Origins of Life and Evolution of Biospheres (2005) 35: 489–506

COMPARATIVE EVALUATION OF RAMAN SPECTROSCOPY AT DIFFERENT WAVELENGTHS FOR EXTREMOPHILE EXEMPLARS

S. E. JORGE VILLAR[1], H. G. M. EDWARDS[2,*] and M. R. WORLAND[3]
[1]*Area de Geodinamica Interna, Facultad de Humanidades y Educacion, Universidad de Burgos, C/Villadiego s/n 09001 Burgos, Spain;* [2]*Chemical and Forensic Sciences, University of Bradford, Bradford BD7 1DP, UK;* [3]*British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET, UK*
*(*author for correspondence, e-mail: h.g.m.edwards@bradford.ac.uk)*

(Received 25 November 2004; accepted in revised form 28 February 2005)

Abstract. Raman spectra have been obtained for extremophiles from several geological environments; selected examples have been taken from hot and cold deserts comprising psychrophiles, thermophiles and halophiles. The purpose of this study is the assessment of the effect of the wavelength of the laser excitation on the ability to determine unique information from the Raman spectra about the specificity of detection of biomolecules produced as a result of the survival strategies adopted by organisms in extreme terrestrial environments. It was concluded that whereas FT-Raman spectroscopy at 1064 nm gave good quality results the time required to record the data was relatively large compared with other wavelengths of excitation but that better access to the CH stretching region for organic molecules was given. Shorter wavelength excitation of biomolecules in the blue-green regions of the visible spectrum using a conventional dispersive spectrometer was more rapid but very dependent upon the type of chemical compound being studied; most relevant biomolecules fluoresced at these wavelengths but carotenoids exhibited a resonance effect which resulted in an improved detection capability. Minerals and geological materials, in contrast, were best studied at these visible wavelengths. In general, the best compromise system for the excitation of the Raman spectra of both geological and biological materials was provided using a 785 nm laser coupled with a dispersive spectrometer, especially for accessing the 1800–200 cm^{-1} wavenumber shift region where much of the definitive analytical information resides. This work will have conclusions relevant to the use of miniaturised Raman spectrometers for the detection of biomolecules in extraterrestrial planetary exploration.

Keywords: biosignatures, extremophile, planetary exploration, Mars, Raman spectroscopy, wavelength laser excitation

Introduction

The Raman effect is a molecular scattering process which is manifest from the interaction between a laser beam and a chemical system from which the shift in wavenumber of the exciting radiation scattered by vibrating molecules and the incident electromagnetic radiation can be related to the structure, composition and identification of the scattering molecules (Long, 2002). The Raman scattering is significantly weaker in intensity than Rayleigh scattering, where the incident electromagnetic radiation is scattered without change in wavenumber. Although the

wavenumber shifts observed in Raman scattering are independent of the excitation wavelength, the scattering intensity is inversely proportional to the wavelength of excitation; hence, with all other instrumental effects remaining the same, a Raman spectrum obtained in the ultraviolet at 250 nm is inherently nearly 300 times stronger than that obtained with near infrared excitation at 1064 nm. Hence, many Raman spectra obtained hitherto have been recorded in the visible region of the electromagnetic spectrum. However, the onset of fluorescence emission, which is several orders of magnitude larger than Raman scattering, occurring at lower wavelengths and higher laser excitation energies, can completely swamp the observation of the weaker Raman bands especially of organic molecules which have low energy electronic states. For this reason, the recording of Raman spectra using longer wavelength laser excitation to obviate the occurrence of potentially troublesome fluorescence has been finding much favour despite the problems caused by detection of weaker spectral features. Improvements in experimentation, especially in sample illumination and in detection of the long-wavelength shifted Raman bands, have now created several possibilities for the recording of good quality Raman spectra from difficult specimens with effective suppression of troublesome fluorescence backgrounds (Edwards, 2004).

Raman spectroscopy has several important characteristics which make it a valuable technique for extraterrestrial exploration. The possibility to analyze both organic and inorganic compounds with little or no sample preparation, the determination of the structural composition of the compounds, data acquisition from macro and micro samples, the capability of *in situ* analyses and adaptability for remote analyses make it in a desirable technique for the potential study of planetary surfaces (Ellery and Wynn-Williams, 2003; Wang *et al.*, 2003; Wang and Haskin, 2000; Dickensheets *et al.*, 2000).

The development of miniature Raman spectrometers together with the selection of suitable laser wavelengths are the most important limits to bear in mind for the inclusion of this technique in future robotic landers for the study of traces of life on the surface of Mars.

Organisms in hostile environments develop adaptation strategies of survival (Cockell, 2001; Cockell, 2000; O'Brien *et al.*, 2004; Rothschild and Cockell, 1999; Rivkina *et al.*, 2004; Warwick *et al.*, 2004). These include changes in the microhabitat, with mobilisation of some phases, mineral transformations (Jorge *et al.*, 2003; Edwards *et al.*, 2003a) and the production of specific organic molecules (Finegold, 1986; Wynn-Williams *et al.*, 2000) which are essential for resistance to the stressed conditions (Vera *et al.*, 2004; Edwards *et al.*, 1998). These give rise to geo- and biomarkers (Edwards *et al.*, 2003a, b, 2004b) of which a knowledge is of vital importance for evaluation of the strategies adopted by the microorganisms for the colonisation of geological strata in extreme conditions (Edwards *et al.*, 2003c, 2004c).

The study of these geo- and bio-markers in a range of extreme terrestrial environments is a necessary pre-requisite for planetary exploration. The selection of the most suitable wavelength for Raman analyses is recognised as critical for the

success of the search for life beyond our planet (Sharma *et al.*, 2003; Bishop *et al.*, 2004; Edwards *et al.*, 2003).

In this work we present the Raman spectroscopic analysis of different extremophiles and their bio- and geomarkers from terrestrial extreme habitats, undertaken with a range of laser excitation wavelengths in the visible and near infrared, all of which are being considered for possible adoption into portable Raman systems for remote chemical analysis: 1064, 785, 633, 488 and 514 nm. The Raman spectra obtained from the different organic and inorganic compounds present in the extremophile systems have been assessed in order to select the most suitable laser excitation for each situation.

Spectroscopy

FT-Raman spectra were recorded using a Bruker IFS66 spectrometer with FRA 106 Raman module attachment and dedicated microscope. The wavelength excitation was at 1064 nm, using a Nd3+/YAG laser. The spectral resolution was 4 cm^{-1} and from 2000 to 4000 scans were accumulated over about 30–60 mins to improve the signal-to-noise ratio.

For analyses with 785, 633, 514 and 488 nm laser excitation a Renishaw InVia Raman Microscope coupled to a Leica DMLM microscope with 20X, 50X objective lenses was utilized. 30–70 accumulations at 10 s exposure time for each scan with a laser power between 0.5 to 50 mW were typically used to collect spectra.

