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Pigmentation as a survival strategy for ancient and modern photosynthetic microbes under high ultraviolet stress on planetary surfaces

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Abstract: Solar radiation is the primary energy source for surface planetary life, so that pigments are fundamental components of any surface-dwelling organism. They may therefore have evolved in some form on Mars as they did on Earth. Photosynthetic microbes are major primary producers on Earth, but are concurrently vulnerable to ultraviolet (UV) damage. Using non-intrusive laser Raman spectroscopy to recognize the component parts of biomolecules, we have shown not only the abundance of microbial photosynthetic and photoprotective pigments *in situ*, but also their spatial distribution within their microhabitat. This essential aspect of their screening or avoidance survival strategies is lost on extraction with solvents. This precise approach is eminently suited to analysis of epilithic (surface) and endolithic (within rocks) communities in Antarctic desert habitats, which are putative analogues of early Mars. Raman spectra for key biomolecules (e.g. the UV screen parietin and the antioxidant β-carotene in epilithic lichens) enable not only the detection of organics in light-stratified habitats, but also the characterization of unknown pigments. Typical biomarkers of astrobiological relevance in our Raman spectral database include scytonemin (a UV screen), chlorophyll (primary photosynthetic pigment), phycocyanin (accessory pigment for shade adaptation) and a hopanoid extracted from 2.5 Gya microbial stromatolite from Australia. This compound dates from the same time period when a wetter Mars could have had a potentially flourishing surface microbial community of its own. Analyses with a laboratory Raman instrument have been extended to a novel miniature Raman spectrometer, operating at the same optimal excitation wavelength (1064 nm) via an In-Ga-As detector. After evaluation in Antarctica, this instrument will be space-qualified for a proposed Mars rover mission to detect biomolecules in the near-surface sediment profile of palaeolakes, using experience with Antarctic biomarkers to interpret alien spectra of fundamental components, without the need for prior knowledge of the identity of the target compounds.

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Key words: Antarctic, Mars, photosynthetic, pigments, planetary, Raman spectroscopy, survival and ultraviolet.

Introduction

Before microbes could evolve on Earth, habitats within the physico-chemical constraints of the lithosphere had to become suitable for life. At the end of the Hadean period ($\sim$ 4.2 Gya), the lithosphere was cooling to the known limits of present-day thermophilic bacteria (90–120 °C) and could have become habitable (Russell & Hall 1997). We assume that life on certain terrestrial planets such as Earth probably originated in geothermal habitats in which organic material was created in anaerobic ecosystems. These derived energy from electron transfer along redox gradients involving ferric sulphides and other key minerals at relatively high pH (Russell & Hall 1997; Kelley *et al*. 2001). We also support the corollary of Russell & Hall (1999) that a similar origin of life on Mars was inevitable. Although heterotrophic microbes may have evolved to feed on the organic input from meteorites, any autotrophic (self-sufficient) microbes living on Earth $\sim$ 3.8 Gya would probably have been chemolithotrophic organisms. These are dependent on redox gradients for obtaining energy in the hot, deep, sediment-laden water of the early oceans. Present-day *Pyrolobus*, one of the Archaea obtained at over 120 °C from a marine hydrothermal vent, has a growth temperature of 113 °C and can tolerate autoclaving at 121 °C for an hour (Blöchl *et al*. 1997). Microbial ecosystems follow physico-chemical gradients not only for extracting energy, but also for migrating

into thermally favourable habitats. Microbial infrared sensors for suitable geothermal niches could have evolved into light sensors for migrating towards the most efficient and ubiquitous energy source of all, photosynthetically active solar radiation, PAR (Nisbet & Fowler 1999).

Meanwhile, Mars would have cooled to a suitable temperature for a biosphere sooner than the Earth for several reasons. Its 50% smaller diameter gives a fourfold larger surface-area-to-volume ratio for heat loss. This smaller volume resulted in the cessation of volcanic and tectonic activity, unlike the Earth which is still active. Its greater distance from the Sun provided less thermal input and it was not subject to the hiatus of the Moon-creating collision that re-melted the crust of the Earth. After a potential geothermal origin of life, McKay (1997) considers that the surface of Mars was probably a wet habitable zone from $\sim$ 3.8 Gya. During this period, the atmospheres of the Earth and Mars contain little oxygen and had a negligible ozone layer to screen out the UVB (280–320 nm) and UVC ($<$ 280 nm) that accompanied PAR. We consider that the selective pressure of solar radiation in surface habitats would have been highly influential on the direction of any microbial evolution (Garcia-Pichel 1998; Cockell & Horneck 2001). This would have had two effects:

(1) biological selection for pigments capable of harnessing photosynthetically active radiation (PAR, 400–750 nm) as a more efficient and ubiquitous energy source than chemosynthetic redox systems;
(2) selection for organisms able to synthesize ultraviolet- (UV) screening and energy-quenching pigments to protect vital molecules such as proteins and nucleic acids.

Pigments would therefore have been key components of any surface-dwelling microbes, whether photosynthetic, such as the Archaean bacterium *Chloroflexus* (Blankenship 1992), or heterotrophic like. *Deinococcus radiodurans* (Battista 1997). They could have received concurrent UV-protection and antioxidant activity from early multi-purpose carotenoids such as bacteriorhodopsins (Mulkidjanian & Junge 1997). The isoprenoid backbone of carotenoids would have provided rigidity for early membranes in hot habitats, whilst acting as accessory pigments for chlorophyll during their gradual emergence from darkness.

