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Functional biomolecules of Antarctic stromatolitic and endolithic cyanobacterial communities

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For activity and survival in extreme terrestrial Antarctic habitats, lithobiontic cyanobacteria depend on key biomolecules for protection against environmental stress and for optimization of growth conditions. Their ability to synthesize such molecules is central to their pioneering characteristics and major role as primary producers in Antarctic desert habitats. Pigmentation is especially important in protecting them against enhanced UVB damage during stratospheric ozone depletion (the Ozone Hole) during the Antarctic spring and subsequent photoinhibition in the intense insolation of the summer. To be effective, especially for the screening of highly shade-adapted photosystems of cyanobacteria, protective pigments need to be located strategically. Antarctic lithic cyanobacterial communities are therefore stratified, as in soil biofilms of Alexander Island, the benthic stromatolitic mats of ice-covered hypersaline lakes in the McMurdo Dry Valleys, and the endolithic communities within translucent Beacon sandstone outcrops of Victoria Land. The protective pigments include scytonemin, carotenoids, anthroquinones and mycosporine-like amino acids. To detect and locate photoprotective pigments *in situ* in free-living cyanobacteria and cyanolichens from hot and cold desert habitats, we have used Fourier-transform Raman micro-spectroscopy. With appropriate power inputs for labile molecules, this high-precision, non-intrusive laser-based technique can not only identify biomolecules in their natural state but also locate them spatially within the habitat relative to the components of the community, which require protection. In conjunction with direct and epifluorescence microscopy it provides a spatial and functional description of the protective strategy of a community. We present the unique Raman spectrum of scytonemin and use its primary and corroborative peaks to identify it within the plethora of other biochemical constituents of several natural cyanobacterial communities, including an Antarctic endolith. The remote-sensing aspect of this technique makes it suitable not only for spatial biochemical analysis of present and palaeontological Antarctic communities but also for analogous putative habitats on Mars.

Key words: Antarctic, cyanobacteria, endolithic, lichens, lithic, pigments, Raman, scytonemin, spectroscopy, ultraviolet

Introduction

A major challenge for studies of the microbial ecology of cyanobacterial communities is to identify and quantify *in situ* the functional molecules which protect them from the environmental stresses they tolerate as part of their pioneering characteristics. Although molecules which protect against photoinhibition (e.g. carotenoids) and UV photodamage (e.g. scytonemin) can be extracted from the community with solvents for quantification (Downes *et al.*, 1993), to determine their small-scale distribution within the functional stratification of the light-dependent community requires specialized and tedious procedures. The terrestrial Antarctic contains a variety of cyanobacteria-dominated communities. Cyanobacteria not only form biofilms on the soil surface (Wynn-Williams, 1996) but also form benthic mats in Antarctic Dry Valley streams (Vincent *et al.*, 1993), stromatolitic mats in hypersaline ice-covered lakes (Wharton, 1994) and cyanobacterial zones in endolithic microbial communities inside translucent Beacon sandstone (Friedmann & Ocampo-Friedmann, 1988). They also enter symbiotic associations resulting in cyanolichens. Although these are not found south of Mars Oasis at 71°52′S on Alexander Island (R. I. Lewis-Smith, personal communication) several lichen species are common on the Antarctic Peninsula and adjacent islands (Redon, 1985; Kappen, 1993). *Nostoc* spp. are the most common cyanobionts, but members of the genera *Scytonema*, *Gloeocapsa* and *Stigonema* occur as well. The physical microenvironment of cyanobacteria in lithic habitats, whether free-living or symbiotic, enables them to optimize their metabolic capacity whilst minimizing the effects of extreme environmental stress, especially the effects of photoinhibition and enhanced UVB during the spring depletion of stratospheric ozone (Farman *et al.*, 1985; Wynn-Williams, 1994).

Cyanobacteria are characteristically pioneer micro-organisms which drive microbial ecosystems by their photosynthetic primary productivity. However, their dependence on solar irradiance restricts them to near-

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surface habitats and renders them vulnerable to a broad range of environmental stresses (Wynn-Williams, 1999*b*). These include UVB photodamage (Garcia-Pichel & Castenholz, 1994), photo-inhibition (Donkor & Häder, 1996), desiccation (Potts, 1994), extremes of high and low temperature often straddling freezing point (Melick & Seppelt, 1994), high osmotic stress in surface evaporites (Grant *et al*., 1998) and inorganic oligotrophy (Wainwright *et al*., 1993). Tolerance of these stresses may depend on the protective effect of strategically located key biomolecules such as photoprotectants, accessory pigments, water-replacement molecules, compatible solutes and acids or chelators to mobilize inorganic nutrients (Edwards *et al*., 1997). The spatial location of photoprotectants in the moribund upper matrix of cyanobacterial mats is often vital for the survival of highly shade-adapted organisms underneath (Vincent *et al*., 1993), especially during UVB enhancement within the Ozone Hole (Häder, 1993).

There is therefore a need to analyse the distribution of key molecules in undisturbed field-fresh material, not only to detect and quantify them but also to locate them. It is also of fundamental micro-ecological importance to be able to characterize these molecules in hydrated field conditions *in situ* while they are still associated with biological ultrastructures such as proteins (e.g. phycobiliproteins), membranes and thylakoids. Their conformational state and degree of hydration may be of biochemical importance, as in the two distinct hydration states of calcium oxalate found in desiccating lichens (Russell *et al*., 1998; Wynn-Williams *et al*., 1997). Although pigments can occasionally be solvent-extracted from lichens without incurring serious damage (Solhaug & Gauslaa, 1996), their study outside the community potentially loses important ultrastructural and biochemical information. A powerful technique offering microscale resolution is epifluorescence microscopy (EFM) combined with image analysis. It can be used to show the distribution of autofluorescent photopigments, such as the light-harvesting phycobiliprotein, phycocyanin, by using selective excitation wavelengths (green light at 560 nm to stimulate fluorescence at 655 nm: Wynn-Williams, 1988). However, this approach cannot be used quantitatively nor confirm molecular configuration. Neither can EFM be used for calcium oxalate, a non-fluorescent compound, which is functionally important in the bioweathering of rock and modifies the microbial habitat for endolithic symbioses (Edwards *et al*., 1997).