Specimens

There is a need to evaluate the wavelength of excitation of Raman spectra for extremophiles; this has led directly to our selection of a range of suitable samples from different terrestrial origins:

- Endolithic community in an orange Beacon sandstone, from Antarctica, the most extreme cold desert on Earth.
- Endolithic community in a pale cream coloured sandstone with a white hard crust from Antarctica.

  The Antarctic samples were collected by one of us (MRW) during field expeditions to Mars Oasis and Battleship Promontory, Antarctica, in the Summer season, January/February 2002. They were stored at $-25\,^{\circ}C$ until required for analysis.
- Transparent gypsum crystal with colonies of *Gloecapsa* cyanobacteria situated inside the exfoliation planes; a halotroph from the Haughton meteorite impact crater, Devon Island, Canadian Arctic. The sample was collected by Dr. John Parnell (Edwards *et al.*, 2005) during the 2003/4 season and maintained at ambient temperature until required for use.

- Biofilm from Tatio Geysers, in the Atacama desert, collected at 4000 metres altitude.
- Epilithic lichen (*Acarospora cf. schleichera*) from the hot, dry desert of Atacama, Chile, collected at 2400 metres altitude.
- Epilithic lichen (*Xanthomendoza mendozae*) collected in the North of the Atacama desert at 4500 metres altitude.

The Atacama Desert samples were collected by one of us (SEJV) during a field expedition in the spring/summer of 2004. The samples were maintained at ambient temperature until required for use.

The spectroscopic identification of Raman band signatures from geological and biological materials was effected through comparison with spectra of standard materials which have been characterised in the literature and stored in our database; examples of the key features which can be used to recognise the presence of these biochemicals and minerals in their Raman spectra are given particularly in the literature, such as Edwards *et al.*, 1998, 2003a; Edwards, 2004, Villar *et al.*, 2003, Wynn-Williams and Edwards, 2000.

It is stressed that no special sample preparation was undertaken for the Raman spectroscopic analysis other than breaking a section of rock to expose the inner stratification; the detachment of organisms from their environment was not attempted since this would normally destroy the vital information that could be obtained from the *in situ* examination and analysis, from which the dependence of the biology upon the geological substrate can be determined.

Endolith in Orange Sandstone

This specimen shows visually a white band, containing the organisms, between the orange crust and a darker orange band (Figure 1).

Raman spectra achieved on this crust with 1064 nm laser excitation gives bands at 128, 206, 355, 465, 542, 696, 795, 807, 1064, 1081, 1161 and 1227 cm^{-1}; these are all assignable to quartz and no signatures of other compounds appear. The same results were obtained in the spectra collected from the red band.

Spectra achieved with 785 nm laser excitation of the red band below the surface crust shows signatures at at 223, 291, 404, 495, 609 cm^{-1}, assigned to haematite. This iron oxide is responsible for the red colour of the sample. A very weak signature at 290 cm^{-1} in the spectrum from the orange crust is indicative that haematite is present but in low concentration (Figure 2). No results from the red zone are achieved with either green or blue lasers. Haematite is known as a protective inorganic pigment against the UV-radiation (Clark, 1998) and it is speculated that the organism may induce the depletion of this mineral in a protective strategy.

The black band containing the organisms was analysed using 488, 785 and 1064 nm laser excitation. With the blue laser (488 nm), bands at 1525, 1156, 1190 and 1004 cm^{-1} from β-carotene, a broad signature centered at 1350 cm^{-1}, assigned to

Figure 1. Endolithic community in an orange sandstone; the depletion of iron oxides around the organism is clearly visible.

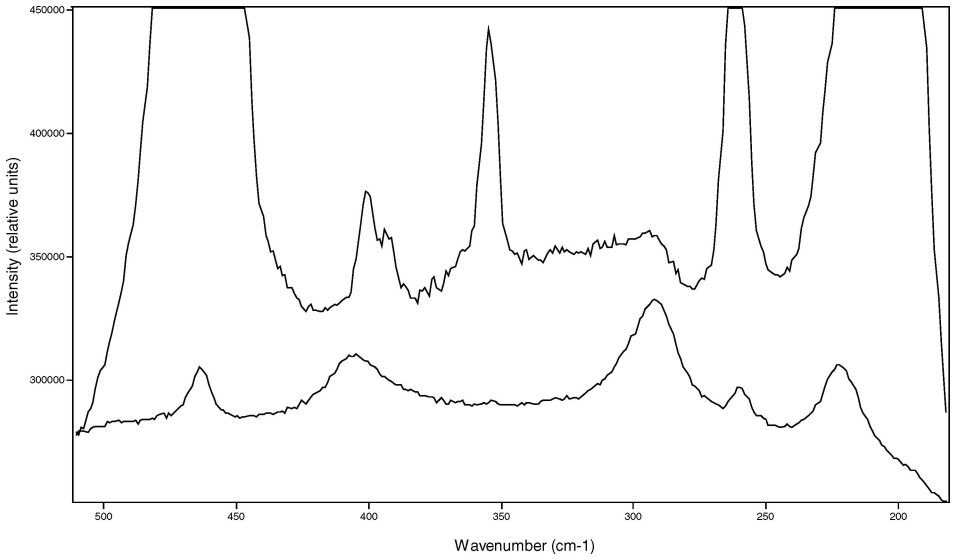

Figure 2. Raman spectra achieved from the specimen shown in Figure 1 with a 785 nm laser wavelength. The spectrum from the orange crust (upper) is compared with the spectrum of haematite (lower).

chlorophyll, and one broad band at 1600 cm^{-1} (probably scytonemin) appear in the Raman spectrum (Figure 3).

Two analyses from different areas were obtained using 1064 nm excitation. The spectrum achieved from the black band shows bands at 499, 542, 687, 1313, 1338,

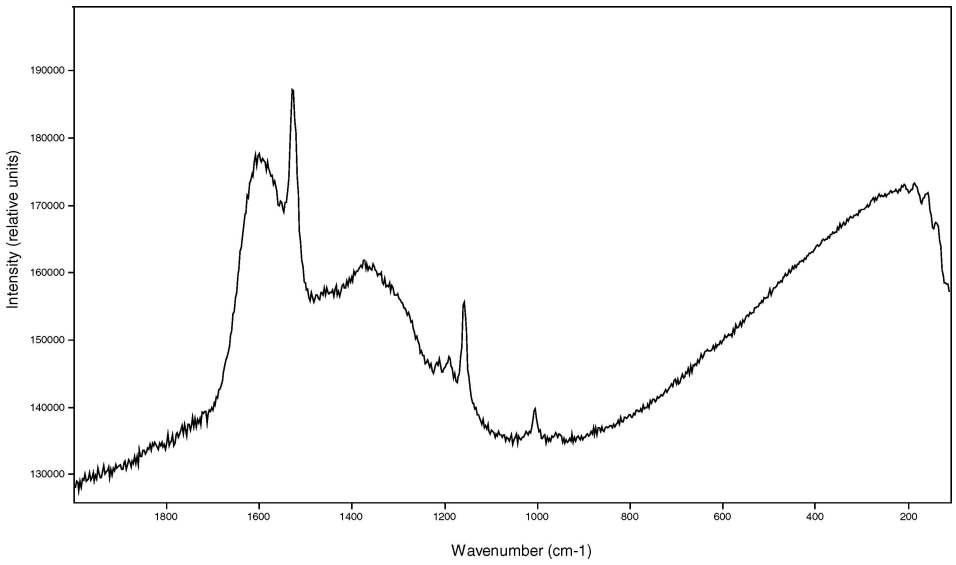

Figure 3. Raman spectrum of the organic band in the endolith in the orange sandstone from Figure 1 (488 nm laser excitation).

