evropský sociální fond v ČR · EVROPSKÁ UNIE · MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY · OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Raman spectroscopy of phototrophic microbial communities from extreme Earth environments: stories from the Atacama Desert and Mojave Desert

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Tato akce se koná v rámci projektu:
Vybudování vědeckého týmu environmentální metabolomiky a ekofyziologie a jeho zapojení do mezinárodních sítí (ENVIMET; r.č. **CZ.1.07/2.3.00/20.0246**) realizovaného v rámci Operačního programu Vzdělávání pro konkurenceschopnost.

Research interests

- **Life in extreme Earth environments**

- **Raman spectroscopy of biomarkers related to extremophilic microorganisms**

  - **analytical/methodical studies with artificially prepared materials**

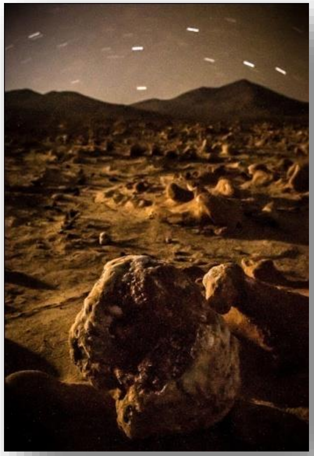
  - **native specimens – geobiological systems from extreme desert environments**

Research interests

- Biosynthesis of pigments as a response to variable light conditions and stress factors (UV- radiation)

    - Scytonemin – a cyanobacterial UV-screening pigment in halite from the Atacama Desert

    - Carotenoids in extremophiles



Research interests

- **Evaluation of miniaturized Raman instrumentation with relevance for future astrobiological investigation of Mars (e.g. ExoMars mission, NASA 2020 mission)**

ESA/AOES Medialab artist's concept

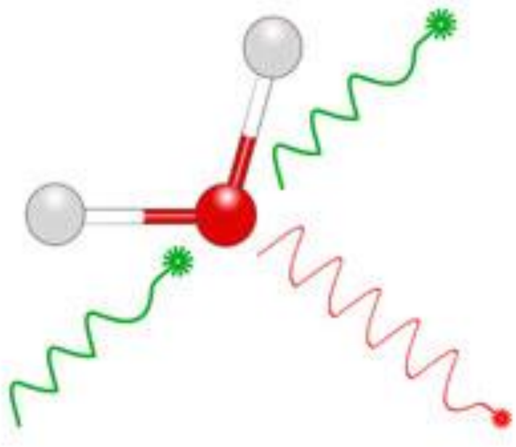
Raman spectroscopy

- Vibrational spectroscopic technique
- Non-destructive
- Rapid data acquisition
- Detection of solid, liquid and gas phases
- Detection of both inorganic and organic compounds