Laser-based Fourier-transform Raman spectroscopy (FTRS) does meet these requirements and has been applied successfully to stratified Antarctic endolithic microbial communities (Russell *et al*., 1998) and differential pigment distribution in epilithic lichens (Edwards *et al*., 1998). Each molecule is identifiable because its Raman spectrum is the unique synthesis of the vibrational states of all the interatomic bonds constituting a specific molecule, so that they scatter light at specific wavelengths other than that of the incident laser (Williams & Batchelder, 1994). Even the different hydration state of calcium oxalate monohydrate and dihydrate results in distinctly different Raman spectra (Wynn-Williams *et al*., 1997). The usually complex structure of pigments, such as the anthraquinone parietin, gives them distinct Raman spectra with key diagnostic peaks (Edwards *et al*., 1998). These are recognizable not only in pure samples but also amongst all the other compounds that constitute a cell, a community, or even the inorganic habitat in which they are situated.

This paper focuses on a key photoprotective pigment for cyanobacteria in UV-stressed lithic habitats – scytonemin. We show how the Raman spectrum for the pure compound can be used to detect and locate the molecule in field-fresh cyanobacteria *in situ* in a wide range of habitats.

Materials and methods

Scytonemin purification

Pure scytonemin (Prouteau *et al*., 1993) was obtained from natural samples of intertidal cyanobacterial mats, dominated by *Lyngbya* cf. *aestuarii*. 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.

Field-collected samples

To determine whether key Raman peaks attributable to scytonemin could be detected in nature, samples from the following cyanobacterial communities were analysed. (1) An upper intertidal cyanobacterial mat dominated by *Lyngbya* cf. *aestuarii* from subtropical Laguna Ojo de Liebre, Guerrero Negro, Baja California Sur, Mexico. This mat is known to contain very high amounts of scytonemin, the bulk of which is found in the upper 250 μm (Garcia-Pichel & Castenholz, 1991). (2) A cyanobacterially 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, Utah, USA. The bulk areal concentrations of scytonemin had been determined by standard methods to be 335 mg m^{-2}. Those of chlorophyll *a* were 32 mg m^{-2}. (3) A soil lichen of the genus *Collema*, containing *Nostoc* sp. as a photobiont, from the same location as sample 2, containing typically 1.34 mg of scytonemin g^{-1} dry lichen biomass (Büdel *et al*., 1997). (4) A cyanobacterial culture belonging to the genus *Chlorogloeopsis* (strain O-89-Cgs(1)). This was isolated from a walkway pavement in Eugene, Oregon, USA, and grown in the laboratory with or without exposure to UVA radiation and therefore containing either 15 mg of scytonemin g^{-1} dry biomass, or un-

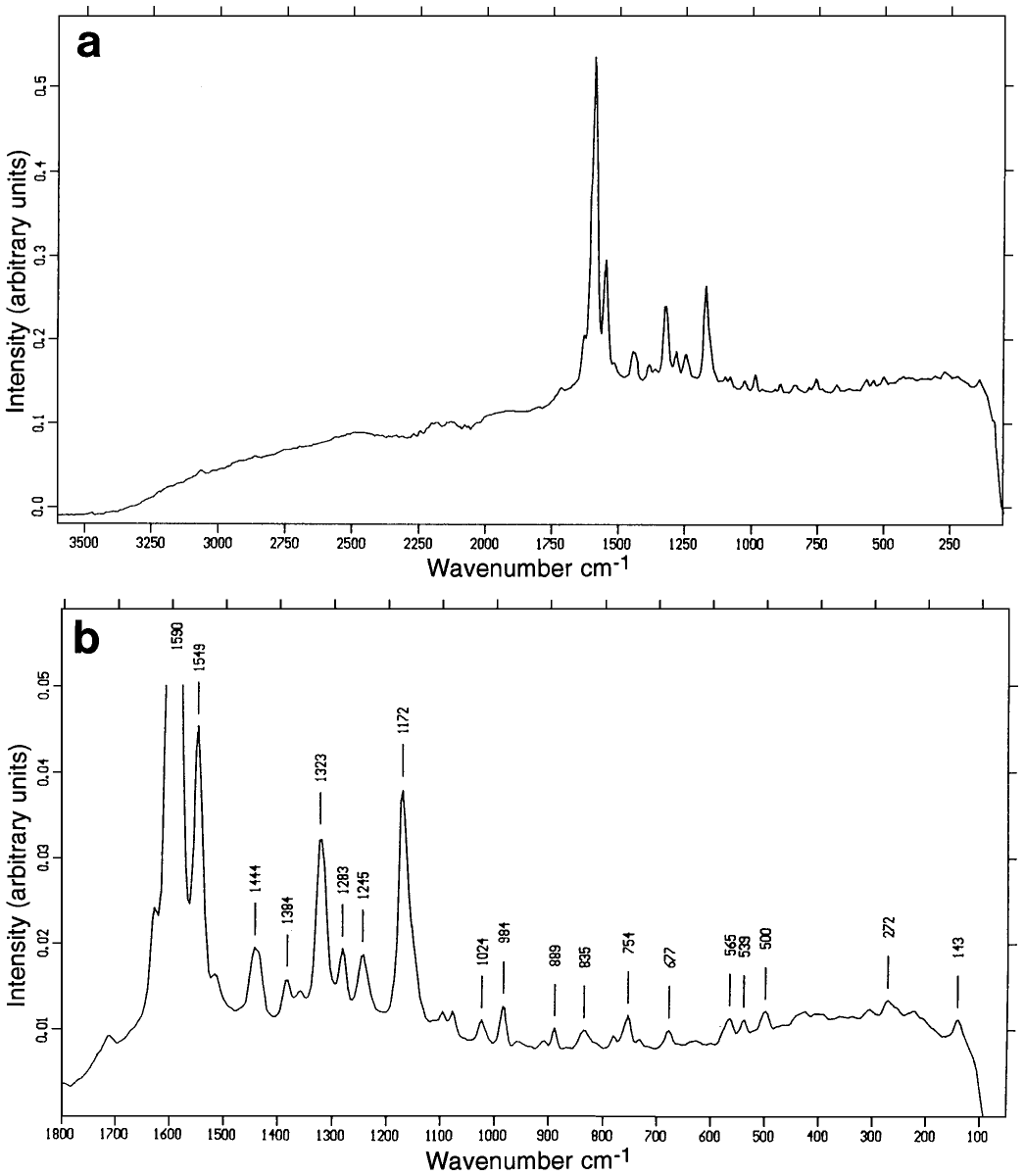


Fig. 1. (*a*) FT-Raman spectrum (1064 nm excitation) of pure scytonemin (oxidized form) in the wavenumber range 100–3500 cm^{-1}, at low sensitivity. (*b*) FT-Raman spectrum of pure scytonemin (oxidized form) in the range 100–1800 cm^{-1}, at higher sensitivity showing a strong resonance at 1590 cm^{-1} and weaker but distinct resonances at 1549, 1323 and 1172 cm^{-1}.

