INTRODUCTION

Raman spectroscopic approach to analytical astrobiology: the detection of key geological and biomolecular markers in the search for life

1. Prologue

The compilation of this Theme Issue of Philosophical Transactions of the Royal Society on the theme of Raman spectroscopic analysis of materials from extreme environments is timely for several reasons, perhaps the most significant of these being the realization in the last decade that this technique can provide unique information about the limits of survival of biological organisms in terrestrial locations, where hitherto the prospects of finding life were thought to be minimal. The year 2009 marked the bicentenary of the birth of Charles Darwin and the sesquicentenary of the publication of his monumental work, On the Origin of Species by Natural Selection in November 1859. This book ran to six editions and it would be superfluous to comment here on the astounding debates that raged in the second half of the nineteenth century involving all aspects of arts and sciences and religion. Although today we associate Darwin’s work with evolution, it is interesting that this was only mentioned in the final and sixth edition, which also contained his first recognition of and counter argument against the claims then being made by theologians. Although Darwin himself did not have knowledge then of the extreme-tolerant, ‘extremophilic’, organisms that today we regard as ubiquitous, the analogy with the survival by natural selection of those organisms that were best suited to survive predatory attacks or environmental changes independently proposed by Alfred Russell Wallace and Darwin is quite similar in evolutionary terms to the birds, beetles and lizards that caused so much wonder from Darwin’s observations in the Galapagos Islands in the Beagle expedition of the 1830s.

It is clear from the analytical data that have been obtained in recent years that the strategies being adopted by organisms in stressed environments, examples of which are tabulated in table 1, in life-threatening scenarios are critical for their survival and evolutionary behaviour. The key factors that have emerged suggest that the synthesis of particular suites of protective biochemicals that act as protectants and repair agents when damage has occurred, often in multi-functional roles, is critical for survival in these ‘limits of life’ situations. The
Table 1. Terrestrial extremes for the survival of extremophilic organisms.

<table>
<thead>
<tr>
<th>Extreme Parameter</th>
<th>Extremophile</th>
<th>Survival Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td><em>Pyrolobus fumarii</em></td>
<td>113°C (Volcano Island, Italy)</td>
</tr>
<tr>
<td></td>
<td><em>Chroococcidiopsis</em> sp.</td>
<td>−15°C (Mars Oasis, Antarctica)</td>
</tr>
<tr>
<td>Pressure</td>
<td></td>
<td>1200 bar</td>
</tr>
<tr>
<td>Radiation</td>
<td><em>Deinococcus radiodurans</em></td>
<td>5 Mrad (5000 times the fatal dose for humans)</td>
</tr>
<tr>
<td>Underground Depth</td>
<td></td>
<td>3.2 km</td>
</tr>
<tr>
<td>Acidic pH</td>
<td></td>
<td>pH 0.0</td>
</tr>
<tr>
<td>Basic pH</td>
<td></td>
<td>pH 13.0</td>
</tr>
<tr>
<td>Longest in Space</td>
<td><em>Bacillus subtilis</em></td>
<td>6 yr NASA satellite</td>
</tr>
<tr>
<td>Saltiest</td>
<td><em>Haloarcula</em></td>
<td>30% salt</td>
</tr>
<tr>
<td>Smallest</td>
<td><em>Picoplankton</em></td>
<td>0.1 μm</td>
</tr>
</tbody>
</table>

correlation of the extreme terrestrial survival by Archaean cyanobacterial colonies that have had to adjust and evolve from a primitive and hostile Earth some 3.8 Gyr ago with the stresses of possible similar colonizations on our neighbouring planets and their satellites is now at the forefront of multidisciplinary research efforts to understand and evaluate these possibilities using unmanned planetary rovers and accompanying sensor instrumentation.

The recognition of extinct or extant life signatures in the terrestrial geological record is fundamentally dependent upon the understanding of both the structural morphology and chemical composition of relict biomaterials; the identification of cyanobacterial colonies that have adapted biogeologically their mineral matrices in early evolutionary processes is a fundamental step in the acquisition of analytical data from remote planetary probes designed for life-detection experiments, particularly on Mars and on the planetary satellite moons Europa and Titan. A key factor in the assessment of early life signatures is the molecular presence of chemicals designed to protect the emerging organisms from the damaging effect of radiation exposure and of desiccation and temperature changes; in this respect the non-destructive capability of Raman spectroscopy to delineate the interfacial interactions between substrates and endolithic biology is now deemed an essential part of the ExoMars life-detection suite of instrumentation planned by the European Space Agency (ESA) in the Aurora programme. A description of the scientific basis for the biogeological discrimination offered by Raman spectroscopy between organic and inorganic moieties in specimens from terrestrial Mars analogue sites is a recurrent theme in the original research papers presented in this Theme Issue.