Modern carotenoids (e.g. β-carotene) not only absorb UVA (320–380 nm) and quench free radicals produced by UV damage, but also absorb in the UVC range, which is lethal to DNA that absorbs optimally at 254 nm. Similarly, the UVB-absorbing pigment scytonemin of cyanobacterial sheaths can also absorb UVC (Dillon & Castenholz 1999). This is paradoxically irrelevant today as the dense atmosphere of modern Earth screens out all UVC before it reaches the biosphere. However, this capability would have been vital on early Earth (and Mars?) and may be a significant relic of the early evolution of microbial pigmentation. This has taken two protective routes:

(1) UV absorption by screening pigments or the quenching of UV-generated free radicals;
(2) an avoidance strategy entailing growth at very low light levels in stratified habitats. This requires accessory pigments such as phycocyanin to harvest scarce photons in support of the chlorophyll that is central to the terrestrial photosynthesis (Quesada & Vincent 1997). Both strategies entail stratification of communities so that the spatial distribution of pigments has a cumulative effect of ecological significance. However, pigmentation strategies are not obligatory as some microbes have highly efficient DNA-repair mechanisms as an alternative to damage prevention (Battista 1997).

The original surface microbial colonists of early Earth could have resembled present-day green photosynthetic bacteria (e.g. *Chloroflexus*), purple sulphur bacteria (e.g. *Chromatium*) and non-purple sulphur bacteria (e.g. *Rhodopseudomonas*) (Blankenship 1992). However, their restriction to anoxic habitats and relatively inefficient anaerobic metabolism dependent on redox gradients for energy would have limited their potential for colonization. The evolution of their oxygenic photosynthesis enabled cyanobacteria to colonize any illuminated habitats where free water was available as an electron donor by photolysis. Pigmentation was therefore a fundamental aspect of the evolution of life on Earth as it resulted in an aerobic atmosphere with all the metabolic potential of energy-efficient aerobic respiration to complete the carbon cycle. The uniformity of solar radiation would have resulted in a relatively homogeneous distribution of pigmented microbes in Martian palaeolakes, so that a rover transect of $\sim$ 1 km would result in a statistically good chance of hitting a detectable layer of residual biomolecules buried beneath the surface oxidized zone. Isoprenoid derivatives of carotenoids are found in the Cambrian cyanobacterial fossil record (Wang 1998), confirming their antiquity. Porphyrins derived from chlorophyll are also common in ancient sediments over 145 Ma (Huseby *et al.* 1996).

To enable them to absorb photons, pigments have characteristic functional moieties, such as the common aromatic ring component of scytonemin in cyanobacteria and parietin of epilithic lichen pigments (Edwards *et al.* 1998, 1999). These moieties are in a defined vibrational state that responds to the input of laser light by scattering the radiation at shifted wavelengths relative to the predominant Rayleigh scattering of the incident wavelength. This response, described as Raman scattering, results in a spectrum of vibrational bands that is unique to a given compound by virtue of its components and bonding. After calibration against the components of known pigments, Raman spectroscopy can therefore characterize *in situ* the molecular components of pigments and other significant organic and inorganic constituents of a microbial community within its lithic habitat without prior knowledge of their identity (Wang *et al.* 1998; Wynn-Williams & Edwards 2000). Examples of these spectra obtained *in situ* to date include cyanobacteria, algae and fungi within Antarctic endolithic sandstone communities (Russell *et al.* 1998) and their epilithic UV-exposed neighbours (Edwards *et al.* 1998; Holder *et al.* 2000).

This paper will address the occurrence and spatial distribution of pigmentation necessary for photosynthesis UV protection in extreme Antarctic habitats with implications for

Mars analogues. We present, in an ecological context, the Raman spectra and absorption peaks of key microbial pigments concerned with these processes on Earth and potentially on early Mars, as revealed by its biochemical fossil record.

Materials and methods

Lichen and cyanobacterial samples were collected primarily in Antarctic deserts but were compared with material collected elsewhere.

Material from the McMurdo Dry Valleys region, Ross dependency

Endolithic microbial communities

Beacon sandstone was sampled in 1982–83 and 1995–96 for cryptoendolithic communities at six diverse sites in Victoria Land as described in Russell *et al.* (1998). Original data for these sites are compared with reported data for the Linnaeus Terrace site, where intensive research on this habitat has been conducted by Friedmann and others (Friedmann 1982; Nienow & Friedmann 1993). The present study included sites at Beacon Heights overlooking the head of Taylor Valley (Friedmann 1982) and Battleship Promontory in the Convoy Range (Friedmann *et al.* 1988). Endolithic communities were also sampled from Beacon sandstone outcrops on a plateau at Timber Peak overlooking the Priestley Glacier in central Victoria Land. These three sites were compared with sandstone outcrops on Mt Mackintosh, one of three locations where cryptoendolithic communities were not evident despite prolonged searching. The geology of Beacon Heights is described and mapped in McElroy & Rose (1987), and that of Battleship Promontory is described and mapped in Pocknall *et al.* (1994). From each site, four samples were analysed for structural microbial and organic content.