detectable amounts of scytonemin (Garcia-Pichel *et al.*, 1992). (5) A cryptoendolithic cyanobacterial community in Beacon sandstone from Battleship Promontory (76°54′S, 160°57′E) in the Convoy Range, southern Victoria Land, Antarctica (Friedmann *et al.*, 1988). The geology of this region is described and mapped in Pocknall *et al.* (1994) and is compared with other endolithic microbial habitats in Russell *et al.* (1998). The site is a sheltered north-facing plateau at 2000 m asl, *c.* 8 km from the polar plateau. The grain composition of the quartz-based sandstone, as determined by image analysis of gently crushed samples, was as follows. Size distribution (mean diameter, $n = 36$): 82% < 250 μm, 17% at 250–500 μm and 1% at < 500 μm. The translucent sandstone, stored in the dark at −20 °C since sampling in 1995, was fractured vertically to expose the crypto-endolithic community for Raman spectroscopy. The presence of scytonemin in this community had not been determined by alternative methods. All the samples were in a naturally dry state, although this is not a requirement for the technique.

Principles and application of Fourier-transform Raman spectoscopy

Fourier-transform Raman spectroscopy (FTRS) is based on the scattering of monochromatic laser radiation by its interaction with the molecular vibrations and rotations in the constituents of an untreated sample. The monochromatic 1064 nm near-infrared laser beam is focussed to the required 'footprint', which can be as small as 5 μm. Elastic collisions between the photons and the organic and inorganic compounds comprising the field sample result in radiation scattered mostly at the incident frequency

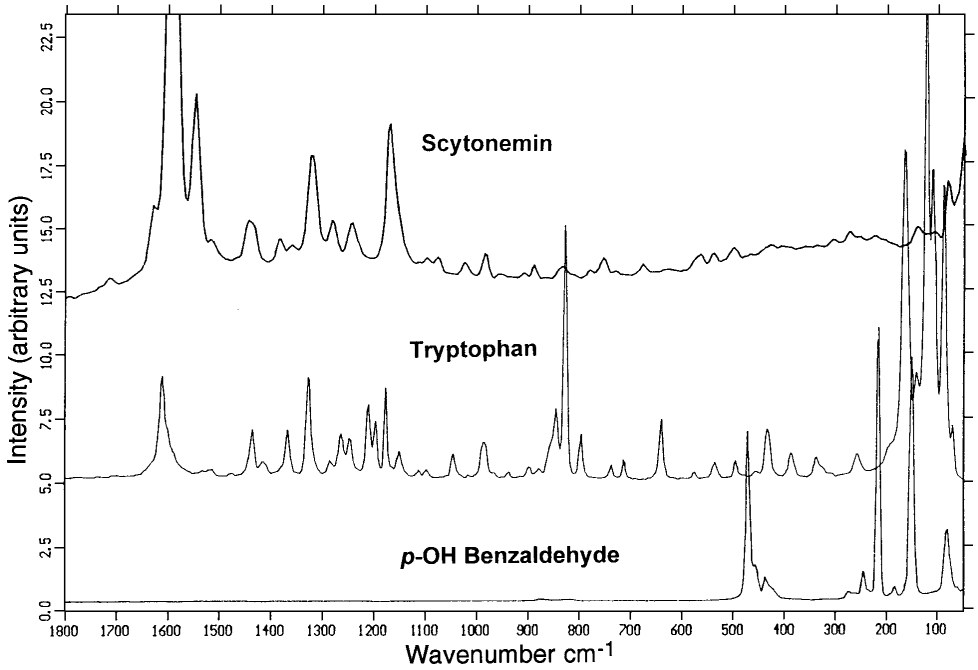


Fig. 2. Comparative FT-Raman spectra in the range 100–1800 cm^{-1}, of pure scytonemin and its moieties tryptophan and *p*-hydroxybenzaldehyde.

(Rayleigh scattering). However, concurrent inelastic collisions resulting from vibrational transitions of chemical bonds in the compounds produce a small fraction of the scattered radiation with shifted frequencies. Spectral lines which are shifted to energies lower than that of the laser source (Stokes lines) are produced by ground-state molecules, whilst lines at higher frequencies (anti-Stokes lines) are due to molecules in higher excited vibrational states. In Raman spectroscopy, the frequencies (Hz) of light of different wavelengths (λ) are traditionally expressed as the equivalent wavenumber (cm^{-1}). This is the number of waves per centimetre path in a vacuum (*c.* 15 000 cm^{-1} for red light) and numerically is $1/\lambda$. To present the Raman spectrum, the Rayleigh scatter line at the excitation wavelength is standardized to a wavenumber = 0. The Raman spectrum of a given compound consists of a unique fingerprint of all its atoms, groups and bonds and their interactive effect (stretching, deformation, rotating) on each other. Characteristic corroborative groups of peaks from this spectrum can be used to identify the compound amongst others in a mixed sample.

Spectra were recorded using a Fourier-Transform Bruker IFS66 (Bruker IR Analytische, Karlsruhe, Germany) 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 this was coupled via a TV camera to a Raman microscope with a $\times 40$ objective giving a resolution of *c.* 40 μm at the sample. About 10 000 scans at 4 cm^{-1} resolution were needed to obtain good spectra with wavenumbers accurate to ± 1 cm^{-1} or better.

For the surface crust communities and mats, three replicate samples were scanned directly without preparation. For the endolithic community from Battleship Promontory, 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. Compilation spectra given in the figures are based on the means of the triplicate spectra per zone. The objective of the Raman analyses presented here is to detect the presence of target biomolecules and their relative proportions and spatial distribution *in situ* rather than their absolute concentrations, determination of which requires calibration – a technique under development.

