evaporation pond (Eilat, Israel). *Hydrobiologia* **626**, 15-26.

Osterloo, M. M., Hamilton, V. E., Bandfield, J. L., Glotch, T. D., Baldridge, A. M., Christensen, P. R., Tornabene, L. L., Anderson, F. S. (2008). Chloride bearing materials in the southern highlands of Mars. *Science* **319**, 1651-1654.

Osterrothová, K., Jehlička, J. (2009). Raman spectroscopic identification of usnic acid in hydrothermal minerals as a potential Martian analogue. *Spectrochimica Acta A* **73**, 576-580.

Pentecost, A. (1993). Field Relationships Between Scytonemin Density, Growth, and Irradiance in Cyanobacteria Occuring in Low Illumination Regimes. *Microbial Ecology* **26**, 101-110.

Perreault, N. N., Andersen, D. T., Pollard, W. H., Greer, C. W., Whyte, L. G. (2007). Characterization of the Prokaryotic Diversity in Cold Saline Perennial Springs of the Canadian High Arctic. *Applied and Environmental Microbiology* **73**, 1532-1543.

Petry, R., Schmitt, M., Popp, J. (2003). Raman Spectroscopy – A Prospective Tool in the Life Sciences. *Chemphyschem* **4**, 14-30.

Poulet, F., Bibring, J.-P., Mustard, J. F., Gendrin, A., Mangold, N., Langevin, Y., Arvidson, R. E., Gondet, B., Gomez, C., and Omega Team (2005). Phyllosilicates on Mars and implications for early martian climate. *Nature* **438**, 623-627.

Proteau, P. J., Gerwick, W. H., Garcia-Pichel, F., Castenholz, R. (1993). The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. *Experientia* **49**, 825-829.

Radax, Ch., Gruber, C., Stan-Lotter, H. (2001). Novel haloarchaeal 16S rRNA gene sequences from Alpine Permo-Triassic rock salt. *Extremophiles* **5**, 221-228.

Raman, C. V., Krishnan, K. S. (1928). A new type of secondary radiation. *Nature* **121**, 501-502.

Reiser, R., Tasch, P. (1960). Investigation of the viability of osmophile bacteria of great geological age. *Transactions of the Kansas Academy of Science* **63**, 31-34.

Rettberg, P., Eschweiler, U., Strauch, K., Reitz, G., Horneck, G., Wanke, H., Brack, A., Barbier, B. (2002). Survival of microorganisms in space protected by meteorite material: Results of the experiment 'EXOBIOLOGIE' of the PERSEUS mission. In: Space life sciences: Extraterrestrial organic chemistry, UV radiation on biological evolution, and planetary protection (Eds: Bernstein, M. P., Rettberg, P., Mancinelli, R. L., Race, M. S.), Pergamon-Elsevier Science Ltd., Oxford, Book series: *Advances in Space Research* **30**, 1539-1545.

Rettberg, P., Rabbow, E., Panitz, C., Horneck, G. (2004). Biological space experiments for the simulation of Martian conditions: UV radiation and Martian soil analogues. In: Space life sciences: Search for signatures of life, and space flight environmental effects on the nervous system (Eds: Horneck, G., Levasseur-Regourd, A. C., Rabin, B. M., Slenzka, K. B.), Pergamon-Elsevier Science Ltd., Oxford, Book series: *Advances in Space Research* **33**, 1294-1301.

Rieder, R., Gellert, R., Anderson, R. C., Brückner, J. Clark, B. C., Dreibus, G., Economou, T., Klingelhöfer, G., Lugmair, G. W., Ming, D. W., Squyres, S. W., d'Uston, C., Wänke, H., Yen, A., Zipfel, J. (2004). Chemistry of rocks and soils at Meridiani Planum from the alpha particle X-ray spectrometer. *Science* **306**, 1746-1749.

Roach, L. H., Mustard, J. F., Murchie, S., Weitz, C. M., Ehlmann, B. L., Pelkey, S., Seelos, F. P., Bibring, J. P., the CRISM team (2007). Sulfate identification in east Candon, Valles Marineris with CRISM visible-infrared spectra. Lunar and Planetary Science XXXVIII, Abstract 2106.

Rontó, G., Bérces, A., Lammer, A., Cockell, C. S., Molina-Cuberos, G. J., Patel, M. R., Selsis, F. (2002). Solar UV Irradiation Conditions on the Surface of Mars. *Photochemistry and Photobiology* **77**, 34-40.

Rothschild, L. J. (1990). Earth Analogs for Martian Life. Microbes in Evaporites, a New model System for Life on Mars. *Icarus* **88**, 246-260.

Rothschild, L. J., Giver, L. J., White, M. R., Mancinelli, R. L. (1994). Metabolic activity of microorganisms in evaporites. *Journal of Phycology* **30**, 431-438.