Raman spectroscopy

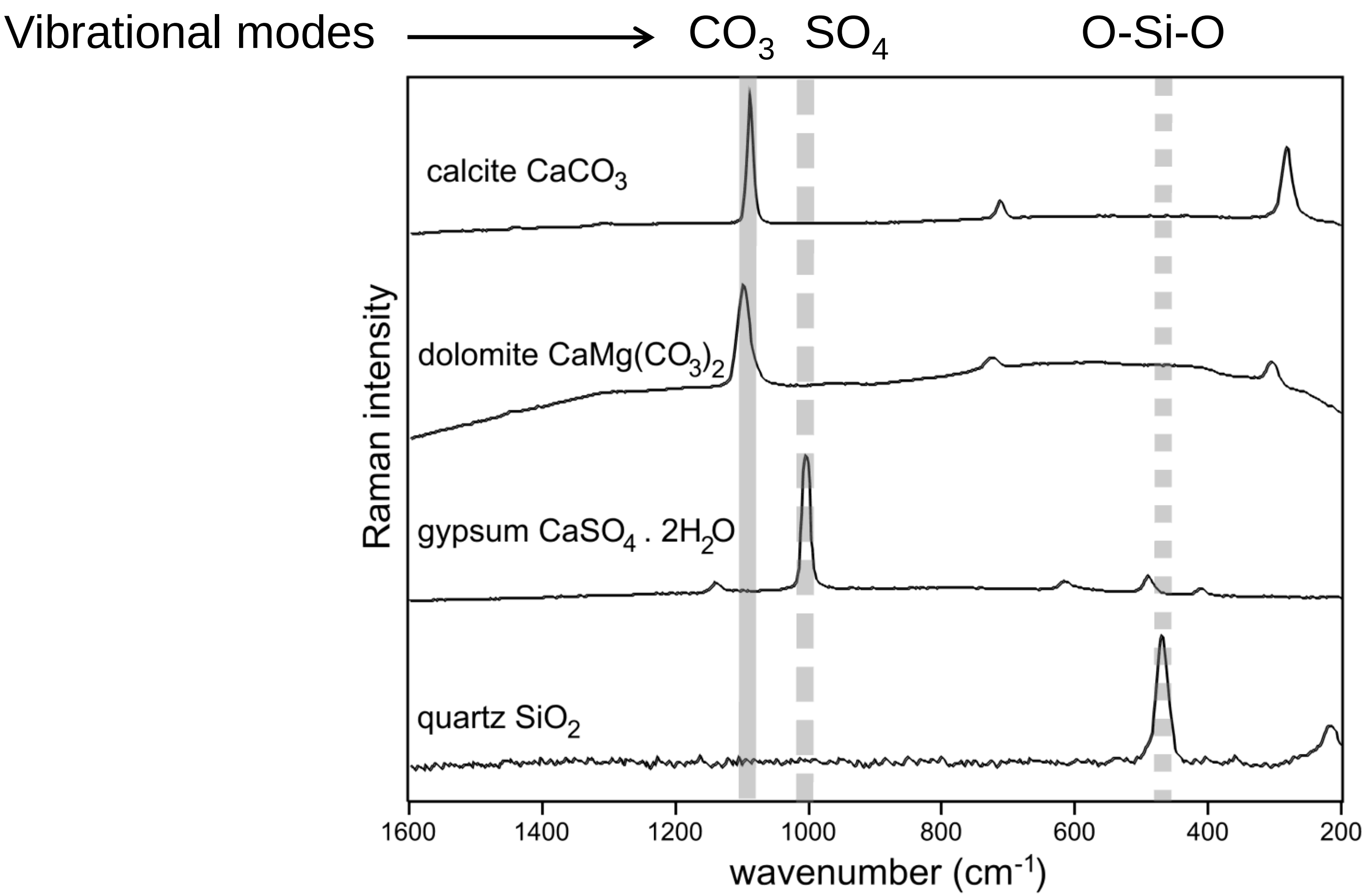
Raman scattering

inelastic diffusion of light



Raman spectroscopy

Raman bands



Evaporites as habitats for microorganisms

Halophiles in recent environments

Cyanobacteria

Halophilic archaea

Halophilic bacteria

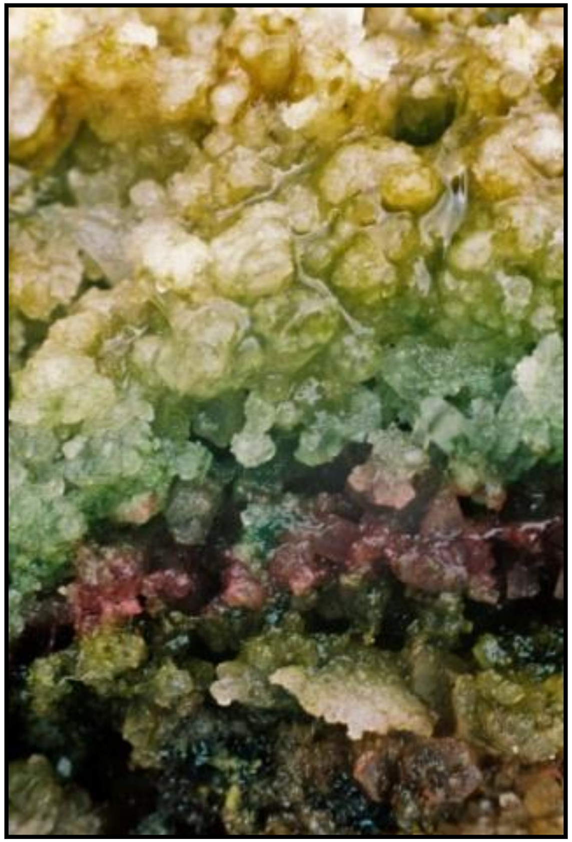
Halophilic algae

Evaporites as habitats for microorganisms

in brines

Evaporites as habitats for microorganisms

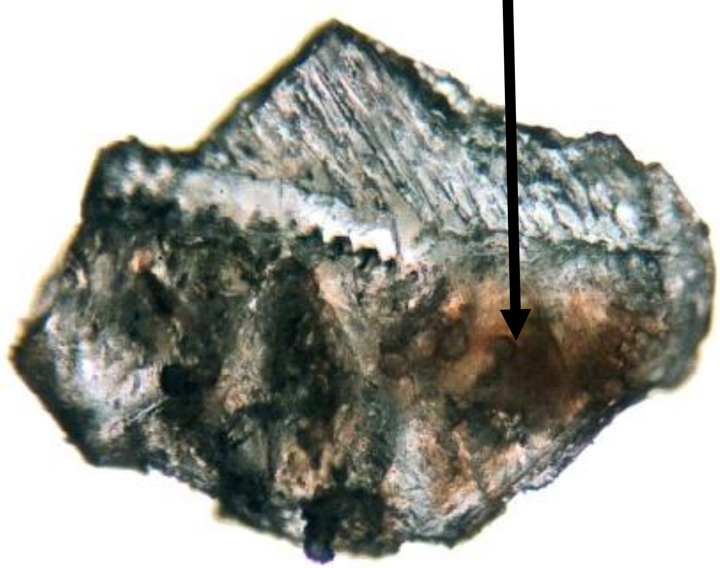
in pore spaces of evaporitic crusts



Evaporites as habitats for microorganisms

incorporated within the evaporitic minerals – especially halite, gypsum

Halobacterium salinarum



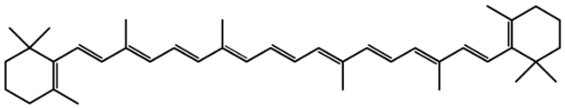
Evaporites as habitats for microorganisms

- **Viable isolates of halophilic bacteria and archaea found within halite inclusions of permian age (~250 million years old)**

  (Vreeland et al. 2000, Stan-Lotter et al. 2004)

- **1.46 million years old carotenoids in halite (NaCl) from Death Valley**

  (Winters et al. 2013)