Results

The unique Raman spectrum of pure scytonemin (Fig. 1*a*, *b*) shows a distinctive high peak at a wavenumber of 1590 cm^{-1} with clear but lower intensity peaks at 1549, 1323 and 1172 cm^{-1}. The peaks at 1590 and 1172 cm^{-1} are the main diagnostic peaks but all are corroborative. Comparative spectra for tryptophan and *p*-hydroxybenzaldehyde, which are model moieties of the scytonemin molecule, show different patterns of peaks (Fig. 2), illustrating the uniqueness of each spectrum, which is not merely the sum of component parts. The conspicuous pattern of scytonemin peaks has the potential to act as a biomarker for the molecule in pure or mixed cyanobacterial communities. To test this hypothesis, a culture of *Chlorogloeopsis* and a variety of natural communities known to contain scytonemin-rich cyanobacteria were

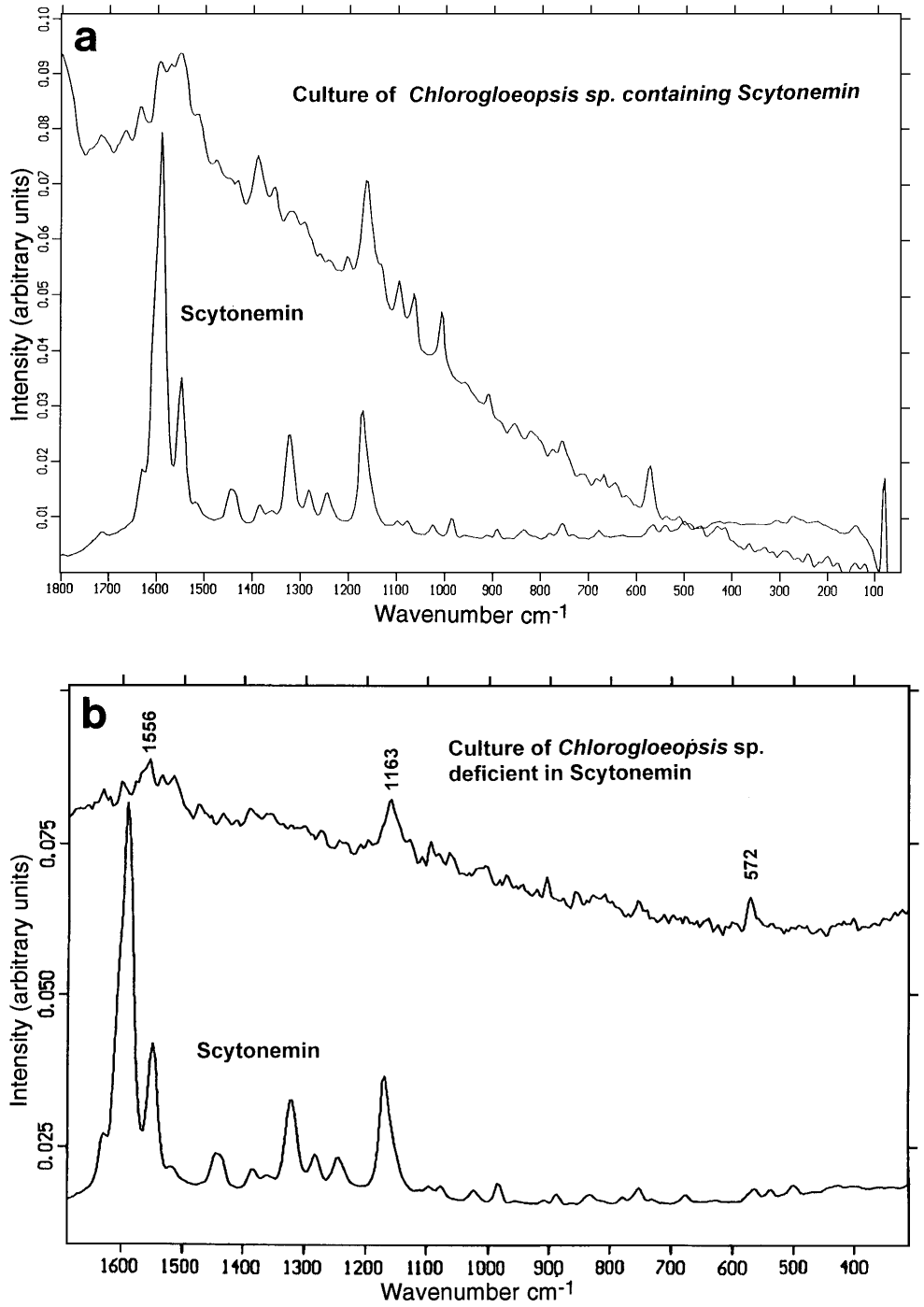


Fig. 3. (*a*) Comparative FT-Raman spectrum of pure scytonemin with the cyanobacterium *Chlorogloeopsis* containing scytonemin. (*b*) Comparative FT-Raman spectrum of pure scytonemin with the same strain of *Chlorogloeopsis* as in (*a*) but lacking scytonemin.

scanned for these peaks (Figs 3–5) relative to pure scytonemin and a strain of the same *Chlorogloeopsis* known to be deficient in scytonemin (Fig. 3*b*).

In the strain of *Chlorogloeopsis* grown under conditions promoting the synthesis of scytonemin, the 1590 cm^{-1} peak was clearly evident but was partially masked by other bands in the 1500–1550 cm^{-1} range, although the shoulder at 1549 cm^{-1} was detectable. The corroborative peak at 1172 cm^{-1} was distinct. In the control culture, lacking scytonemin, all four scytonemin resonances were absent (Fig. 3*b*). The conspicuous peaks which were present at wavenumbers of 1556, 1163 and 572 cm^{-1} were distinctly different (at a wavenumber resolution of ± 1 cm^{-1}) from those of scytonemin.

All the desert samples showed all four scytonemin peaks to a greater or lesser degree. The Raman spectrum of the intertidal cyanobacterial *Lyngbya* mat showed the primary peak at 1590 cm^{-1} corroborated by the secondary peak at 1172 cm^{-1} (Fig. 4*a*). There was also a shoulder at 1549 cm^{-1} which was further indicative of scytonemin. In the desert crust containing *Nostoc* and *Scytonema* (Fig. 4*b*) the 1590 cm^{-1} peak was distinct, with a clear 1172 cm^{-1} secondary peak.