Crustose lichens from Victoria Land

Xanthoria elegans (Link) Th. Fr. is a bright orange–red crustose lichen obtained from a sheltered east-facing coastal slope ($\sim$ 100 m asl) of Harrow Peaks, Victoria Land, Antarctica, and was collected in 1995–96. Harrow Peaks (74° 06′ S, 164° 51′ E) consist of Granite Harbour Intrusives comprising two-mica granite and tonalite. The exposed granodiorite outcrops sampled for lichens faced ENE at 100 m altitude $\sim$ 0.5 km from the coast of Wood Bay. Crustose yellow-pigmented thalli of *Acarospora chlorophana* (Wahlenb.) Massal. were collected at Football Saddle (72° 31′ S, 169° 46′ E), northern Victoria Land, Antarctica in 1995–96. The substratum at Football Saddle consists of McMurdo volcanics comprising alkalibasalt, trachyandesite and phonolite from the late Cenozoic–Quaternary. The lichens were encrusting a pitted south-facing gentle slope ($< 10°$) that was at $\sim$ 800 m altitude and $\sim$ 10 km from the coast. The temperature at nearby Cape Hallett (72° 19′ S, 170° E) ranged from -28 °C in winter to $+5.6°$ in summer, with a mean annual temperature of -15 °C (Campbell & Claridge 1987). At this altitude and distance from the coast with receipt of katabatic winds down the Tucker Glacier, the annual precipitation would have been nearer the 45 mm water equivalent recorded for the Vanda Station (77° 14′ S) dry valley site than the 120 mm recorded at the exposed coastal site of Cape Hallett (Campbell & Claridge 1987). The Antarctic material was stored under ambient conditions and transported at -20 °C without pre-drying.

Scytonemin-containing cyanobacterial communities

Pure scytonemin (Prouteau *et al.* 1993) was extracted from natural samples of intertidal cyanobacterial mats, dominated by *Lyngbya* cf. *aestuarii* by Dr F. Garcia-Pichel (Edwards *et al.* 1999). Dried mats were ground under liquid nitrogen in a mortar and extracted with ethyl acetate. Scytonemin was purified by precipitation according to the 'bulk procedure' of Garcia-Pichel & Castenholz (1991), and additionally by semi-preparative thin-layer chromatography. Purity was checked spectrophotometrically and by TLC co-chromatography against a standard. To determine whether key Raman peaks attributable to scytonemin could be detected in nature, samples from the following cyanobacterial communities were analysed, including a cyanobacteria-dominated sandy soil crust ('black crust type') containing surface populations of scytonemin-producing *Nostoc* and *Scytonema* spp. (Garcia-Pichel & Belnap 1996) from aridlands in Arches National Park, Moab, UT, USA. The bulk areal concentrations of scytonemin were determined by standard methods to be 335 mg m^{-2}, whilst the chlorophyll$_a$ concentration was 32 mg m^{-2}.

Raman spectroscopy

Raman spectroscopy depends on the scattering of laser radiation at a wavelength (λ) shifted from that of the incident light by its interaction with the molecular vibrations and rotations in the constituents of an untreated sample. The shift is expressed as the equivalent wavenumber (cm^{-1}), which is $1/\lambda$. The Raman spectrum of a given compound consists of a unique fingerprint of all its components. Characteristic corroborative groups of bands in this spectrum can be used to identify the compound in a mixed sample. Near-infrared (IR, 1064 nm) excitation is optimal for minimizing interference by autofluorescence of pigments, but typically requires an interferometer to analyse the spectra. However, the development of an InGaAs detector now permits the miniaturization of our Raman spectrometer for fieldwork and planetary missions whilst retaining the optimal 1064 nm laser wavelength. This makes the technique eminently suitable for the remote characterization of biomolecules at planetary surfaces (Wynn-Williams & Edwards 2000).

Laboratory FT Raman spectrometer system

For laboratory studies of Antarctic lithic samples and a Mars meteorite, spectra were recorded using a Fourier transform Bruker IFS66 (Bruker IR Analytische GmbH, Karlsruhe) instrument and FRA 106 Raman module attachment with 350 mW Nd/YAG laser excitation at 1064 nm and a liquid-nitrogen-cooled germanium detector. For spectroscopy of rock profiles and surface crusts, the instrument was coupled via a TV camera to a Raman microscope with a $\times 40$ objective

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Table 1. *Evolution of early terrestrial habitats*

Time period (Ga)	Global oxygen & ozone	Incident surface UV	Redox environment	Early planetary habitat
< 4.2	None	A, B and C	Volcanic	Hadean:
> 4.2	None	A, B and C	Anaerobic origins	High pH, deep water/geothermal mud
< 4.0	None	A, B and C	Anaerobic, biotic	Deep water/geothermal mud
< 3.8	Minimal	A, B, some C	Anaerobic; Locally oxygenic	Mud subsurface stromatolites
< 3.8	None	A, B, some C	Micro-aerophilic	Mud subsurface
< 3.1	O_2 & O_3	A and B	Aerobic. Freedom from anaerobic mud	Mud surface mats Stromatolites Endoliths
< 2.2	O_2 & O_3	A and B	Aerobic. Anywhere wet and illuminated	Mud surface mats Water column Porous rock
< 1.5	O_2 & O_3	A and B	Aerobic. Ozone layer/hole emergence	Rock surface Rock profile Soil biofilms Evaporites

Table 2. *Probable early microbial colonists of the Earth*

Time period (Ga)	Early planetary habitat	Primary colonists	Present-day microbial examples
	Hadean		
< 4.2	Deep water/geothermal mud	Pre-biotic	NA
	Precambrian		
< 3.8	Deep water/geothermal mud	Thermophilic chemo-lithotrophic bacteria	*Pyrolobus*
	Mud subsurface	Green photosynthetic bacteria	*Chloroflexus* *Primaevofilum*
	Mud subsurface	Photosynthetic purple sulphur bacteria	*Chromatium*
	Mud subsurface stromatolites	Proto-cyanobacteria	*Primaevofilum?* *Synechococcus?*
	Mud subsurface	Photosynthetic purple non-sulphur bacteria	*Rhodobacter*, *Rhodospirillum*
< 3.1	Mud surface mats Stromatolites	Cyanobacteria	*Nostoc*, *Phormidium*
< 1.5	Endoliths		*Chroococcidiopsis*
	Mud surface mats	Cyanobacteria	*Chlamydomonas*
	Water column	Algae, diatoms	*Pinnularia*
	Porous rock	Endolithic algae	*Trebouxia*
	Rock surface	Epilithic lichens	*Acarospora*
	Rock profile	Endolithic lichens	*Buellia*
	Soil biofilms	Chromogenic bacteria	*Deinococcus*
	Evaporites	Halophilic bacteria	*Haloarcula*
	Meteorites	Endoliths microbes?	*Chroococcidiopsis*
		Chromogenic bacteria?	*Deinococcus*