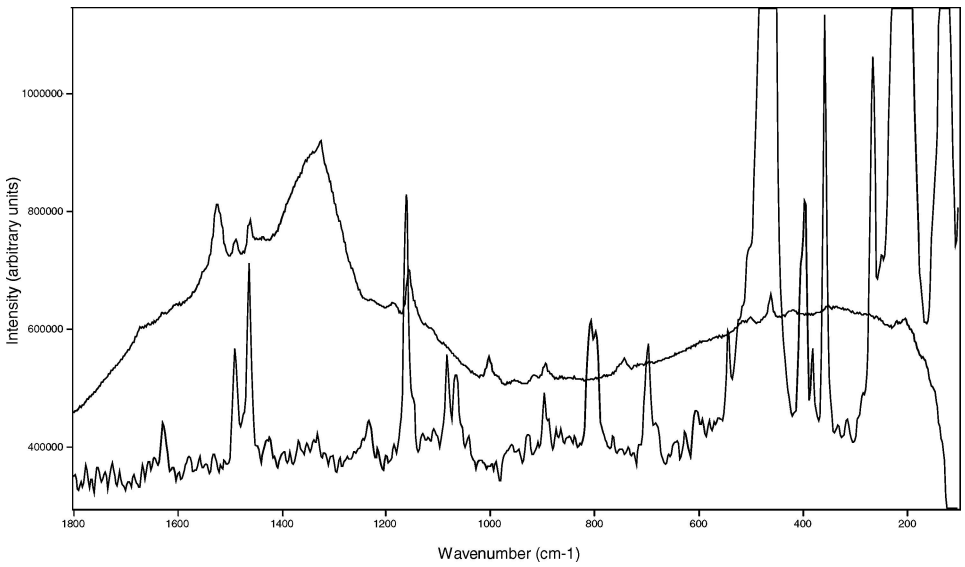

Figure 4. Raman spectra achieved with 785 nm (upper) and 1064 nm laser excitations from the organic band in the orange sandstone endolith. The upper spectrum shows signatures of carotene, chlorophyll and calcium oxalate monohydrate; the lower spectrum shows bands of calcium oxalate monohydrate and quartz.

1474, 1560, 1620 cm^{-1} from a compound with a chlorophyll-like structure; the second spectrum shows bands at 1490, 1463, 896, 502 and 206 cm^{-1}, characteristic of calcium oxalate monohydrate.

The results achieved using a 785 nm laser excitation show several differences compared with the 1064 nm wavelength. Calcium oxalate monohydrate is still visible, but now a broad signature centered at 1324 cm^{-1} and the bands at 914, 744 and 517 cm^{-1} are all more easily recognized and assigned to chlorophyll; furthermore, the characteristic bands of β-carotene are again visible (Figure 4).

The main difference between the conventional near-infrared Raman and FT-Raman spectroscopy (785 and 1064 nm, respectively) is the time required to collect spectra from these samples. With conventional spectroscopy, about 15 min is required to obtain a good quality spectrum in the wavenumber region of 1800–0 cm^{-1} whereas for FT-Raman spectroscopy it is necessary to acquire data for about 2 h in the wavenumber region of 4000–50 cm^{-1} to achieve spectra of similar quality.

Endolith in Pale Cream Sandstone

This specimen (Figure 5) does not show any trace of iron oxide but has a thin crust of gypsum, which serves as a cement between the quartz grains and which is easily recognized by the Raman signatures at 1137, 1009, 618 and 415 cm^{-1} (Figure 6). Gypsum has been proven as an efficient protective mechanism against UV-radiation (Mancinelli, 1998; Edwards *et al.*, 2000). In this endolith the organism may have used a different mechanism to protect itself against the potentially lethal UV radiation, which is very strong in Antarctica because of the atmospheric depletion of ozone at high latitudes, the "ozone hole". No other mineral is present in the rock (Figure 7).

The spectra achieved from the black zonal region with 785 and 1064 nm lasers show in both cases, bands of scytonemin (1713, 1632, 1603, 1592, 1552, 1547, 1520, 1446, 1386, 1324, 1282, 1247, 1168, 1096, 1024, 984, 888, 839, 753, 677, 540 and 497 cm^{-1}). The best quality spectrum was collected using the 785 nm wavelength.

Beneath the black area a light green band appears. Several analyses were made with different laser wavelengths. With 488 nm laser excitation, the spectrum shows bands at 1521, 1214, 1193, 1157 and 1004 cm^{-1} which have been assigned to a carotene. Quartz bands also appear. The same result was obtained using the 514 nm wavelength (Figure 8), but with the 785 nm laser carotene is not detected in the spectrum and the large, broad band centered at 1335 cm^{-1} has been assigned to chlorophyll.

The green band in the specimen has a very light colour which indicates only a small quantity of pigment. The detection of carotene only by the blue and green lasers (488 and 514 nm, respectively) is attributed to a resonance Raman scattering effect. Chlorophyll only appears when the spectrum is collected with 785 nm excitation.

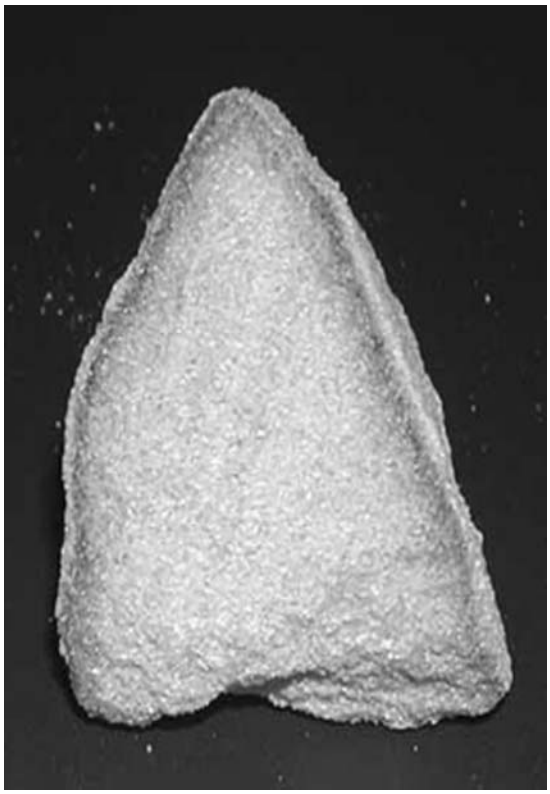

Figure 5. Endolithic community from Antarctica in a pale cream sandstone.