Although the epilithic desert crust cyanolichen *Collema*

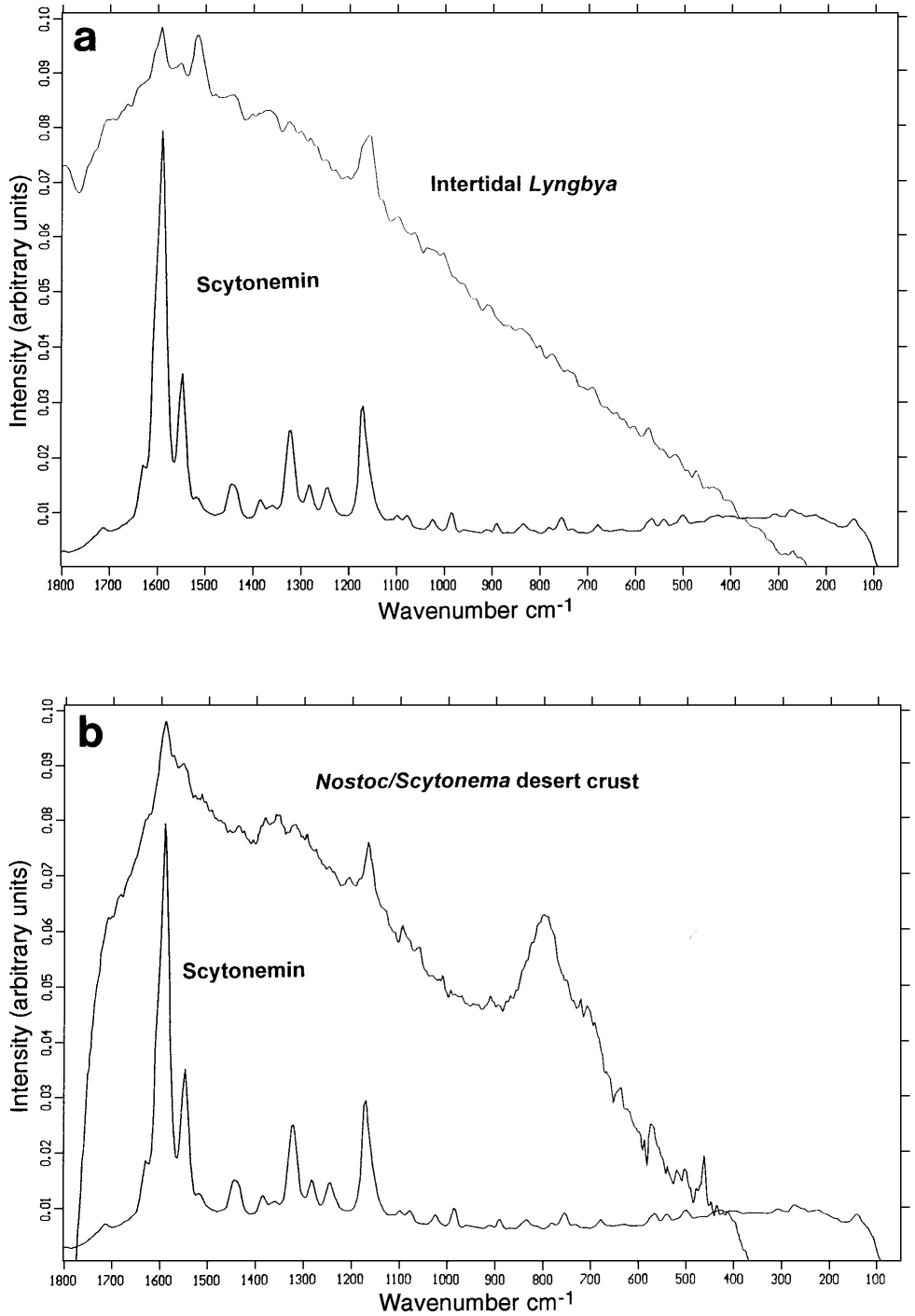


Fig. 4. (*a*) Comparative FT-Raman spectrum of pure scytonemin with an intertidal cyanobacterial mat dominated by *Lyngbya* containing scytonemin. (*b*) Comparative FT-Raman spectrum of pure scytonemin with a cyanobacterial desert crust from Utah dominated by *Nostoc* and *Scytonema* containing scytonemin.

(Fig. 5*a*) showed all the peaks, only the 1590 and 1172 cm^{-1} peaks were conspicuous. Earlier Raman analyses of endolithic lichen communities, including those at Battleship Promontory (Russell *et al.*, 1998), showed peaks which were derived from unidentified pigments. In the cyanobacterial zone of this community at *c.* 4 mm depth, an unidentified pigment peak detected at 1590 cm^{-1} (Fig. 5*b*) is consistent with scytonemin. Direct light microscopy of this stratified community showed dark melanized fungi in the lichen zone at *c.* 1 mm depth (Fig. 6). Epifluorescence microscopy with green excitation (560 nm) confirmed the presence of interstitial coccoid cyanobacteria at *c.* 4 mm depth by the autofluorescence (at 635 nm) of their phycocyanin (Fig. 7).

Discussion

We conclude that the occurrence of a corroborative pattern of a primary FT-Raman spectral peak at 1590 cm^{-1} together with a secondary peak at 1172 cm^{-1} and others at 1549 and 1323 cm^{-1} is indicative of the photoprotective pigment scytonemin in a sample, as the results were