Ruban, A. V., Pascal, A. A., Robert, B., Horton, P. (2001) Configuration and dynamics of xantophylls in light harvesting antennae of higher plants. *Journal of Biological Chemistry* **276**, 24862-24870.

Russell, N. C., Edwards, H. G. M., Wynn-Williams, D. D. (1998). FT-Raman spectroscopic analysis of endolithic microbial communities from Beacon sandstone in Victoria Land, Antarctica. *Antarctic Science* **10**, 63-74.

Saito, T., Miyabe, Y., Ide, H., Yamamoto, O. (1997). Hydroxyl radical scavenging ability of bacterioruberin. *Radiation Physics and Chemistry* **50**, 267-269.

Schubert, B. A., Lowenstein, T. K., Timofeeff, M. N. (2009). Microscopic Identification of Prokaryotes in Modern and Ancient Halite, Saline Valley and Death Valley, California. *Astrobiology* **9**, 467-482.

Schubert, B. A., Timofeeff, M. N., Lowenstein, T. K., Polle, J. E. W. (2010a). *Dunaliella* Cells in Fluid Inclusions in Halite: Significance for Long-term Survival of Prokaryotes. *Geomicrobiology Journal* **27**, 61-75.

Schubert, B. A., Lowenstein, T. K., Timofeeff, M. N., Parker, M. A. (2010b). Halophilic Archaea cultured from ancient halite, Death Valley, California. *Environmental Microbiology* **12**, 440-454.

Scott, D. H., Dohm, J. M., Rice, J. W. Jr (1995). Map showing channels and possible paleolake basins. *US. Geological Survey Miscellaneous Investigations Series* Map I-2461.

Segura, T. L., Toon, O. B., Colaprete, A., Zahnle, K. (2002). Environmental Effects of Large Impacts on Mars. *Science* **298**, 1977-1980.

Seibert, N. M., Kargel, J. S. (2001). Small-scale Martian polygons: liquid surface water. *Geophysical Research Letters* **28**, 899-902.

Shahmohammadi, H. R., Asgarani, E., Terato, H., Saito, T., Ohyama, Y., Gekko, K., Yamamoto, O., Ide, H. (1998). Protective Roles of Bacterioruberin and Intracellular KCl in the Resistance of *Halobacterium salinarum* against DNA-damaging Agents. *Journal of Radiation Research* **39**, 251-262.

Siefermann-Harms, D. (1987). The light-harvesting and protective functions of carotenoids in photosynthetic membranes. *Phisiologia Plantarum* **69**, 561-568.

Sinha, R. P., Klisch, M., Gröniger, A., Häder, D. P. (1998). Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. *Journal of Photochemistry and Photobiology B – Biology* **47**, 83-94.

Sinninghe Damsté, J. S., Koopmans, M. P. (1997). The fate of carotenoids in sedimants: An overview. *Pure and Applied Chemistry* **69**, 2067-2074.

Smith, P. H., Tamppari, L. K., Arvidson, R. E., Bass, D., Blaney, D., Boynton, W. V., Carswell, A., Catling, D. C., Clark, B. C., Duck, T., DeJong, E., Fisher, D., Goetz, W., Gunnlaugsson, H. P., Hecht, M. H., Hipkin, V., Hoffman, J., Hviid, S. F., Keller, H. U., Kounaves, S. P., Lange, C. F., Lemmon, M. T., Madsen, M. B., Markiewicz, W. J., Marshall, J., McKay, C. P., Mellon, M. T., Ming, D. W., Morris, R. V., Pike, W. T., Renno, N., Staufer, U., Stoker, C., Taylor, P., Whiteway, J. A., Zent, A. P. (2009). H_2O at the Phoenix landing Site. *Science* **325**, 58-61.

Stan-Lotter, H., Radax, C., McGenity, T., Legat, A., Pfaffenhuemer, M., Wieland, W., Gruber, C., Denner, E. B. M. (2004). From Intraterrestrials to Extraterrestrials – Viable Haloarchaea in Ancient Salt Deposits. In: Halophilic Microorganisms (Ed: Ventosa, A.), Springer-Verlag, Heidelberg, pp 89.

Stivaletta, N., Barbieri, R. (2009). Endolithic microorganisms from spring mound evaporite deposits (southern Tunisia). *Journal of Arid Environments* **73**, 33-39.

Storrie-Lombardi, M. C., Hug, W. F., McDonald, G. D., Tsapin, A. I., Nealson, K. H. (2001). Hollow cathode ion lasers for deep ultraviolet Raman spectroscopy and fluorescence imaging. *Review of Scientific Instruments* **72**, 4452-4459.