giving a resolution of ~ 40 μm at the sample. With objectives of higher magnification, the laser 'footprint' can be as precise as 5 μm. About 10000 scans at 4 cm^{-1} resolution were needed to obtain good spectra with wavenumbers accurate to ± 1 cm^{-1} or better. For surface samples, three replicates were scanned directly without preparation. For endolithic communities, three replicate rock samples were fractured vertically to expose the community. Fifteen individual point spectra were taken down a 10 mm profile of each sample. This gave three replicate spectra per zone. Spectra of the face of the surface crust and the zone of iron accumulation were obtained using the FT-Raman spectrometer in macroscopic mode with a sample footprint of 100 μm. Compilation spectra given in the figures later are based on the mean of the triplicate spectra per zone.

Miniature Raman spectrometer for field use and on Mars landers

The miniature 852 nm confocal microscope and Raman spectrometer (CMaRS), developed by Montana State University has a small probe head (< 100 cm^3) that houses the confocal microscope and Raman filters. The confocal microscope comprises a silicon micro-electromechanical (MEMS) bi-axial scanning mirror, precision-moulded aspheric lenses and piezoelectric focus control. Confocal imaging occurs at video rates of 30 frames/s. The light source for both confocal imaging and Raman spectroscopy is an 852 nm distributed Bragg reflector diode laser. Rayleigh-scattered light is detected to form the confocal image, while the Raman-shifted light is separated by a Raman filter set and detected with a dispersive CCD-based compact spectrometer. Spectral resolution is

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Table 3. *Potential evolution of photosynthetic and photoprotective pigmentation on Earth*

Period (Gya)	Primary microbial colonists	Photosynthetic pigmentation	Typical photoprotective pigments	Primary survival strategy
> 4.2	Pre-biotic	NA	NA	NA
< 4.2	Thermophilic chemo-lithotrophs	Infrared sensors	NA	Avoidance
< 3.8	Green photosynthetic bacteria	Bacterio-chlorophyll	Carotenoids	Avoidance
			Carotenoids	Quenching
	Purple sulphur photosynthetic bacteria	Bacterio-chlorophyll	Carotenoids	Avoidance
				Quenching
	Proto-cyanobacteria	$Chlorophyll_a$	Carotenoids	Avoidance
		Phycocyanin	Scytonemin	Quenching
	Purple non-sulphur photosynthetic bacteria		Carotenoids	Avoidance
		Bacterio-chlorophyll	Mycosporines	Quenching
3.1	Cyanobacteria	$Chlorophyll_a$	Carotenoids	Quenching
		Phycocyanin	Scytonemin	Screening
		Phycoerythrin	Mycosporines	Avoidance
2.2	Cyanobacteria	$Chlorophyll_a$	Carotenoids	Quenching
	Algae, diatoms	$Chlorophyll_a$	Carotenoids	Screening
	Endolithic algae	$Chlorophyll_a$	Carotenoids	Avoidance
	Endolithic cyanobacteria	$Chlorophyll_a$	Carotenoids	Avoidance
		Phycocyanin	Carotenoids	Avoidance
1.5	Endolithic lichens	$Chlorophyll_a$	Carotenoid, Atranorin	Quenching Quenching
	Chromogens	None	Carotenoids	All
	Halophiles	None	Carotenoids	All
	Epilithic lichens	$Chlorophyll_a$	Carotenoids, parietin, rhizocarpic acid	All

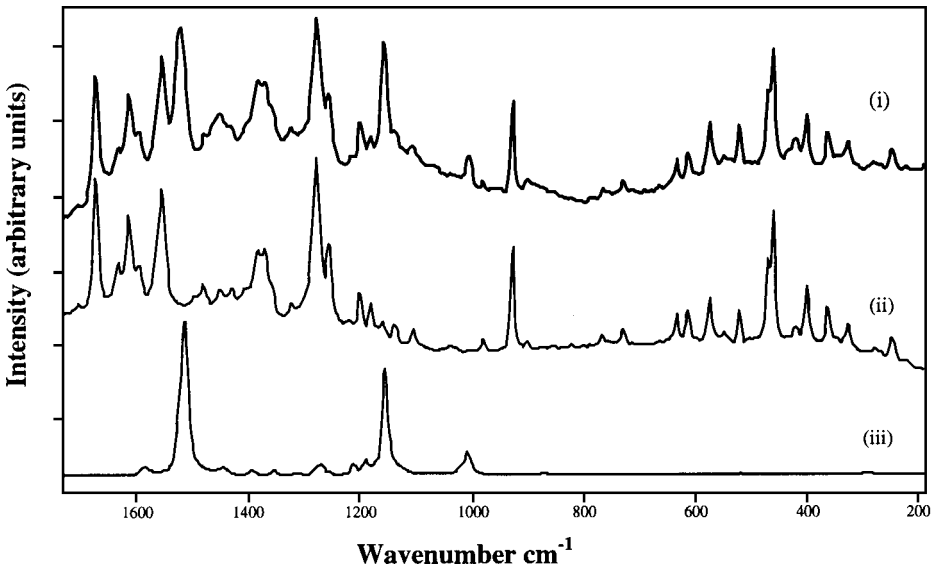

Fig. 1. FT-Raman spectra of (i) *Caloplaca saxicola*, a crustose lichen strongly pigmented with parietin and β-carotene. Collected from an exposed cliff site at Crater Cirque, northern Victoria Land, Antarctica, (ii) pure parietin, a orange-coloured UV-screening anthraquinone pigment and (iii) β-carotene, a red-coloured energy-quenching isoprenoid pigment.