The search for life signatures in early evolutionary processes informs the need for the addressing of basic questions about the origins of life arising from a prebiotic world (Edwards 2007). In our terrestrial geological history, the recognition of the presence of fossilized relict cyanobacterial organisms through their morphological traces is a key factor in the ‘top-down’ approach to an understanding of the early biological colonization and the prebiological chemistry of the evolving geosphere.

Cyanobacterial colonization of geological substrates has been identified in our earliest terrestrial geological formations, extending from some 3.8 Gyr, and it is clear that an understanding of the survival strategies and protection mechanisms
being adopted by these organisms can provide some important clues as to the conditions that were operating on early Earth and in their adaptation to the evolutionary conditions (Schopf et al. 2002; Brasier et al. 2004).

The survival of cyanobacterial extremophiles in stressed terrestrial environments is fundamentally dependent upon their synthesis of a specialized suite of protective biochemicals in response to desiccation, low-wavelength high-energy radiation insolation, extremes of pH, temperature and pressure and high concentrations of toxic heavy metal ions (Cockell & Knowland 1999; Wynn-Williams & Edwards 2000a,b). Our understanding of the roles of these chemical protectants reveals a sophistication in design for specific functions; for example, the ultraviolet radiation screening effectiveness of scytonemin in cyanobacterial sheaths, which selectively absorbs in the UVA, UVB and UVC regions of the electromagnetic spectrum, while permitting the transmission of the longer wavelength photosynthetically active radiation that is necessary for the operation of chlorophyll in photosynthetic colonies. Other protectants, such as trehalose, which is synthesized in Antarctic cyanobacterial mats colonizing salt-rich lakes, are dualistic in their roles; trehalose is used as a water-replacement intracellular molecule, which also combats osmotic stress caused by abnormally large concentrations of soluble salts such as calcium chloride, and a range of carotenoids serve as light-harvesting pigments and also as cellular DNA damage repair agents.

It is generally accepted that life started on Earth around 3800 Myr, when the environmental conditions on the surface were drastically different from the current situation. At that time, it is predicted that Mars had similar conditions to those pertaining on Earth and this has led to the idea that life could also have appeared on Mars (Davis & McKay 1996; McKay 1997; Cockell et al. 2000; Holm & Andersson 2005). As the external conditions were changing on early Mars, therefore as on Earth, organisms needed to adapt to new habitats and colonize even the most extreme niches using a range of response strategies. Those organisms that were able to survive in such hostile environments can be considered to have left signatures of their presence on Mars; it is fundamentally important, therefore, to analyse our terrestrial analogues of possible Mars organisms (Raulin & McKay 2002; Simoneit 2004; Delaye & Lazcano 2005).

Cold deserts, such as Antarctica or the Arctic, have been proposed as the closest Martian analogues on Earth (Wynn-Williams 2000). The extreme low temperatures, highly dry atmospheres, strong UV radiation, low nutrient availability or long periods without sunlight have not been an obstacle for the adaptation and proliferation of many different types of micro-organisms. The survival adaptations of micro-organisms in these environments extend to both inorganic and organic strategies (Jorge Villar et al. 2003, 2005; Edwards et al. 2005), especially involving the use of a wide range of radiation protective pigments (Mueller et al. 2005; Jorge Villar et al. 2006).

2. What can we learn from astrobiology?

Astrobiology has been described as the only scientific discipline that has no formal evidence for its own existence; that still awaits the analytical data that will emerge from remote planetary probes and eventually exploration of our Solar System and

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Beyond. The key to this question must address initially the compounds that are to be identified in meteorites and cosmic material harvested on Earth and their relevance to prebiotic synthesis elsewhere. Clearly, the material found on Earth resulting from meteoritic infall has been subjected to the stresses of atmospheric entry following aeons of exposure to the low temperatures and high-energy radiation in deep space. We are currently unable to assess the influence of these conditions upon the chemistry of organic compounds contained within meteorites, but recent experiments performed under project STONE, a ESA-sponsored effort resulting in the first artificial meteorite experiment designed to understand the alteration of geological and biological materials subjected to a space environment and their subsequent atmospheric re-entry, have made significant progress in this direction (Brack et al. 2006; Cockell et al. 2007). However, it should be recognized that the origin of meteorites formed from ejecta resulting from impact events on planetary surfaces could have involved conditions and processes that would have altered the residual chemical signatures of any organisms that could have existed on the planetary surface prior to the impact collision. It is believed therefore that the presence or otherwise of key organic biomolecular signatures in meteorites or cosmic debris can only represent one part of the astrobiological scenario and that a major thrust would necessitate the examination of the geological record of the original planet. This is the current thinking behind the use of novel analytical instrumental techniques for the determination of chemical life signatures in the robotic search for life on planetary surfaces prior to human exploration, as exemplified by the NASA and ESA Aurora missions.