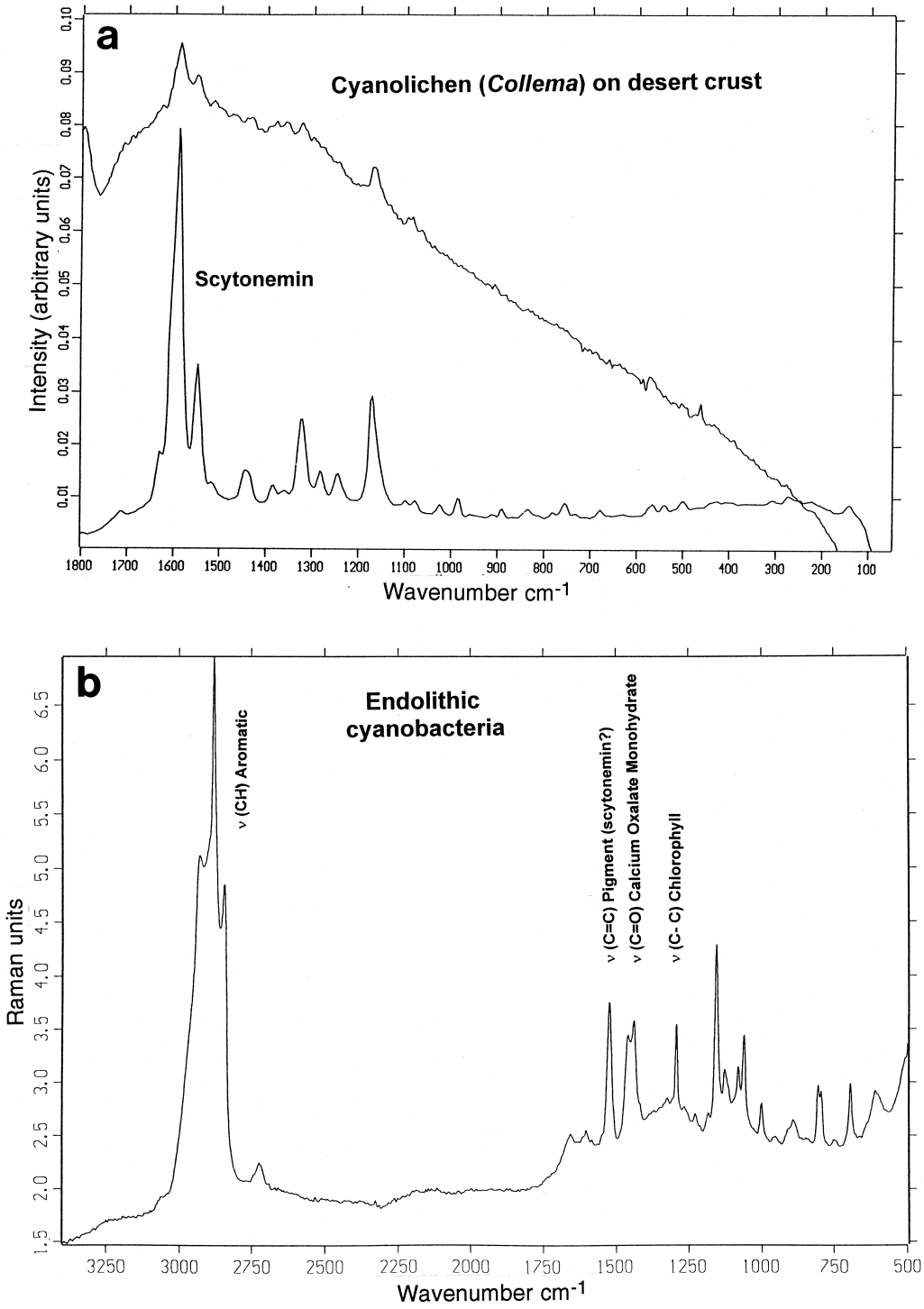
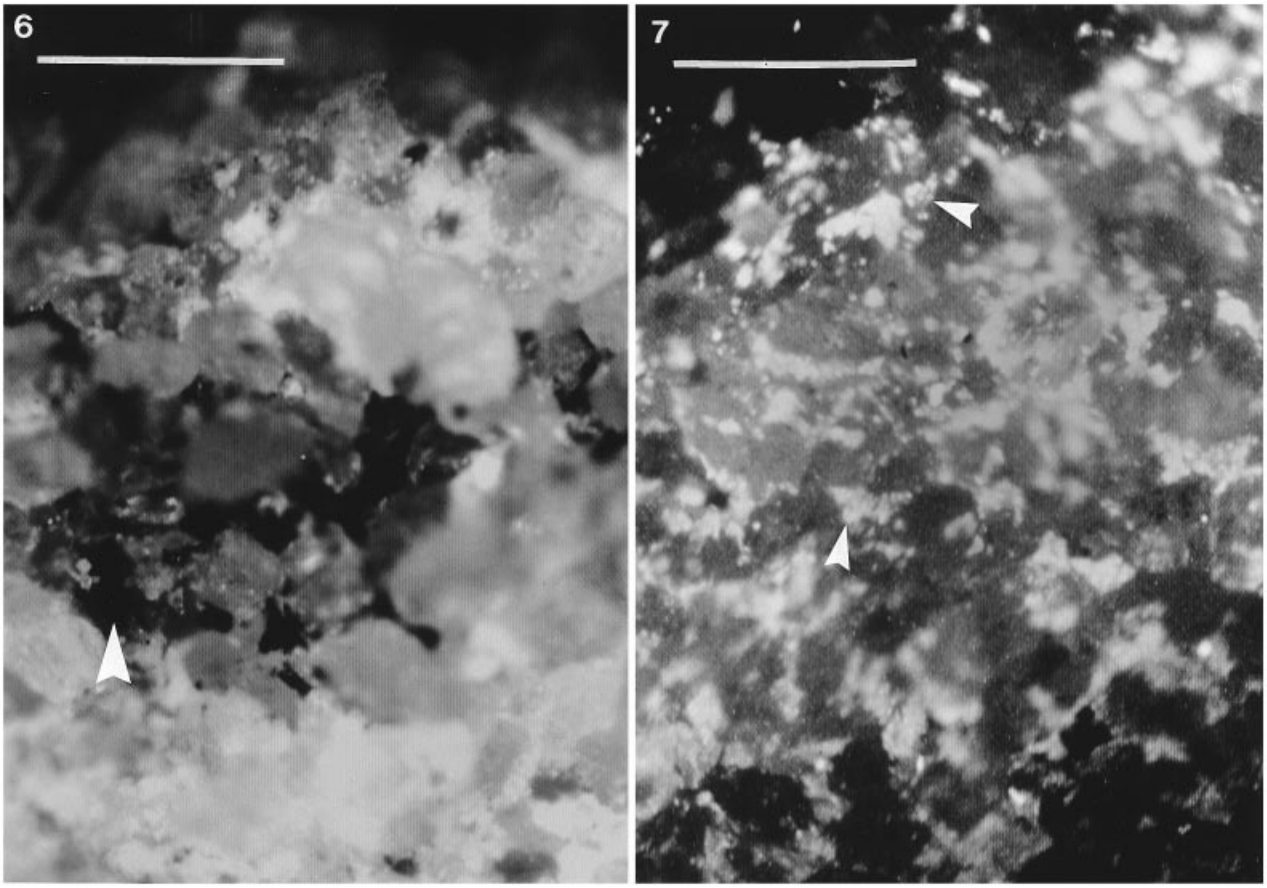


Fig. 5. (*a*) Comparative FT-Raman spectrum of pure scytonemin with *Collema*, a cyanolichen growing on a desert crust in Utah, whose cyanobiont, *Nostoc*, contains scytonemin. (*b*) FT-Raman spectrum of the cyanobacterial zone of a cryptoendolithic microbial community in Beacon sandstone from Battleship Promontory, Antarctica (after Russell *et al.*, 1998) showing a peak at 1590 cm^{-1} consistent with the presence of scytonemin.