8 cm^{-1} over a range from 400 to 1800 cm^{-1}. Raman spectra may be obtained over a variable field-of-view by controlling scanning in the microscope, from a minimum spot size of 1 μm to full field of 250 μm × 250 μm. The operating range for the 852 nm system is 400–1800 cm^{-1}. It will weigh less than 500 g with a volume of under 1000 cm^3, using less than 5 W of power.

The 1064 nm version of the CMaRS, with a dispersive spectrometer/indium-gallium-arsenide (InGaAs) linear diode array detector, has shown better and broader Raman spectra than the prototype 852 system with reduced fluorescence. The current prototype 1064 nm system, developed by Micron Optical Systems, Inc. of Norfolk, Virginia, has a range from 500 to 3500 cm^{-1} and a resolution of 8 cm^{-1}. It is a fixed

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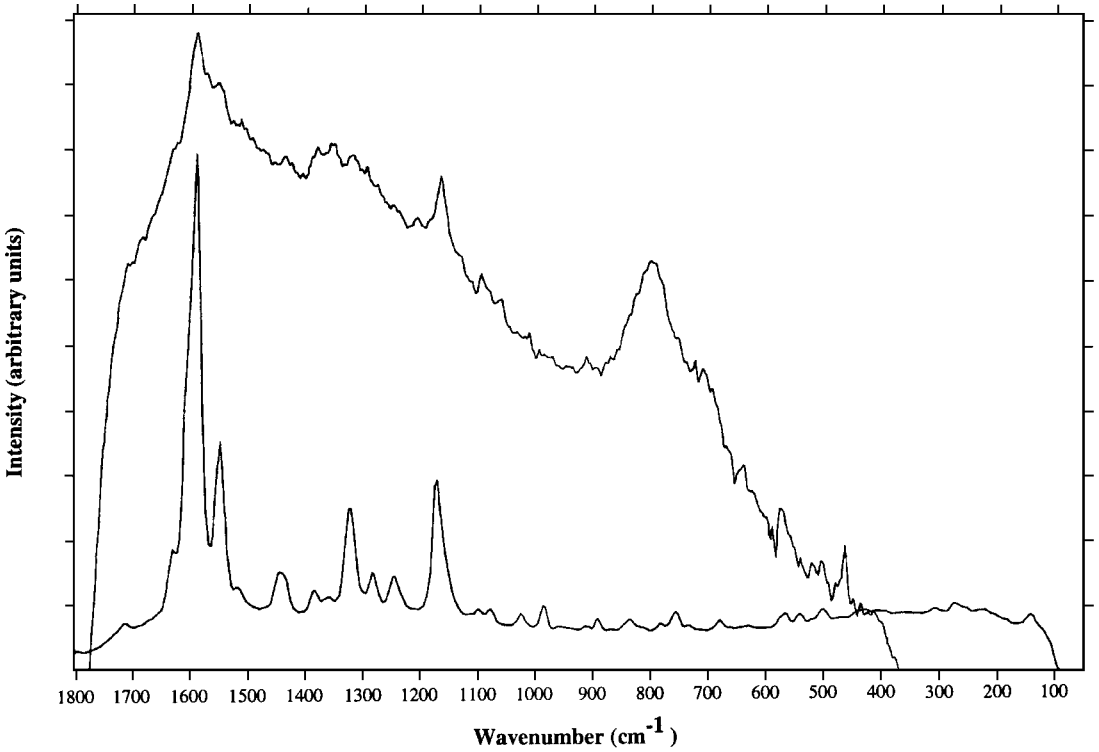

Fig. 2. Comparative FT Raman spectrum of the cyanobacterial UV-screening pigment Scytonemin (bottom) and a desert crust of the cyanobacterium *Nostoc* (top). The corroborative bands of the Scytonemin at 1590, 1549, 1323 and 1172 cm^{-1} are evident in the cyanobacterial crust (after Wynn-Williams *et al*. 1999).