Methodical work

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Analyzed mixtures

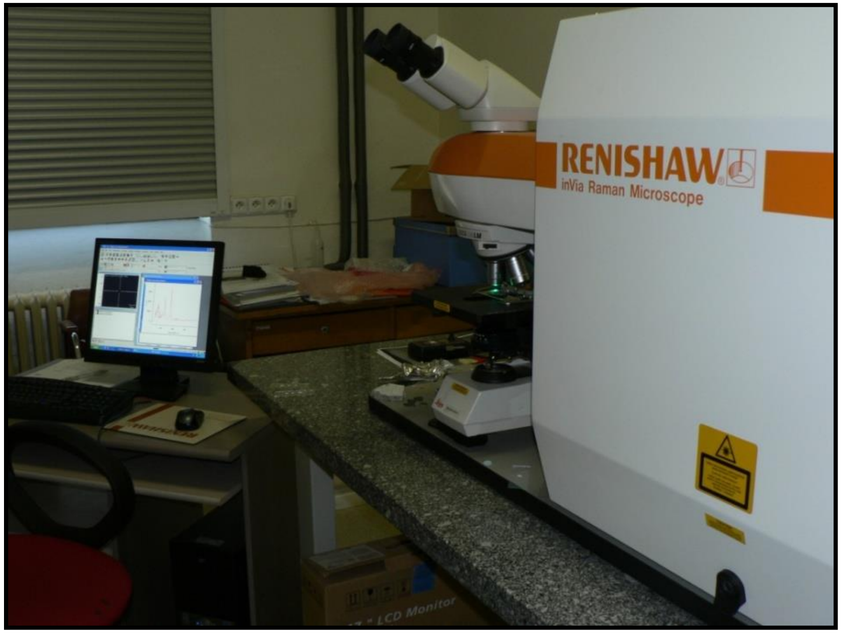
β-carotene/halite

β-carotene/gypsum

β-carotene/epsomite

Raman spectroscopy

Benchtop vs. portable

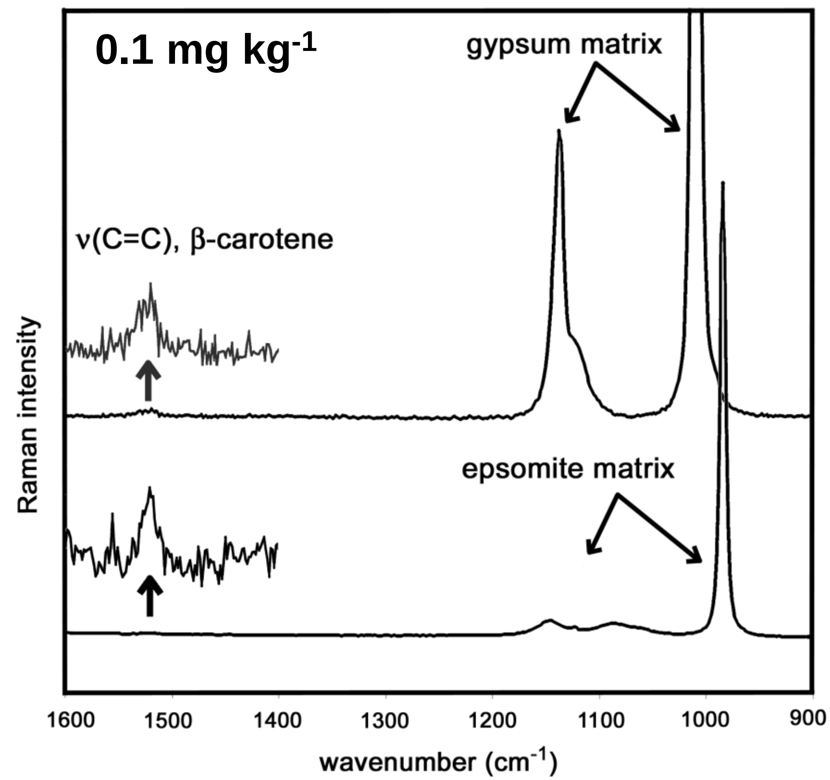


The lowest concentration detected

In sulphates

gypsum and epsomite

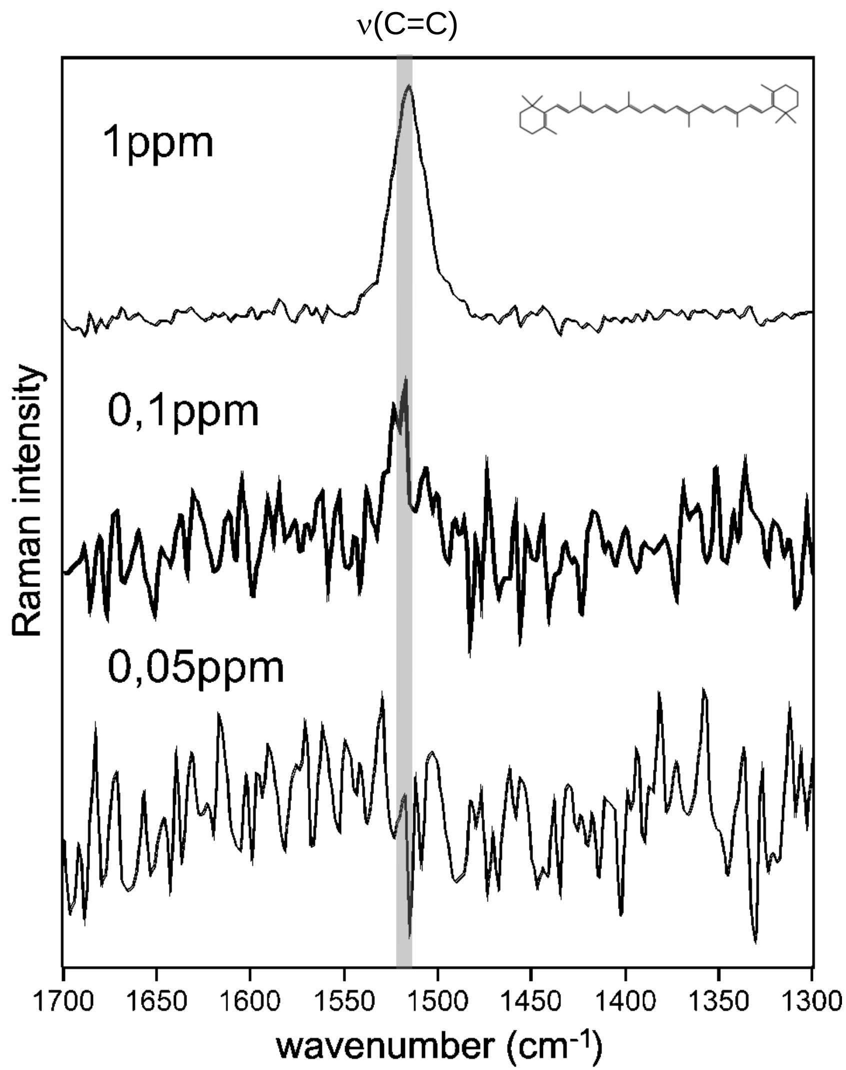
(514.5nm)