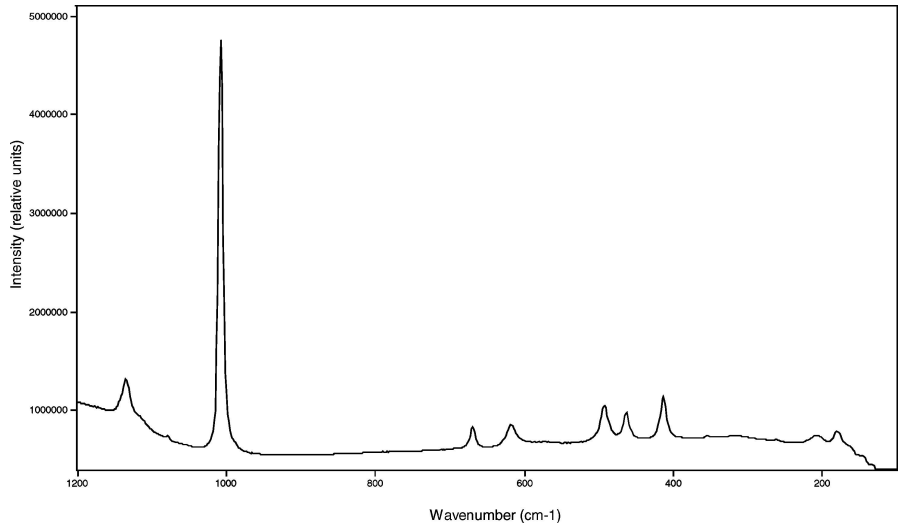

Figure 6. Raman spectrum collected using a 785 nm laser excitation from the crust of the endolith in pale cream sandstone shown in Figure 5. The Raman spectral signatures are characteristic of gypsum.

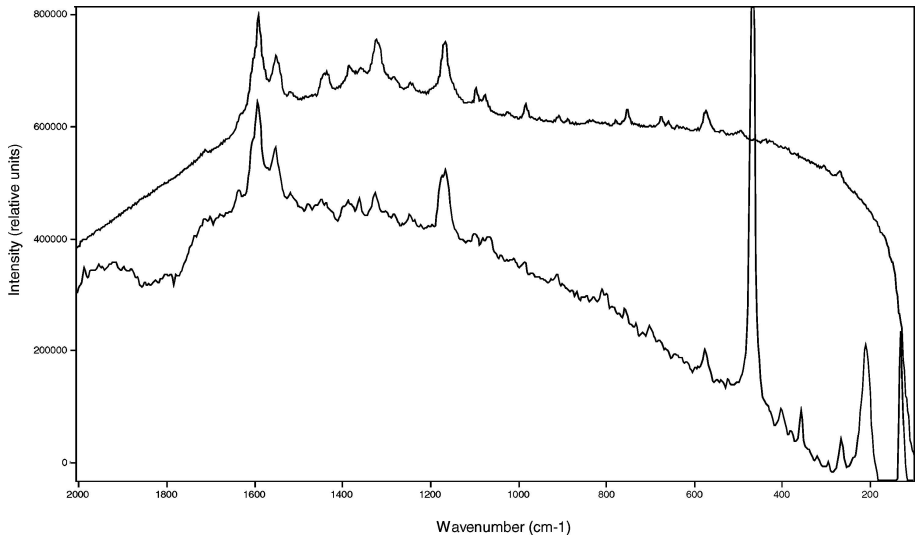

Figure 7. Raman spectra achieved with 1064 nm (lower) and 785 nm (upper) laser excitation from the organic band in endolith in the the pale cream sandstone specimen. Signatures of scytonemin are clearly seen in both spectra but the signal-to-noise ratio is better with the 785 nm laser.

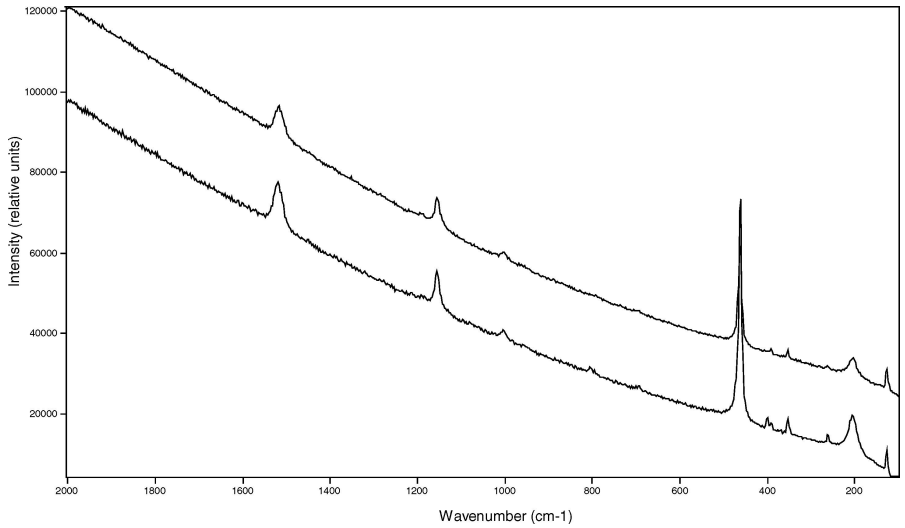

Figure 8. Spectra collected from the green endolith region in the pale cream sandstone with 514 nm (upper) and 488 nm (lower) laser excitation. Only quartz and carotene Raman signatures are visible.

Spectroscopic differences between the black area (fungal hyphae) and the green area (algal) are obvious in relation to the pigments observed. Scytonemin is the only pigment in the black zone but carotene and chlorophyll appear in the algal zone. The black band is the shallower and is nearer to the surface, so it is reasonable to

propose that the algal organism may need extra protection against the incident UV radiation.

Halophilic Cyanobacteria

All laser excitations used give good quality spectra of a gypsum crystal with bands at 1139, 1008, 619, 492, 413 209, 179 and 131 cm^{-1}.

The spectra achieved of the organism with 1064 nm laser excitation are poor and no conclusive results can be obtained. The best results from the cyanobacterial colonies achieved by imaging and focussing through the gypsum crystal were obtained using the green (514 nm) and the near infrared (785 nm) laser excitations.

With the 514 nm excitation wavelength (Figure 9) two different colonies of cyanobacteria have been discovered. Several analyses on the same strains show strong bands at 1517, 1157 and 1006 cm^{-1} from carotene and broad bands at 1630, 1598, 1552, 1454, 1379 and 1281 cm^{-1} assigned to scytonemin. The second strain gives a spectrum with broad bands at 1671, 1575 (with a shoulder at 1595 cm^{-1}) 1197, 914 and 464 cm^{-1}, assigned to parietin, and a very strong band centred at 1340 cm^{-1} characteristic of chlorophyll; weak bands of carotene also appear here.