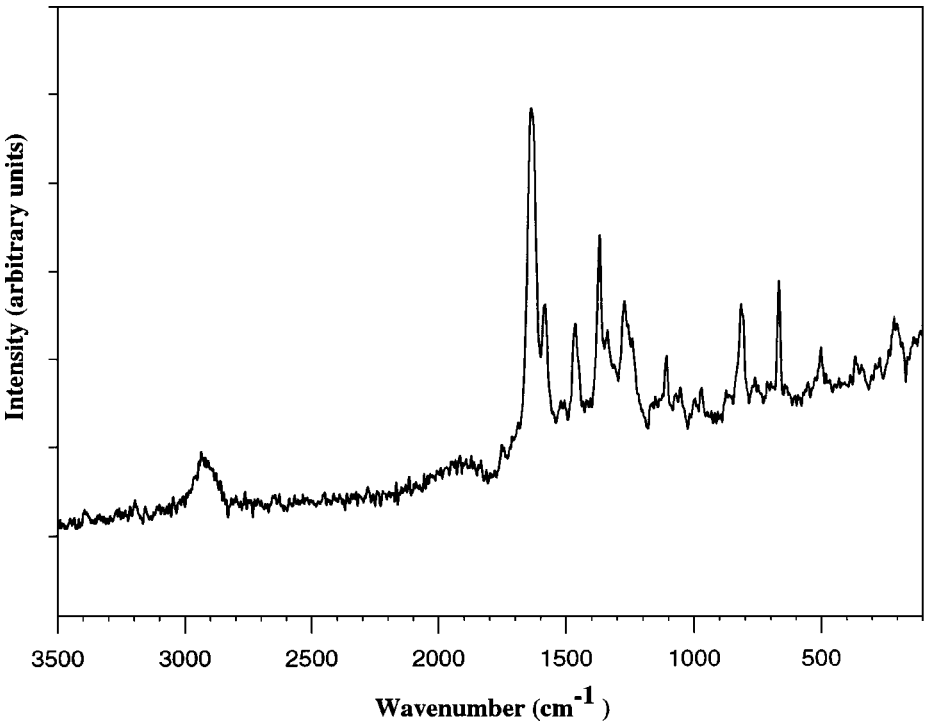

Fig. 3. FT Raman spectrum of the accessory photosynthetic pigment c-phycocyanin extracted from the cyanobacterium *Spirulina* (Sigma). See the chromophore structure in Fig. 4 and the vibrational assignments in Table 5.

grating (i.e. no moving parts), incorporating a linear axis. This linear axis provides insensitivity to temperature fluctuations as well being rugged and compact. The combination of the diode-pumped solid-state laser with the InGaAs linear diode array has a patent pending.

Absorption spectroscopy

Absorption spectra were obtained from ethanolic extracts of lichen pigments using a Pye Unicam SP 800 spectrometer in the 200–900 nm range (Holder 1998).

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Results and discussion

A synthesis of the probable evolution of surface microbial habitats on Earth is given in Table 1. This shows the gradual transition from a UVC-stressed anaerobic regime to an oxygenic environment protected from UVC but still vulnerable to UVB and UVA. Table 2 summarizes the dominant photosynthetic microbial groups capable of colonizing these habitats. There are numerous present-day examples, some of which are also reputed to be found in the fossil record, such as *Primaevofilum* (Schopf 1993). These organisms either produce screening and quenching pigments or adopt the avoidance strategy requiring accessory pigments for shade adaptation (Table 3). The diversity of pigmentation in cyanobacteria, the potential pinnacle of evolution on Mars, is summarized in Wynn-Williams *et al*. (1999) and Wynn-Williams (2000). This summary shows the commonality of carotenoids and the diversity of other pigments, which, between them, afford comprehensive UV protection.

Their Raman spectral bands can be detected *in situ* in a mixed pool of biochemicals where they act complementarily for the benefit of the whole community. Lichens are formally structured as a symbiotic system of photobiont and mycobiont, but looser communities such as algal biofilms, stromatolites and endoliths show the same mutually beneficial interactions. The use of corroborative groups of Raman bands to characterize pigments *in situ* within undisturbed field fresh material is illustrated in Fig. 1, which shows the spectra for pure β-carotene and the UV-screening pigment parietin relative to the spectrum for a whole lichen, *Caloplaca saxicola*, containing both pigments. Several strong corroborative bands for parietin are clearly visible in the *Caloplaca* spectrum,

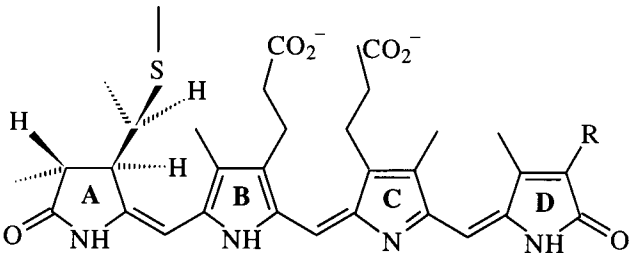

Fig. 4. Structural formula of the phycocyanobilin chromophore. R=C_2H_5 in phycocyanin and allophycocyanin (after Debreczeny *et al*. 1992).

especially in the 1520–1680, 1370–1385 and 925 cm^{-1} wavenumber range. The strongest band is for its anthraquinone component at 1672 cm^{-1}. Similarly, the strong band at 1524 cm^{-1} owing to L(C=C) stretching with corroborative bands at 1157 owing to L(C=C) stretching and 1000 cm^{-1} owing to isoprenoid (C—Ch_3) moieties show the presence of β-carotene in the lichen thallus. This combination suggests a synergistic interaction of a high concentration of a UV-screening pigment at the exposed surface as a primary protection against UV damage, with a secondary strategy of quenching the free radicals and singlet oxygen generated by UV radiation that does penetrate the thallus.

Raman spectroscopy confirmed that the underside of such crustose lichens (e.g. closely related *Xanthoria parietina* from Harrow Peaks, Antarctica) are characteristically devoid of parietin, whilst still containing an amount of β-carotene similar to the exposed upper surface (Edwards *et al*. 1998). Energy-quenching activity is needed throughout the thallus. Although its primary functions are quenching and absorbing UVA, β-carotene has a strong absorption band at 283 nm and

Table 4. *FT Raman spectral band assignments for c-phycocyanin extracted from the cyanobacterium Spirulina* (*Sigma*)

ν (cm^{-1})	Tentative assignment	Additional information
2938 mw,br	ν (*CH*)	
		1680: (C=C) A–B[a]
1638 vs	ν (*C=O*) *Amide I*, α-*helix*	1642: extended chromophores
		1625: folded chromophores on denaturation with acid[b]
1582 m,sh	ν (*C=C*) *conjugated*	1625: (C=C) D ring[a]
		1597: (C=C) D ring[b]
		1550–1600: pyrrole vibrations of B & C rings[a]
1463 m	δ (CH_2)	
1369 ms	δ (CH)	
1338 m,sh	δ (CH)	
1272 m	δ (*CNH*) *Amide III*, α-*helix*	β sub-unit bands above 1250[a]
1241 mw,sh	Amide III random coil	α sub-unit bands 1200–1250[a]
1106 mw	ν (C—N)	
1058 w	ν (C—N)	
970 w	ν (C—C)/ρ (C—H)	
815 m	ν (C—C) backbone	
666 mv	(*C—S*) *of the C—S—S—C group*	
501 mw	ν (*S—S*)	
365 w	Skeletal bending	
212 mw,br	Skeletal bending	

[a] Schneider *et al*. (1993).
[b] Debreczeny *et al*. (1992).