The ν(C=C) band of β-carotene was detected at the **0.1 mg kg^{-1}**

Vítek et al., 2009, Anal. Bioanal. Chem.

Performance of miniaturized Raman

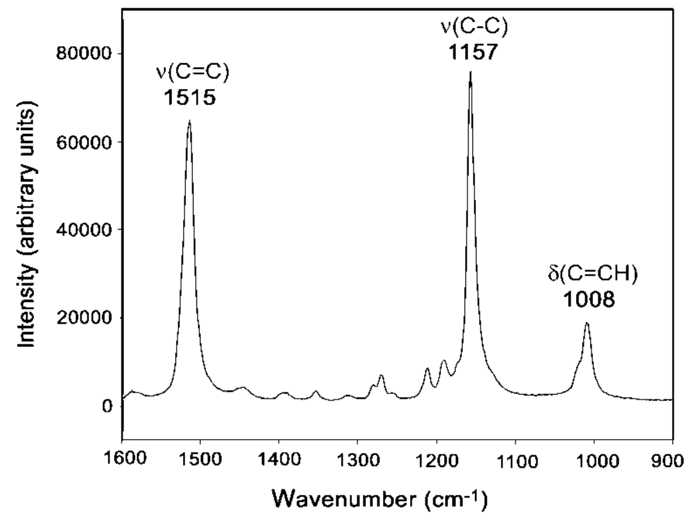


β-carotene in gypsum (532 nm)

Concentration limits of detection:

532 nm → 0.1 ppm

785 nm → 1 ppm

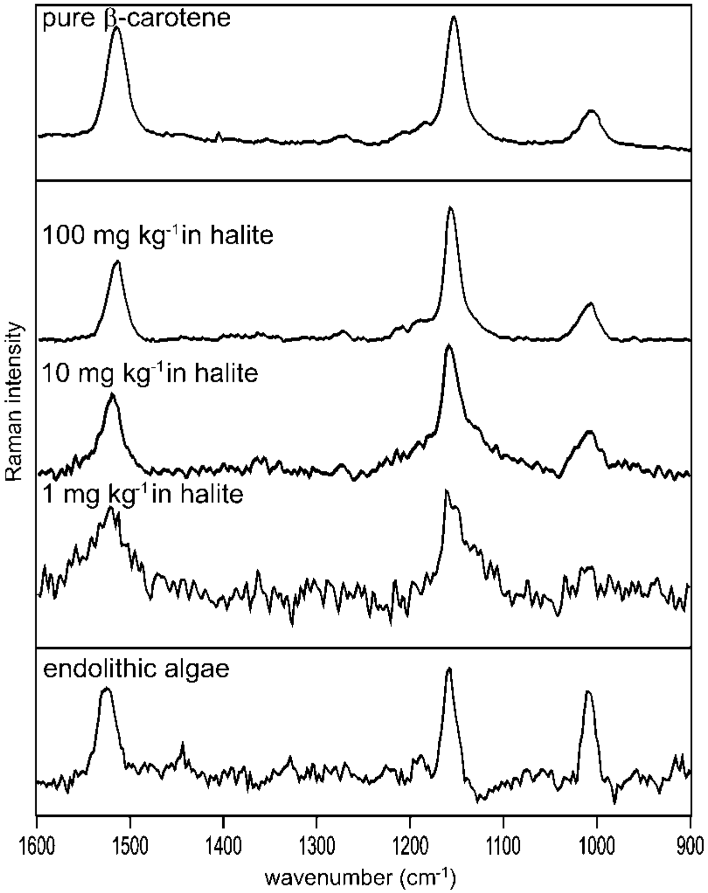


Raman spectra of β-carotene

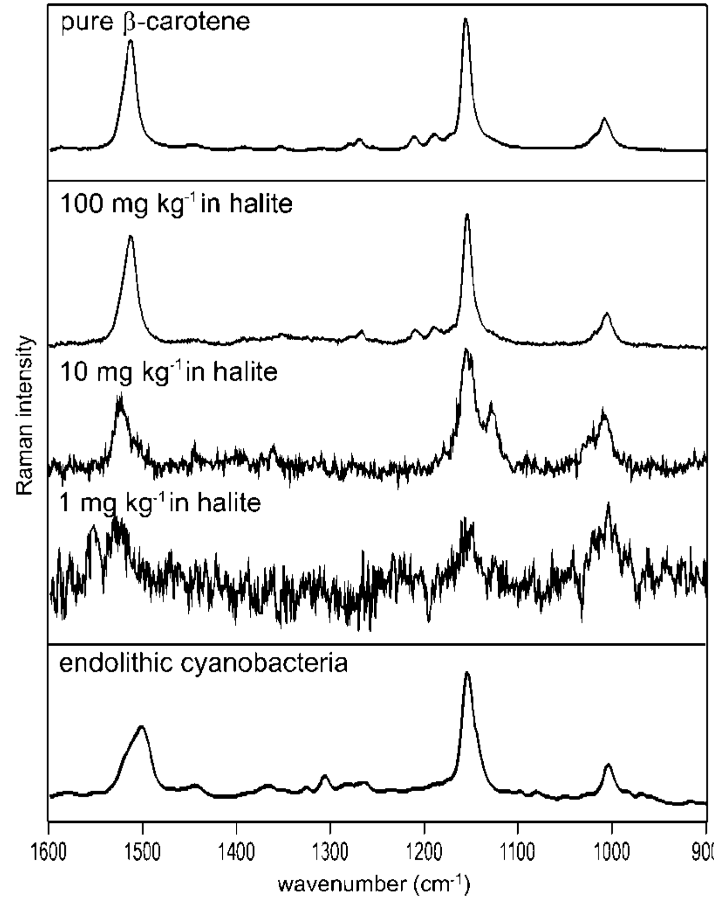
Artificially prepared mixture

Limits of detection

bench-top vs portable



Portable instrument



Bench-top instrument

Vandenabeele et al., Planetary and Space Science, 2012

Real geobiological systems

$\longrightarrow$

Studied sites

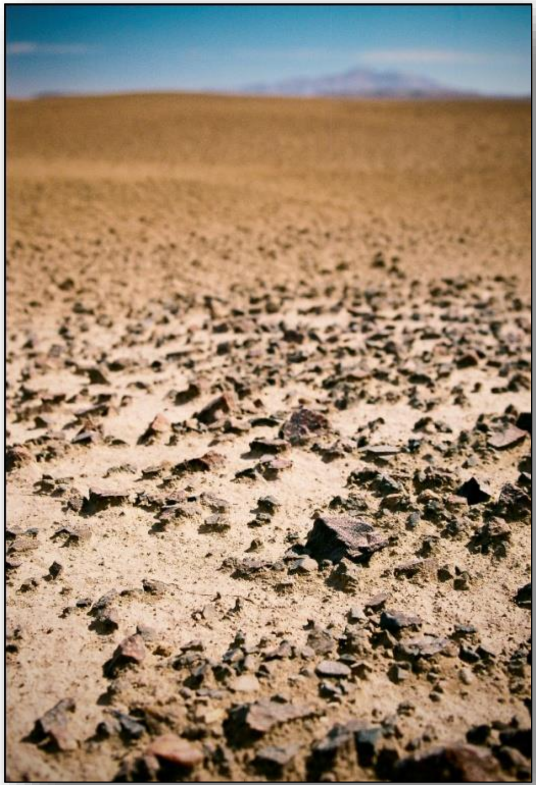
Atacama Desert - expeditions 2011 and 2013