A set of spectra at different depths were taken on a vertical transect through the gypsum from the surface until the organism was detected using 514 and 785 nm

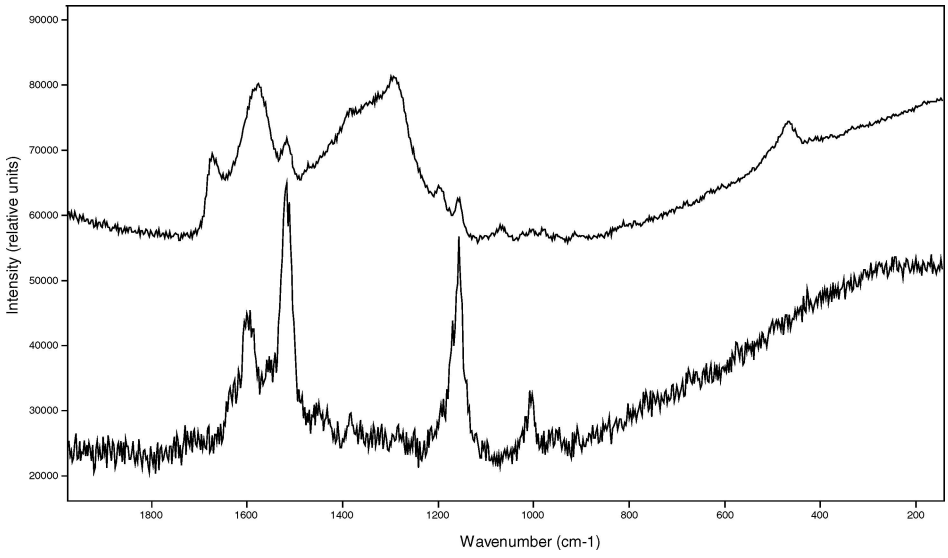

Figure 9. Raman spectra collected from cyanobacterial colonies in gypsum crystal. Although carotene is present in both spectra, the presence of different Raman spectral biosignatures is indicative of two different species. Spectra werecollected using a 514 nm laser excitation.

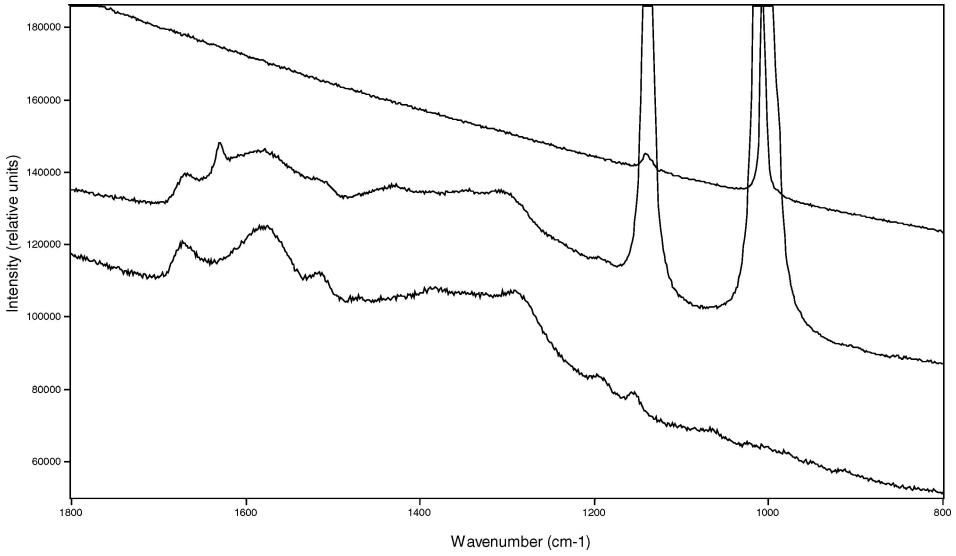

Figure 10. Raman spectra on a vertical transect through a gypsum crystal containing a halophile colony, recorded using 5145 nm excitation. From the top, gypsum spectrum from the surface, spectrum of the internal cyanobacterial colony with gypsum bands, spectrum from the identical cyanobacterial colony with the gypsum spectrum subtracted.

laser excitation (Figure 10). After several analyses, in which only the gypsum signatures were observed, some broad bands appear in the spectrum approximately 5 mm below the crystal surface. Comparing the spectrum obtained here with that achieved directly from the organism itself it is clear that the Raman bands of the cyanobacterial colony can be identified inside the gypsum crystal.

Biofilm

This sample was selected from a geyser with sulphurous water (Figure 11). The organisms are observed as watery red and green coloured mucus biofilms.

The spectra achieved with 1064 nm laser excitation on the red material give bands at 353, 342, 220, 192, 182 and143 cm^{-1} characteristic of realgar, an arsenic sulfide (Figure 12). The spectrum collected from the green region has broad, weak signatures of a carotenoid (1525 and 1157 cm^{-1}). No other bands appear.

Bands of a carotene at 1519, 1190, 1156, 1004, 960 and 881 cm^{-1} appear in the spectrum collected with 514 nm laser excitation but no other organic compound is recognizable. The broad bands observed in the low wavenumber region between 400–100 cm^{-1} are possibly due to some fluorescence or thermal emission effects (Figure 13).

Figure 11. Microorganisms living in the hot water of a geyser from Tatio, in the Atacama Desert, Chile.

Epilithic Lichen at 2400 Metres Altitude

The yellow lichen (*Acarospora cf schleichera*) collected at 2400 metres altitude in the central Atacama desert, was analysed with 1064, 785, 633, and 488 nm laser wavelengths.

With 1064 nm laser excitation, the spectrum collected of the lichen thallial surface shows bands at 1663, 1590, 1491, 1285, 1187 and 1003 cm^{-1}, which have been assigned to rhizocarpic acid and bands at 1524, 1187, 1157 and 1003 cm^{-1} to carotene. The bands at 1187 and 1003 cm^{-1} have a contribution from both, rhizocarpic acid and carotene. Weak bands at 1615 (shoulder), 1372, 1324, 1277, 921 and 636 cm^{-1} are assignable to parietin (Figure 14). The spectrum achieved of the white dust observed below the surface has signatures at 1491, 1462, 896 and 503 cm^{-1} characteristic of calcium oxalate monohydrate, whewellite. Weak bands at 1629, 1593 (both of rhizocarpic acid) and 1526, 1153 cm^{-1} (carotene) are also visible.

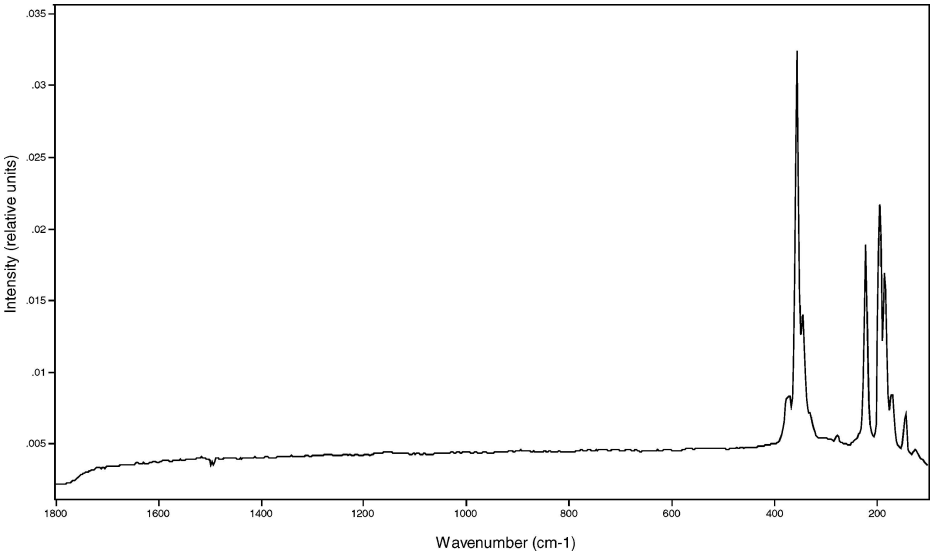

Figure 12. FT-Raman spectra of the red coloured area in a biofilm from the Tatio Geyser in the Atacama Desert, Chile, shown in Figure 11.