consistent with parallel analytical determinations. FTRS has many advantages for ecophysiological studies of cyanobacteria and cyanolichens. Its non-intrusive laser-based principle makes it excellent for remote operation on large samples using fibre-optic cable probes of several metres length. As it does not require extraction with solvents or any other preparation, there is minimal disruption of the microniche. Neither surface reflectivity nor transparency is critical (translucency is adequate), and the signal can be obtained through the walls of transparent containers such as plastic corers. It would therefore be suitable for analysis of intact lake sediment stromatolites (Wharton, 1994) to detect scytonemin and other compounds indicative of environmental change such as fluctuating UVB doses (Leavitt *et al.*, 1997). As the spectrum is derived from the scattering of light rather than absorption, it is independent of path length and its depth of penetration can be selected by adjusting the laser image



Figs 6, 7. Microscopic images for computer analysis of the endolithic microbial community at Battleship Promontory. Fig. 6. Direct light illumination of dark melanized fungi (see arrow) in the lichen zone at *c.* 1 mm depth. Fig. 7. Green (560 nm) epifluorescence illumination of the cyanobacterial zone at *c.* 4 mm depth showing autofluorescence (at 635 nm) of interstitial coccoid cells *c.* 2 μm diameter (see arrows) containing phycocyanin. Scale bars represent 500 μm.

optics. This adjustment is also necessary to avoid excessive heating of dark or labile compounds by absorbed laser energy.

The laser wavelength of 1064 nm results in little or no interference from any water or ice signal (unlike infrared spectroscopy) so that it can be applied to field-fresh, viable natural samples without prior desiccation. FTRS could therefore be used to confirm the presence of cyanobionts in a field-fresh putative cyanolichen, so it has taxonomic potential. The Raman spectrum over the range 200–4000 cm^{-1} covers most vibrational modes, including metal oxides such as mineral substrata, so that the habitat itself can be described, along with organic constituents of the biota. This attribute has been used in analysis of Mars meteorites, not only for organic molecules of potential biological relevance but also to determine the inorganic crystal composition without destruction of the precious sample (Edwards *et al.*, 1999). Normally, the wavenumbers for inorganic materials, such as silica at 457 cm^{-1}, are well separated from those of n(CH) bonds near 3000 cm^{-1} which are characteristic of organic compounds.

The ability to focus the laser beam through the optics of a compound microscope permits precise selection of a microbial or stratal target, with a 'footprint' of as little as 5 μm. This can result in a spectrum from a single cell or trichome. A major advantage of the system is the analysis of biomolecules in their natural state and spatial location within membranes or adsorbed onto minerals. The unique 'fingerprints' of corroborative peaks in the Raman spectra of key functional molecules permit their detection in mixed communities and detection of their fluctuations in concentration with changing environmental stresses. Key functional molecules which have been demonstrated *in situ* include chlorophylls, carotenoids, anthroquinones and oxalates in live cells and fossil biomolecules (Edwards *et al.*, 1997; Wynn-Williams *et al.*, 1997; Russell *et al.*, 1998). The Raman bands assigned to chlorophyll are not visible in all samples because of swamping by background fluorescence of the mineral substratum. This can be decreased by concentrating the laser on a narrow footprint (5 μm, at high magnification) to obtain the signature from a single cell or cyanobacterial trichome without including its background. This refinement of the technique will be part of developing the application of FTRS to natural samples.

Scytonemin is one of a variety of pigments known to protect cyanobacteria from photoinhibition and UV radiation damage (Table 1). Carotenoids (in cyanobacteria and algae) and anthraquinones (in algae) are commonly concerned with photoprotection and the quenching of free

Table 1. Photoprotective pigments found in cyanbacteria, cyanolichens and selected Antarctic endolithic and epilithic microbial communities

Biochemical group	Typical pigment	Examples of occurrence in Antarctic botanical taxa	Mode of protection	Source
Scytonemin	Scytonemin	*Nostoc*, *Calothrix parietina*	UV screening	Garcia-Pichel & Castenholz (1991)
		Cyanolichens, e.g. *Collema* (with *Nostoc*), *Gonohymenia*, *Peltula* (with *Chroococcidiopsis*, *Cyanosarcina*, *Gloeocapsa* and *Myxosarcina*)	UV screening	Lange *et al.* (1992); Büdel *et al.* (1997)
	Gloeocapsin	*Gloeocapsa sanguinea* (Arctic)	UV screening (putative)	F. Garcia-Pichel (unpublished)
Carotenoids	β-Carotene	*Gloeocapsa*	Quenching of reactive species	Buckley & Houghton (1976)
		Nostoc commune	Light absorption	Ehling-Schulz *et al.* (1997)
		Collema (cyanolichen)	Light absorption	Henriksson (1963)
		Trebouxia (endolithic alga)	Light absorption	Culberson (1969)
	Canthaxanthin	*Peltigera praetexta* (*Nostoc* as phycobiont)	Light absorption	Leisner *et al.* (1994); Ehling-Schulz *et al.* (1997)
	β-Carotene, astaxanthin	*Trentepohlia*, *Haematococcus* (green algae)	Screening	Castenholz & Garcia-Pichel (1999)
	Echinenone	*Nostoc commune*	Light absorption	Ehling-Schulz *et al.* (1997)
		Halophilic cyanobacterial mats	Light absorption	Oren *et al.* (1995)
Xanthophylls	Myxoxanthophyll	*Nostoc commune*	Free-radical quenching	Ehling-Schulz *et al.* (1997)
		Chroococcidiopsis sp.	Quenching	F. Garcia-Pichel & Dor (unpublished)
Mycosporine-like	Various mycosporines	Cyanobacteria (e.g. *Nostoc*, *Chlorogloeopsis* sp.)	UV screening	Garcia-Pichel *et al.* (1992); Oren *et al.* (1995)
	Oligosaccharide	*Nostoc commune*	UV screening	Böhm *et al.* (1995)
		Cyanolichens, e.g. *Collema*, *Gonohymenia*, *Peltula*	UV absorption	Büdel *et al.* (1997)
Anthraquinones	Parietin	*Xanthoria parietina* (algal phycobiont)	UV screening	Laudi *et al.* (1969)
		Antarctic lichens	UV screening	Elix (1996)
Para-depsides	Atranorin	*Stereocaulon* spp. (cyanolichen)	UV screening	Elix (1996)
Usnic acids	Usnic acid	*Umbilicaria antarctica* (algal phycobiont)	UV and light screening	Quilhot *et al.* (1991)
Xanthones	Lichexanthone	Antarctic lichens, *Lecanora* spp.	UV screening	Elix (1996)
Pulvinic acids	Calycin	*Lecanora* spp.	UV screening	Elix (1996)
		Xanthoria elegans (algal phycobiont)	UV screening	Holder (unpublished thesis)