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Table 5. *Raman spectral bands and absorption maxima for functional microbial pigments in extreme terrestrial Antarctic habitats* (*after Wynn-Williams et al. 2001*)

Function	Pigment	Pigment type	Raman vibrational bands (wavenumber cm^{-1})					Absorption peaks (nm) UVC < 280	UVB 280–320	UVA 320–400	Visible > 400		Raman data ref. nos.
Ultraviolet	Usnic acid	**Cortical acid**	2930		1607	1322	1289	> 220	290	325	> 400		1, 2, 3
screening	Pulvinic dilactone	**Pulvinic derivative**		1672		1405		> 246	290	367			1, 2
pigments	Parietin	**Anthraquinone**		1675		1099	551	> 257	288		431	422	1, 2, 3, 4
	Calycin	**Pulvinic derivative**			1611		1379	269					1, 2
	Atranorin	***para*-depside**	2942	1666	1303	1294	1266	< 274			> 400		1, 2
	Gyrophoric acid	**Tri-depside**		1661		1290		275	304				1, 2
	Fumarprotocetraric acid	**Depsidone**		1642	1630	1290	1280	273	315				1
	Emodin	**Quinone**		1659					291		440		1
	MAA (*Nostoc*) 7437	**Mycosporine Am. Acid**	2920		1400		820		(> 310)	330			5
	Scytonemin	**8-ring dimer**		1590	1549	1323	1172	252	300	370	> 400		6, 7
Anti-oxidants	β-carotene	**Carotenoid**			1524	1155		> 246	283	384	429	451	1, 4, 8
	Rhizocarpic acid	**Isoprenoid**		1665	1620	1596		> 200					1, 2, 9
Photo-synthesis	Porphyrin	**Tetrapyrrole ring**			1453								8
	$Chlorophyll_a$ (Cyano)	**Tetrapyrrole ring**			1360	1320					680	700	2, 8
	$BChl_a$ (*Rhodopseudomonas*)	**Tetrapyrrole ring**	na								850	870	10
	Chlorobium Chl	**Tetrapyrrole ring**	na								650	660	10
Accessory													
light-harvesting	Phycocyanin	**Phycobilin**		1638	1369						560	620	11, 12
	Phycoerythrin	**Phycobilin**	na								544		

[1] Holder (1998); [2] Wynn-Williams & Edwards (2000); [3] Huneck & Yoshimura (1996); [4] Edwards *et al.* (1998); [5] Garcia-Pichel, personal communication; [6] Wynn-Williams *et al.* (1999); [7] Edwards *et al.* (1999); [8] Wynn-Williams *et al.* (2000); [9] Holder *et al.* (2000); [10] Blankenship *et al.* (1992); [11] Lee *et al.* (1994); [12] Newton, personal communication; [13] McColl (1998).

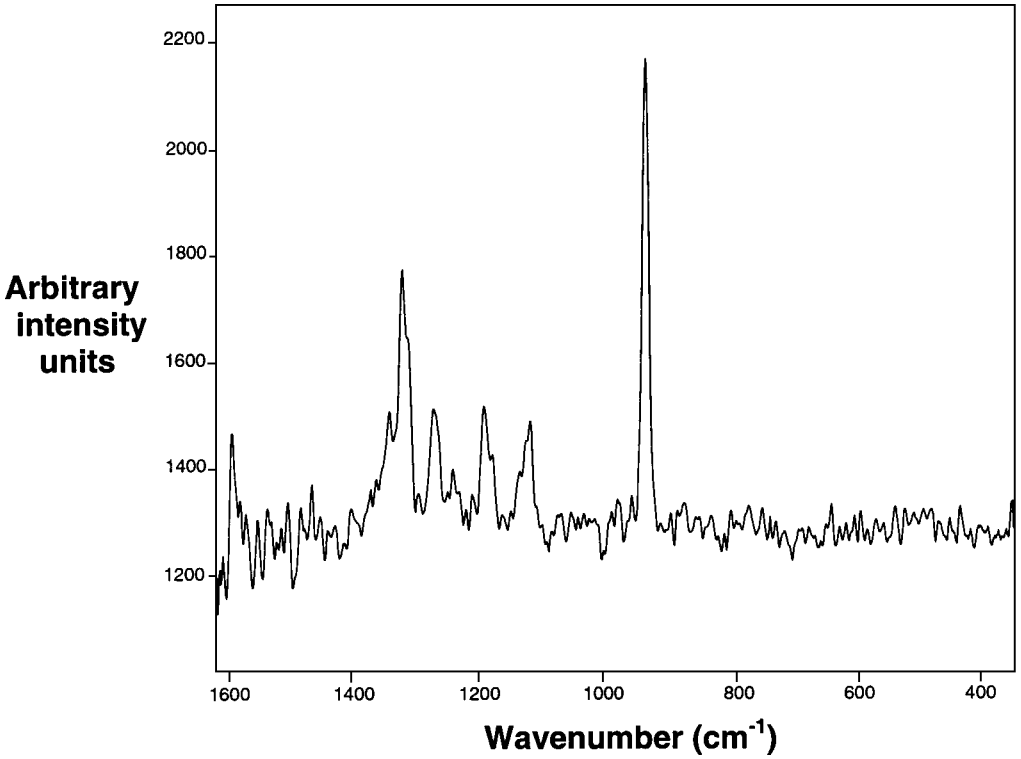

Fig. 5. Raman spectrum (excitation at 852 nm using miniature Raman spectrometer, CMaRS) for a hopanoid (sample 33105) from ancient Apex chert ($\sim$ 2.5 Gya) at Pilbara, NW Australia (sample courtesy of Dr Roger Summons; analysis by Dr David Dickensheets).