Raman spectroscopy is being adopted by ESA as a part of an analytical instrumentation suite for Mars surface exploration, including the ExoMars mission scheduled for 2018. The particular characteristics of Raman spectroscopy that make this technique a valuable instrument for the unambiguous identification of either biological or geological signatures (Jorge Villar & Edwards 2006) are primarily those of non-destructive analysis of specimens, ease of sample examination that does not involve any physical or chemical surface preparation and an ability to collect data remotely via an optical fibre. A crucial part of the analytical procedures will rest with the interpretation of spectral signatures and comparison with laboratory databases of key biochemicals.

The miniaturization of Raman spectrometers involves the consideration of several parameters that have an effect on the spectrum and for the subsequent identification of both organic and inorganic materials. The effects of laser wavelength, spectral resolution, laser power or the selection of a particular wavenumber range have been studied in our laboratory and their impact and consequences for the observed spectral analysis have been assessed.

Different laser wavelengths have different capabilities for the detection of specific biomolecules and minerals and some compounds could not be detected; furthermore, the changes in relative intensities of the characteristic Raman bands related to laser excitation make the unambiguous identification of some molecules difficult, which compromised several databases. Fluorescence and resonance scattering also have an important effect on the observed Raman spectrum in terms of band intensities. A high laser power can induce subtle molecular changes in either mineral and organic molecules and too low a laser power would increase significantly the time necessary for the spectrum accumulation to be
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If a restricted wavenumber region is demanded in the miniaturized instrument, the priority for the identification of bio- or geo-signatures has then to be selected consequent upon the more limited wavenumber region of the Raman spectrum.

Spectroscopic and microscopic data have demonstrated the various protective and adaptive mechanisms and strategies developed by Arctic extremophile microorganisms for their survival in cold desert environments. The use of dark basaltic rocks as a protective niche for endolithic and chasmolithic communities and the development of specialized pigments as survival strategies by psychrophilic snow algae are exemplified in this scenario.

It has been shown (Jorge Villar et al. 2003, 2005, 2006) that in stressed conditions micro-organisms protect themselves through the colonization of light rocks, by living close to the surface, either inside a porous matrix (endolith) or inside a crack (chasmolith), where light and water can be easily supplied. However, it has now been shown that a dark basaltic rock containing endolith- and chasmolith-like communities can also play a protective role and that the restricted light availability in such an environment is not a deterrent for colonization. By using in situ Raman spectroscopy combined with high resolution microscopic and elemental analyses using scanning electron microscopy (SEM), the location and chemical characteristics of these communities have been identified non-destructively and for the first time it has been shown that photosynthetic organisms living in terrestrial dark basaltic rocks can survive and even thrive in such extreme environments (Jorge Villar et al. 2006). The Raman spectra demonstrate the presence of radiation protective scytonemin, a range of carotenoids, accessory photosynthetic pigments such as c-phycocyanin as well as chlorophyll.

In another example, the strategies adopted by snow algae collected from a permanent snow field in the Murchison Fjord area of Spitzbergen (80°N, Norway) have been examined; this scenario would be relevant for Mars polar life detection remote-sensing expeditions. Again, a combination of Raman spectroscopy and SEM techniques have been used for a comprehensive study of the protective strategies adopted by surface-dwelling micro-organisms on glaciers and snow fields, and pigment and other radiation protective compounds such as scytonemin, carotenoids and an anthraquinone similar to emodin have been identified.

Although specifically there is no single terrestrial Mars analogue site, and indeed this would have been too narrow to define, given the broad range of geological niches that have been identified on Mars by the latest NASA rovers, Spirit and Opportunity, the examination of a range of closely related extremophilic scenarios on Earth is of potential relevance to the diverse situations that may be expected on Mars; in this context, a study is currently being undertaken on ancient terrestrial rocks that harbour morphological structures of fossilized early Earth organisms to identify the presence of residual protective chemicals in geological niche sites that would be indicative of early life signatures (Pullan et al. 2008) that could perhaps be indicative of first-pass screening experiments on Mars carried out by planetary surface rover vehicles. The motto of the Royal Air Force, Per Ardua ad Astra, is descriptive of the terrestrial analytical work that is essential for potential astrobiological data to be understood from remote planetary searches for life signals, extinct or extant, and towards that objective the

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compilation of papers in this Theme Issue is dedicated: Raman spectroscopy of extreme environments. A good representation is thereby afforded of the versatility of this analytical technique for the detection of life signatures from materials in terrestrial extreme environments encompassing hot and cold deserts, Arctic and Antarctic regions, deep-sea smokers, glacial sediments, meteorites, volcanic lava flows and strongly acidic locations that will be a foundation for the appreciation of future experiments being undertaken remotely on Mars missions.

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References