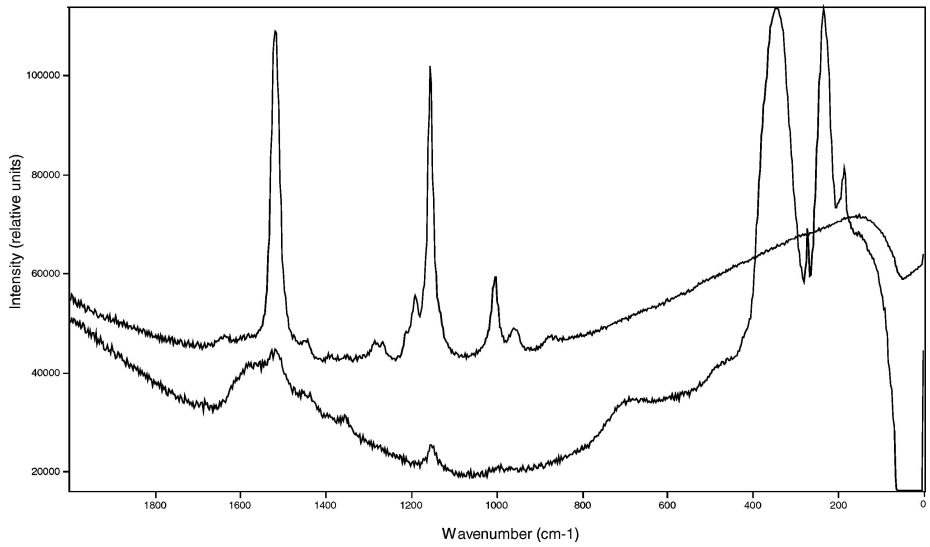

Figure 13. Raman spectrum of the green (upper) and red (lower) areas in the biofilm from the Tatio Geyser, shown in Figure 11.

The same results were obtained when the lichen thallial surface was analysed with 633 and 785 nm wavelengths but the bands at 1524 and 1157 cm^{-1} from carotene are now not so clearly defined because they are compromised with the rhizocarpic and parietin bands.

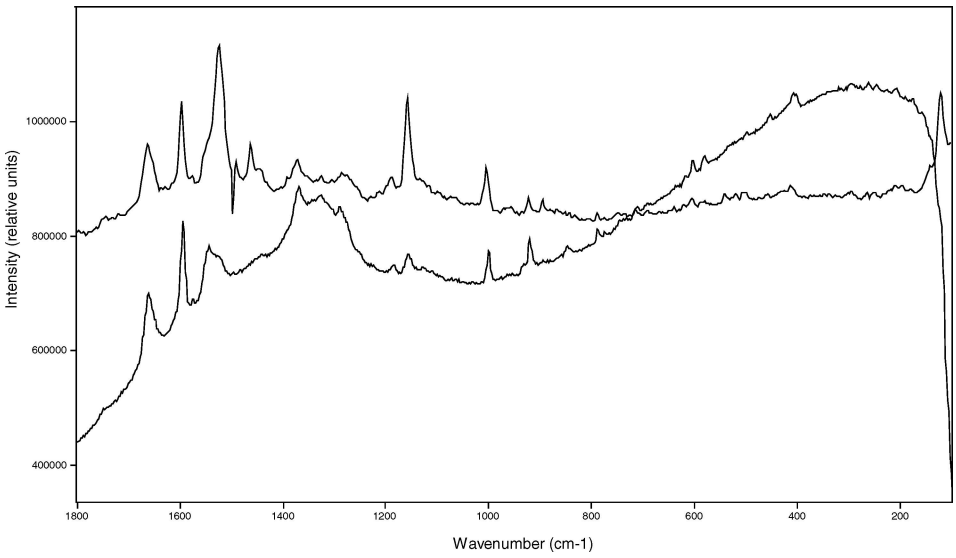

Figure 14. Raman spectra from the top of an epilithic lichen (*Acarospora cf schleichera*) from the Atacama Desert (Chile) recorded using 1064 nm (upper) and 785 nm (lower) laser excitation.

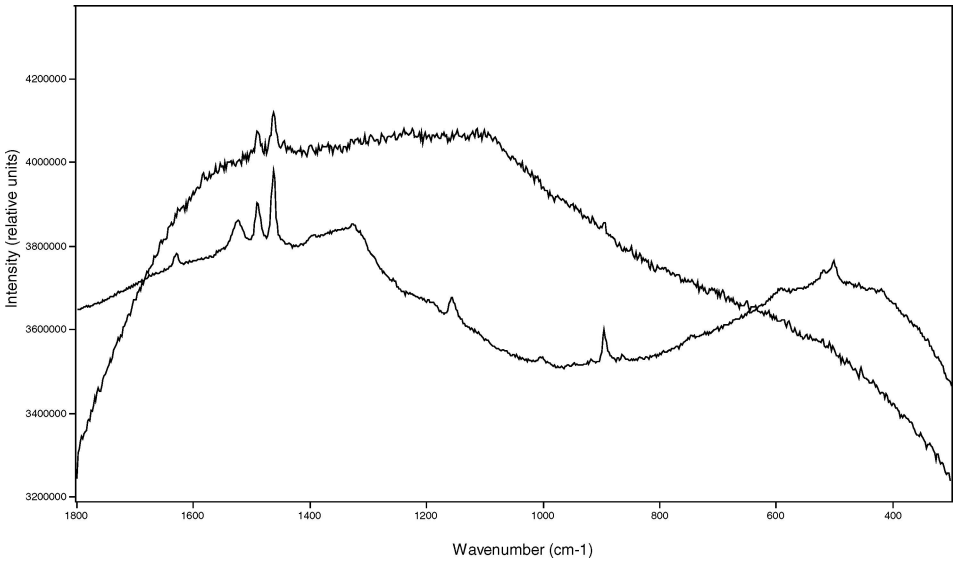

Figure 15. Raman spectra of a white dust from the same lichen (Figure 14) using 633 nm (upper) and 785 nm laser (lower) excitation.

In the spectra collected from the lowest zone (Figure 15), calcium oxalate monohydrate gives signatures with 633 and 785 nm laser excitations; with near-infrared laser excitation carotene bands are also visible.