significant absorption in the UVC range (Holder 1998), which is often overlooked and is also true for cyanobacterial scytonemin (Dillon & Castenholz 1999). A similar screening strategy is evident in crusts of cyanobacteria such as *Nostoc commune*, which lives in hot and cold deserts (Wynn-Williams 2000). It synthesizes a UV-screening pigment, Scytonemin, which accumulates in the sheath of the filaments (Wynn-Williams *et al*. 1999) and which is clearly evident in Raman spectra of whole communities (Fig. 2).

Accessory pigments such as Phycocyanin also give distinctive Raman spectra (Fig. 3) because of the complexity of their chromophore components as shown by the summary of their Raman spectral assignments (Table 4) and the chromophore structure in Fig. 4. The characteristic band at $-3000\,cm^{-1}$, indicative of methyl groups in general biomolecular material, is again evident. The summary of key Raman bands for a range of screening pigments in Table 5 shows that absorption peaks are frequent in the UVC range and common in the UVB range. The Raman band for the quinone ring at $1659\,cm^{-1}$ is a frequent feature in UV protection.

The Raman spectra and absorption maxima in the visible range for primary and accessory photosynthetic pigments (Table 5) are very different. The absorption maxima for phycocyanin and phycoerythrin (560 and 544 nm, respectively) are significantly lower than those for chlorophylls ($>$ 640 nm), as befits their shade-adapted role at the blue end of the spectrum that penetrates deeper into the habitat.

The ability to detect the Raman spectrum of porphyrin derived from chlorophyll (Table 5) permits analysis, not only of present-day pigmentation but also of fossil strata, as potentially found in palaeolakes (Doran *et al*. 1998). This indicates the presence of photosynthetic microbes as ancient as 3.5 Gya (Blankenship 1992) and their hopanoids 2.5 Gya (Fig. 5) in stromatolites.

Conclusions

We have shown that laser Raman spectroscopy can diagnose pigments *in situ* without disrupting the spatial integrity of structured microbial communities or denaturing their biomolecules. By relating these diagnoses to position in the community, we can interpret their role in screening UVA, UVB and UVC or for harnessing PAR at low light levels (Nienow *et al*. 1988). From Mars Orbital Laser Altimetry (MOLA, Smith *et al*. 1999) and Mars Orbiter Camera imagery (MOC, Malin *et al*. 1998), we have evidence of former lacustrine habitats, river beds and even surface drainage channels (Malin & Edgett 2000a, b). There may have even been a northern ocean in Vastitas Borealis (Head *et al*. 1999) with a substantial shallow periphery capable of supporting cyanobacterial communities. These surface aquatic habitats would require protection from solar radiation damage. If microbial evolution started on Mars, there could be basic biochemical structures available from initial organic chemicals which lead

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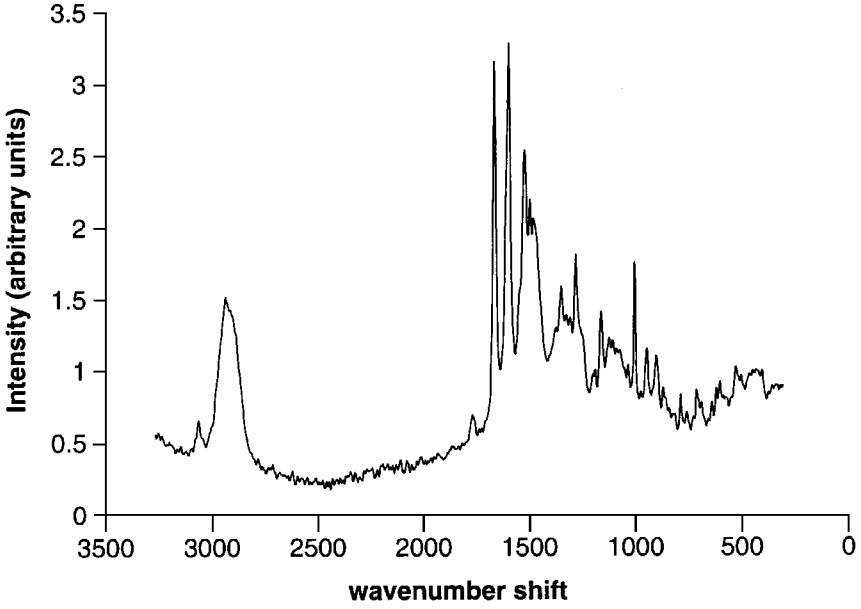

Fig. 6. Raman spectrum of the lichen *Acarospora chlorophana* using a miniature 1064 nm laser Raman spectrometer (CMaRS) with an InGaAs detector, destined for a future Mars lander mission.

to the development of pigments that would make good biomarkers for miniature Raman spectroscopy (Fig. 6) on the surface of Mars in a future lander mission (Dickensheets *et al*. 2000).

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