Squyres, S. W., Grotzinger, J. P., Arvidson, R. E., Bell, J. F. III, Calvin, W., Christensen, P. R., Clark, B. C., Crisp, J. A., Farrand, W. H., Herkenhoff, K. E., Johnson, J. R., Klingelhöfer, G., Knoll, A. H., McLennan, S. M., McSween, H. Y. Jr, Morris, R. V., Rice, J. W. Jr, Rieder, R., Soderblom, L. A. (2004). In situ evidence for an ancient aqueous environment at Meridiani Planum, Mars. *Science* **306**, 1709–1714

Squyres, S. W., Knoll, A. H. (2005). Sedimantary rocks at Meridiani Planum: Origin, diagenesis, and implications for life on Mars. *Earth and Planetary Science Letters* **240**, 1-10.

Szalontai, B., Gombos, Z., Csizmadia, V., Bagyinka, C., Lutz, M. (1994). Structure and Interactions of Phycocyanobilin Chromophores in Phycocyanin and Allophycocyanin from an Analysis of Their Resonance Raman Spectra. *Biochemistry* **33**, 11823-11832.

Tarcea, N., Harz, M., Rosch, P., Frosch, T., Schmitt, M., Thiele, H., Hochleitner, R., Popp, J. (2007). UV Raman spectroscopy - A technique for biological and mineralogical in situ planetary studies. *Spectrochimica Acta A* **68**, 1029-1035.

Taylor, S. R., McLennan, S. M. (1985). The Continental Crust: Its Composition and Evolution, Blackwell, Oxford, pp 312.

Telfer, A., Pascal, A., Gall, A. (2008). Carotenoids in Photosynthesis. In: Carotenoids (Eds: Britton, G., Liaaen-Jensen, S., Pfander, H.), Birkhäuser, Basel, vol. 4, pp 265-308.

Tominaga, H., Fukui, F. (1981). Saline lakes at Syowa Oasis, Antarctica. *Hydrobiologia* **82**, 375-389.

Tosca, N. J., McLennan, S. M., Clark, B. C., Grotzinger, J. P., Hurowitz, J. A., Knoll, A. H., Schröder, C., Squyres, S. W. (2005). Geochemical modeling of evaporation processes on Mars: Insight from the sedimentary record at Meridiani Planum. *Earth and Planetary Science Letters* **240**, 122-148.

Vago, J. L. and the ExoMars Project Team, Planetary Science Decadal Survey: Mars Panel Meeting, 10 September 2009, Arizona State University, Tempe (USA).

Varnali, T., Edwards, H. G. M., Hargreaves, M. D. (2009). Scytonemin: molecular structural studies of a key extremophilic biomarker for astrobiology. *International Journal of Astrobiology* **8**, 133-140.

Varnali, T., Edwards, H. G. M. (2010). *Ab Initio* calculations of scytonemin derivatives of relevance to extremophile characterization by Raman spectroscopy. *Philosophical Transaction of the Royal Society A* **368**, 3057-3221.

Villar, S. E. J., Edwards, H. G. M., Cockell, Ch. S. (2005a). Raman spectroscopy of endoliths from Antarctic cold desert environments. *Analyst* **130**, 156-162.

Villar, S. E. J., Edwards, H. G. M., Worland, M. R. (2005b). Comparative evaluation of Raman spectroscopy at different wavelengths for extremophile exemplars. *Origins of Life and Evolution of Biospheres* **35**, 489-506.

Villar, S. E. J., Edwards, H. G. M., Benning, L. G. (2006). Raman spectroscopic and scanning electron microscopic analysis of a novel biological colonisation of volcanic rocks. *Icarus* **184**, 158-169.

Villar, S. E. J., Edwards, H. G. M. (2006). Raman spectroscopy in astrobiology. *Analytical and Bioanalytical Chemistry* **384**, 100-113.

Villar, S. E. J., Benning, L. G., Edwards, H. G. M. and AMASE team (2007). Raman and SEM analysis of a biocolonised hot spring travertine terrace in Svalbard, Norway. *Geochemical Transactions* **8**, 8.

Vítek, P., Osterrothová, K., Jehlička, J. (2009). Beta-carotene—A possible biomarker in the Martian evaporitic environment: Raman micro-spectroscopic study. *Planetary and Space Science* **57**, 454-459.

Vítek, P., Jehlička, J., Edwards, H. G. M., Osterrothová, K. (2009). Identification of β-carotene in an evaporitic matrix—evaluation of Raman spectroscopic analysis for astrobiological research on Mars. *Analytical and Bioanalytical Chemistry* **393**, 1967-1975.

Vítek, P., Edwards, H. G. M., Jehlička, J., Ascaso, C., De los Ríos, A., Valea, S., Villar, S. E. J., Davila, A. F., Wierzchos, J. (2010). Microbial colonization of halite from the hyper-arid Atacama Desert studied by Raman spectroscopy. *Philosophical Transactions of the Royal Society A* **368**, 3205-3221.