Carotene is the only compound shown with 488 nm laser excitation; again, this is attributable to a resonance Raman scattering effect.

Epilithic Lichen at 4500 Metres Altitude

Carotene (1522, 1156 and 1004 cm^{-1}) and parietin (Figure 16) (1669, 1612, 1551, 1367, 1326, 1273, 1255, 1215, 1179, 927, 521, 462 and 400 cm^{-1}) both appear in the spectra achieved with 785 and 1064 nm laser wavelengths. However, the spectrum collected with 488 nm laser excitation (Figure 17) shows bands of carotene (1513 and 1153 cm^{-1}), broad bands at 1591, 1452, 1321, 1284 and 1166 cm^{-1}, which can be assigned to scytonemin, and another band at 1378 cm^{-1} which is as yet unassigned. No bands of parietin appear in this spectrum.

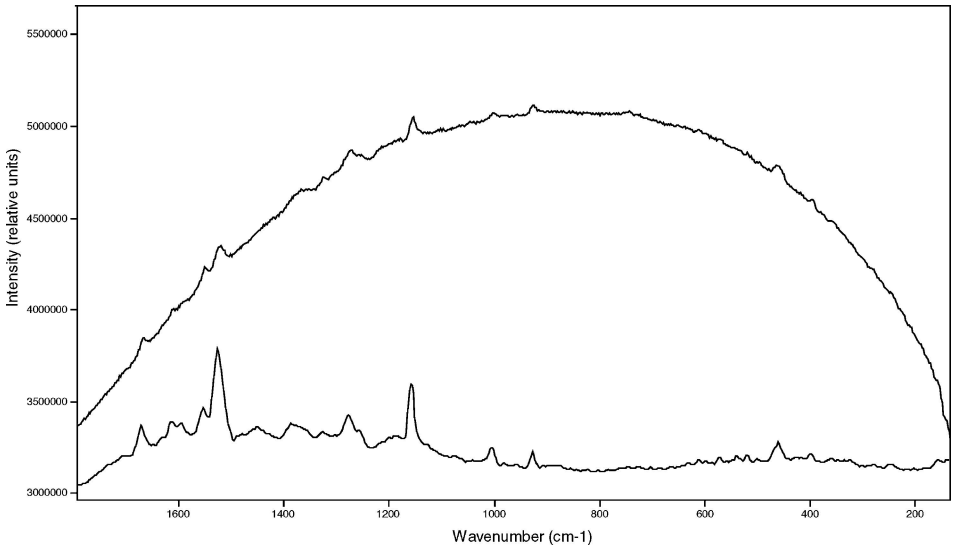

Figure 16. Raman spectra of an epilithic lichen (*Xanthomendoza mendozae*) from the Atacama Desert (Chile) recorded using 785 nm (upper) and 1064 nm (lower) laser excitation.

Conclusions

FT-Raman spectroscopy, working with 1064 nm laser excitation, usually records spectra of organic compounds with a high quality in the macro mode but requires from one to two hours minimum to achieve data in the wavenumber region of 4000-100 cm^{-1}, especially if the biomolecules in the specimen appear in a relatively low concentration.

Conventional spectroscopy needs less time to achieve spectra, generally from one to twenty minutes in the wavenumber region of 1800–0 cm^{-1}; however, the

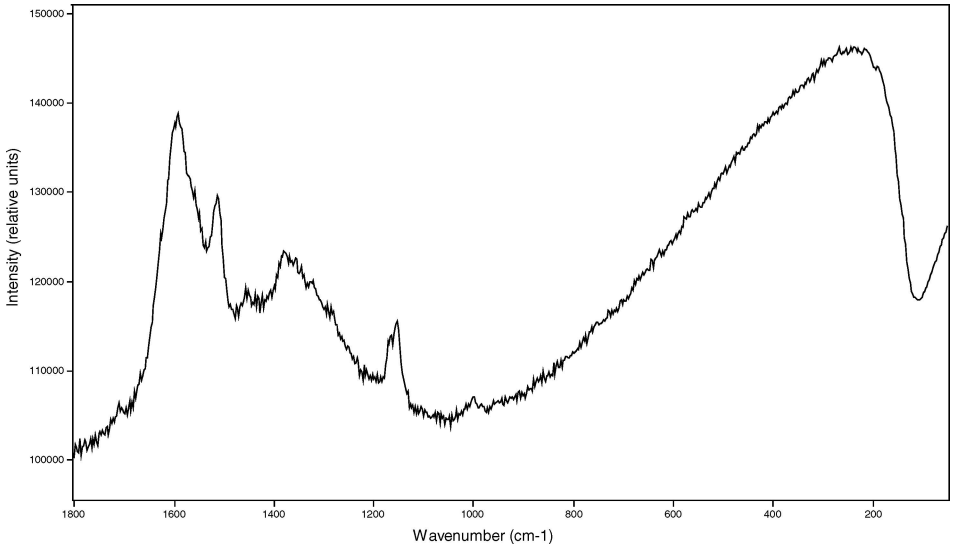

Figure 17. Raman spectrum of the epilithic lichen *Xanthomendoza mendozae* recorded with 488 nm laser excitation.

spectral biosignatures are found to be broader at these visible wavelengths, particularly in the bacterial analysis, compared with those obtained using FT-Raman spectroscopy in the near-infrared (1064 nm). We attribute this to thermal emission and the onset of fluorescence emission. Not all visible lasers wavelengths give the same results. Spectral data using 514 nm (green) and 488 nm (blue) laser excitation are excellent and provide indicators of carotene but with other organic compounds the results obtained are normally very poor. With these wavelengths few other biomarkers can be identified; whereas it is usually possible to assert that there is an organic compound present in the sample but it is generally quite difficult to make unambiguous assignments because of the poor signal-to-noise ratios and the width of the bands. The results obtained with carotene are attributed directly to the resonance Raman effect, particularly with blue and green wavelength excitation.

Blue and green minerals are best studied with 514 and 488 laser wavelengths, but these are not suitable for most of the geomarkers. Whilst spectra of white minerals (such as some carbonates, quartz, gypsum, etc) achieved with a 1064 nm laser have a very good quality, dark coloured minerals are generally very difficult to study. We conclude that 785 nm wavelength studied here is the laser wavelength with the best potential for identification of minerals.

Several spectra from different biomarkers have been collected using 785 nm laser excitation with very good results and the identification of the compound is generally unquestionable (such as parietin, scytonemin, rhizocarpic acid, carotene, chlorophyll) whatever the habitat of the organism (endolith, epilith, salt, biofilm . . .). Furthermore, it is only necessary to collect data in the wavenumber region of

1800-100 cm^{-1} from between one to fifteen minutes to obtain sufficiently good signal-to-noise ratios for spectral biomolecular identification.