radicals resulting from exposure to UV radiation. One of these, the anthroquinone parietin, has been clearly identified by its unique Raman spectrum within field-fresh samples of the algal lichen *Xanthoria*, both in the Antarctic and in more temperate habitats (Edwards *et al.*, 1998). In this case, vibrational assignments for parietin were clearly detected at 1672 and 1613 cm^{-1} in *X. parietina*, *X. elegans* and *X. mawsoni* together with a double peak of chlorophyll at 1369 and 1384 cm^{-1}. Their carotenoid content was also revealed by characteristic peaks at 1521 and 1157 cm^{-1} and it was evident that construction of a pigmentation 'fingerprint' catalogue for lichens of different species and from different habitats was possible for an assessment of their taxonomy, biogeography and tolerance to environmental stress. Zeaxanthin is potentially a key cyanobacterial biomarker in natural communities (Overmann *et al.*, 1993; Oren *et al.*, 1995) and its Raman spectrum will be included in our rapidly expanding database of spectral fingerprints.

As whole organisms and communities are composed of an immense diversity of biochemical moieties, the Raman spectrum of a natural sample shows many potentially diagnostic bands. However, with the objective of detecting a known biomolecule, there is only a need to identify its major, preferably corroborative bands determined from the unique spectrum of the pure biomolecule. Other than their influence on the shape of the diagnostic bands, all the other bands are irrelevant to the identity of the target molecule.

There are several species of cyanolichens in the Antarctic (Table 2). However, none has been found on the continent nor south of Mars Oasis on Alexander Island where the *Nostoc* lichens *Leptogium puberulum*, *Pannaria hookeri* and *Placopsis contortuplicata* have been found in moist areas which also contain free-living *Nostoc commune* (R. I. Lewis-Smith, personal communication). These symbioses will be amenable to assessing their scytonemin content as a potential indicator of their response to enhanced UVB during the spring Ozone Hole (Farman *et al.*, 1985; Wynn-Williams, 1994). Many cyanobacteria from highly exposed habitats accumulate intracellular mycosporines (Garcia-Pichel & Castenholz, 1993), probably used as UVB sunscreens (Garcia-Pichel *et al.*, 1993), as do exposed cyanolichens (Büdel *et al.*, 1997). *Nostoc commune* also produces a range of extracellular mycosporine-like amino acids (Böhm *et al.*, 1995; Ehling-Schulz *et al.*, 1997) which protect against UVB damage. The substituted cyclohexene core structure of mycosporines may make them amenable to FTRS detection as well. Their fluctuations within cyanobacterial cells and/or in their exopolysaccharide sheaths under UVB stress could therefore be detectable in field-fresh material, and under

Table 2. Cyanolichens reported from the Antarctic[a], potentially vulnerable to enhanced UVB radiation within the Ozone Hole

Species or genus	Cyanobiont genus
Cyanobacteria as the main photobiont	
Arctomia sp.	*Nostoc*
Collema sp.	*Nostoc*
Degelia sp.	*Nostoc*
Lempholemma sp.	*Nostoc*
Leptogium puberulum Hue	*Nostoc*
Lichena sp.	*Calothrix* or *Dichothrix*
Massalongia carnosa (Dickson) Koerber	*Nostoc*
Pannaria hookeri (Borrer *ex* Smith) Nylander	*Nostoc*
Parmeliella sp.	*Nostoc*
Peltigera didactyla (Withering) Laundon	*Nostoc*
Peltigera rufescens (Weis) Humboldt	*Nostoc*
Placynthium sp.	*Rivularia* or *Scytonema*
Solorina sp.	*Nostoc* or *Coccomyxa*
Cyanobacteria in localized cephalodia	
Micarea sp.	*Nostoc* or *Stigonema*
Placopsis contortuplicata Lamb	*Nostoc*
Psoroma tenue Henssen	*Nostoc*
Stereocaulon alpinum Laurer	*Nostoc, Stigonema*
Stereocaulon glabrum (Mueller Argoviensis) Vaino	*Stigonema*

[a] Kappen (1993) and R. I. Lewis-Smith (personal communication).

controlled experimental conditions, with concurrent desiccation, low temperature and osmotic stresses such as prevail in Antarctic deserts (Wynn-Williams, 1999*b*).

The role of accessory pigments such as phycocyanin becomes important at the very low light levels which occur in the cyanobacterial zone of stromatolites in ice-covered hypersaline lakes (*c.* 1.5 μmol m^{-2} s^{-1} in aerobic prostrate mats: Parker & Wharton, 1985). These levels are similar in cryptoendolithic communities (0.9–1.7 μmol m^{-2} s^{-1} at Linnaeus Terrace: Nienow *et al.*, 1988). The autofluorescence which is obstructive in Raman spectroscopy based on green laser excitation at *c.* 536 nm (Edwards *et al.*, 1999) is beneficial for epifluorescence microscopy as phycocyanin fluoresces at 635nm (orange-red) under the same conditions. This permits direct correlation of the spatial distribution of the lichen and cyanobacterial constituents of endolithic communities (Figs 6, 7) with the Raman spectra (at 1064 nm excitation) of their composition (Fig. 5*b* and Russell *et al.*, 1998). The remote, non-intrusive nature of the Raman technique makes it suitable not only for detecting living and fossil biomolecules in Antarctic deserts but also for similar analysis of signs of life on Mars (McKay, 1997; Wynn-Williams, 1999*a*).

Even though the application of FTRS to environmental determinations is in its infancy, we believe that its potential is large. It is already usable on field-fresh samples and can potentially be miniaturized for use in the field. Adjustment of the optical conditions can preclude excessive absorption of laser energy causing overheating of labile compounds and can avoid fluorescence of the mineral substratum or irrelevant organics. With these constraints refined, there is the potential to build up a Raman spectral database of commonly occurring biochemicals of ecological relevance in order to interpret complex natural spectra.

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