Vogel, M. B., Des Marais, D. J., Turk, K. A., Parenteau, M. N., Jahnke, L. L., Kubo, M. D. Y. (2009). The Role of Biofilms in the Sedimentology of Actively Forming Gypsum Deposits at Guerrero Negro, Mexico. *Astrobiology* **9**, 875-893.

Volkmann, M., Gorbushina, A. A., Kedar, L., Oren, A. (2006). Structure of euhalothece-362, a novel red-shifted mycosporine-like amino acid, from halophilic cyanobacterium (*Euhalothece* sp.). *FEMS Microbiology Letters* **258**, 50-54.

Vreeland, R. H., Piselli, A. F. Jr., McDonnough, S., Meyers, S. S. (1998). Distribution and diversity of halophilic bacteria in a subsurface salt formation. *Extremophiles* **2**, 321-331.

Vreeland, R. H., Rosenzweig, W. D., Powers, D. W. (2000). Isolation of a 250-million-year-old halotolerant bacterium from a primary salt crystal. *Nature* **407**, 897–900.

Wang, A., Haskin, L. A., Squyres, S. W., Jolliff, B.L., Crumpler, L., Gellert, R., Schröder, C., Herkenhoff, K., Hurowitz, J., Tosca, N.J., Farrand, W.H., Anderson, R., Knudson, A.T. (2006a). Sulfate deposition in subsurface regolith in Gusev crater, Mars. *Journal of Geophysisal Research - Planets* **111**, No.E02S17.

Warren, J. K. (2006). Evaporites: sediments, resources and hydrocarbons. Springer-Verlag, Berlin Heidelberg, 1035 p.

Warren-Rhodes, K. A., Rhodes, K. L., Pointing, S. B., Ewing, S. A., Lacap, D. C., Gómez-Silva, B., Amundson, R., Friedmann, E. I., McKay, C. P. (2006). Hypolithic Cyanobacteria, Dry Limit of Photosynthesis and Microbial Ecology in the Hyperarid Atacama Desert. *Microbial Ecology* **52**, 389-398.

Weitz, C. M., Milliken, R. E., Grant, J. A., McEwen, A. S., Williams, R. M. E., Bishop, J. L. (2008). Light-toned strata and inverted channels adjacent to Juventae and Ganges chasmata, Mars. *Geophysical Research Letters* **35**, L19202.

Wierzchos, J., Ascaso, C., McKay, C. P. (2006). Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology* **6**, 415-422.

Wierzchos, J., Cámara, B., de los Ríos, A., Davila, A. F., Sánchez Almazo, I. M., Artieda, O., Wierzchos, K., Gómez-Silva, B., McKay C. P., Ascaso, C. (2010). Microbial colonization of Ca-sulfate crusts in the hyperarid core of the Atacama Desert: implications for the search for life on Mars. *Geobiology*, in press.

Wiseman, S. M., Arvidson, R. E., Morris, R. V., Murchie, S. L., Seelos, F. P., Andrews-Hanna, J. C., The CRISM team (2009). Hydrated sulfate deposits detected within Schiaparelli Crater, Mars. Lunar and Planetary Science XL, Abstract 1798.

Withnall, R., Chowdhry, B. Z., Silver, J., Edwards, H. G. M., de Oliveira, L. F. C. (2003). Raman spectra of carotenoids in natural products. *Spectrochimica Acta A* **59**, 2207–2212

Wright, S. W., Burton, H. R. (1981). The biology of Antarctic saline lakes. *Hydrobiologia* **82**, 319-338.

Wu, Q., Hamilton, T., Nelson, W. H., Elliot, S., Sperry, J. F., Wu, M. (2001). UV Raman spectral intensities of *E. coli* and other bacteria excited at 228.9, 244.0, and 248.2 nm. *Analytical Chemistry* **73**, 3432-3440.

Wynn-Williams, D. D., Edwards, H. G. M. (2000a). Proximal analysis of regolith habitats and protective biomolecules in situ by laser Raman spectroscopy: overview of terrestrial Antarctic habitats and Mars analogs. *Icarus* **144**, 486-503.

Wynn-Williams, D. D., Edwards, H. G. M. (2000b). Antarctic ecosystems as models for extraterrestrial surface habitats. *Planetary and Space Science* **48**, 1065-1075.

Yen, A. S., Kim, S. S., Hecht, M. H., Frant, M. S., Murray, B. (2000). Evidence that the reactivity of the martian soil is due to superoxide ions. *Science* **289**, 1909-1912.

Zheng-Li, C., Zeng, H., Chen, M., Larkum, A. W. D. (2002). Raman spectroscopy of chlorophyll *d* from *Acaryochloris marina*. *Biochimica et Biophisica Acta* **1556**, 89-91.