We conclude that from the laser wavelengths used in this study, the 785 nm laser wavelength is the most suitable for the analysis of both geo- and biomarkers for a wide range of environmental conditions experienced by these organisms. This will have direct bearing on the selection of Raman spectrometers for extraterrestrial planetary exploration, since it is clear that not all laser wavelengths would be suitable for both geological and biological molecular identification purposes. Naturally, laser wavelength is only one parameter that needs assessment and others such as smalless of mass, robustness and speed of data acquisition are all important factors for the development and adaption of Raman instrumentation on robotic landers for Martian exploration, in particular.

References

Bishop, J. L., Murad, E., Lane, M. D. and Mancinelli, R. L.: 2004, Multiple Techniques for Mineral Identification on Mars: A Study of Hydrothermal Rocks as Potential Analogues for Astrobiology Sites on Mars, *Icarus* **169**, 311–323.

Clark, B. C.: 1998, Surviving the Limits to Life at the Surface of Mars, *Journal of Geophysical Research-Planets* **103**, 28545–28555.

Cockell, C. A.: 2001, The Martian and Extraterrestrial UV-Radiation Environment Part II: Further Considerations on Materials and Design Criteria for Artificial Ecosystems, *Acta Astronautica* **49**(11), 631–640.

Cockell, C. S.: 2000, The Ultraviolet History of the Terrestrial Planets – Implications for Biological Evolution, *Planetary and Space Science* **48**, 203–214.

de Vera, J.-P., Horneck, G., Rettberg, P. and Ott, S.: 2004, The Potential of the Lichen Symbiosis to Cope with the Extreme Conditions of Outer Space II: Germination Capacity of Lichen Ascospores in Response to Simulated Space Conditions, *Adv. Space Res.* **33**, 1236–1243.

Dickensheets, D. L., Wynn-Williams, D. D., Edwards, H. G. M., Schoen, C., Crowder, C. and Newton, E. M.: 2000, A Novel Miniature Confocal Microscope/Raman Spectrometer System for Biomolecular Analysis on Future Mars Missions after Antarctic Trials, *J. Raman Spectroscopy*, **31**(7), 633–635.

Edwards, H. G. M.: 2004, Raman Spectroscopic Protocol for the Molecular Recognition of Key Biomarkers in Astrobiological Exploration, *Orig. Life Evol. Biosphere* **34**, 3–11.

Edwards, H. G. M., Holder, J. M. and Wynn-Williams, D. D.: 1998, Comparative FT-Raman Spectroscopy of *Xanthoria* Lichen-Substratum Systems from Temperate and Antarctic Habitats, *Soil Biol. Biochem.* **30**, 1947–1953.

Edwards, H. G. M., Newton, E. M., Dickensheets, D. L. and Wynn-Williams, D. D.: 2003a, Raman Spectroscopic Detection of Biomolecular Markers from Antarctic Materials: Evaluation for Putative Martian Habitats, *Spectrochim. Acta Part A: Mol. Biomol. Spectroscopy* **59**, 2277–2290.

Edwards, H. G. M., Newton, E. M. and Wynn-Williams, D. D.: 2003b, Molecular Structural Studies of Lichen Substances II: Atranorin, Gyrophoric Acid, Fumarprotocetraric Acid, Rhizocarpic Acid, Calycin, Pulvinic Dilactone and Usnic Acid, *J. Mol. Struct.* **651–653**, 27–37.

Edwards, H. G. M., Newton, E. M., Wynn-Williams, D. D., Dickensheets, D., Shoen, C. and Crowder, C.: 2003c, Laser Wavelength Selection for Raman Spectroscopy of Microbial Pigments *in situ* in Antarctic Desert Ecosystem Analogues of Former Habitats on Mars. *Intern. J. Astrobiol.* **1**, 333–348.

Edwards, H. G. M., Wynn-Williams, D. D. and Jorge Villar, S. E.: 2004a, Biological Modification of Haematite in Antarctic Cryptoendolithic Communities. *J. Raman Spectroscopy* **35**, 470–474.

Edwards, H. G. M., Wynn-Williams, D. D., Little, S. J., Oliveira, L. F. C., Cockell, C. S. and Ellis-Evans, J. C.: 2004b, Stratified Response to Environmental Stress in a Polar Lichen Characterized with FT-Raman Microscopic Analysis, *Spectrochim. Acta Part A: Mol. Biomol. Spectroscopy* **60**, 2029–2033.

Ellery, A. and Wynn-Williams, D. D.: 2003, Why Raman Spectroscopy on Mars? A Case of the Right Tool for the Right Job, *Astrobiology* **3**(3), 565–579.

Finegold, L.: 1986, Molecular Aspects of Adaptation to Extreme Cold Environments, *Adv. Space Res.* **6**, 257–264.

Jorge Villar, S. E., Edwards, H. G. M. and Wynn-Williams, D. D.: 2003, FT-Raman Spectroscopic Analysis of an Antarctic Endolith, *Inter. J. Astrobiol.* **1**(4), 349–355.

Long, D. A.: 2002. *The Raman Effect: A Unified Treatment of the Theory of Raman Scattering by Molecules*, John Wiley and Sons Ltd., Chichester, UK.

Mancinelli, R. L.: 1998, Biopan-Survival I: Exposure of the Osmophiles *Synechococcus SP.* (Nageli) and *Haloarcula SP.* to the Space Environment, *Adv. Space Res.* **22**, 327–334.

O'Brien, A., Sharp, R., Russell, N. J. and Roller, S.: 2004, Antarctic Bacteria Inhibit Growth of Food-Borne Microorganisms at Low Temperatures, *FEMS Microbiol. Ecol.* **48**, 157–167.

Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J., Shcherbakova, V. and Gilichinsky, D.: 2004, Microbial Life in Permafrost, *Adv. Space Res.* **33**, 1215–1221.

Rothschild, L. J. and Cockell, C. S.: 1999, Radiation: Microbial Evolution, Ecology, and Relevance to Mars Missions, *Mutation Res./Fundamental Mol. Mech. Mutagenesis* **430**, 281–291.

Sharma, S. K., Lucey, P. G., Ghosh, M., Hubble, H. W. and Horton: 2003, Stand-off Raman Spectroscopic Detection of Minerals on Planetary Surfaces, *Spectrochim. Acta Part A – Mol. Biomol. Spectroscopy* **59**, 2391–2407.

Vincent, W. F., Mueller, D. R. and Bonilla, S.: 2004, Ecosystems on Ice: The Microbial Ecology of Markham Ice Shelf in the High, *Arctic Cryobiol.* **48**, 103–112.

Wang, A. and Haskin, L. A.: 2000, Development of a Flight Raman Spectrometer for the "Athena" Rover Scientific Instrument Payload for Mars Surveyor Missions, *Microbeam Anal. 2000, Proc. Inst. Phys. Conf. Ser.* **165**, 103–104.

Wang, A., Haskin, L. A., Lane, A. L., Wdowiak, T. J., Squyres, S. W., Wilson, R. J., Hovland, L. E., Manatt, K. S., Raouf, N. and Smith, C. D.: 2003, Development of the Mars Microbeam Raman Spectrometer (MMRS), *J. Geophys. Res.-Planets* **108**(E1): art. No. 5005.

Wynn-Williams, D. D. and Edwards, H. G. M.: 2000, 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.