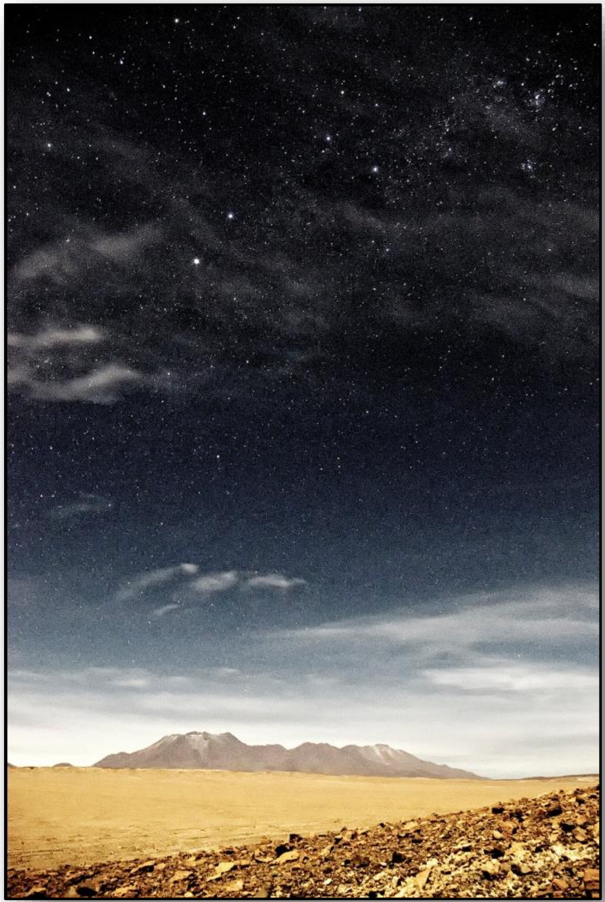
Credit: J. Wierzchos

Mojave Desert – field trips 2011, 2012

Studied sites

Atacama Desert

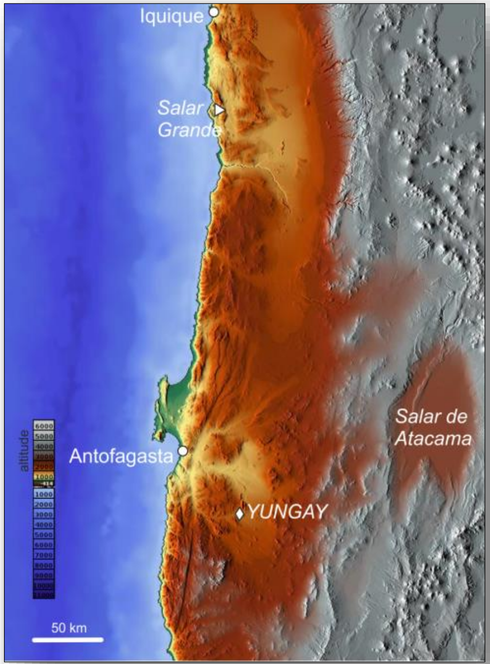




Studied sites

Atacama Desert

- The hyperarid core of the desert is one of the driest areas on Earth
- Dry limit for photosynthesis on Earth *(Warren-Rhodes et al., 2006)*
- “Mars analog“
- Endoevaporitic habitats as a refuge
  - enhances water availability and possess a protection against UV radiation *(Wierzchos et al., 2006, 2011)*



Studied sites

Mojave Desert

Studied sites

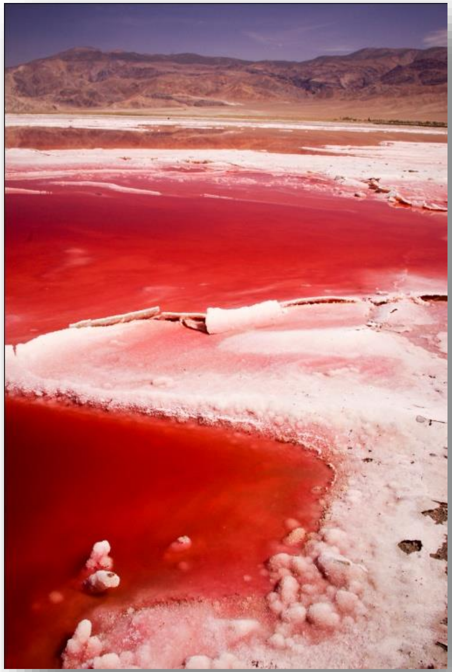
Mojave Desert

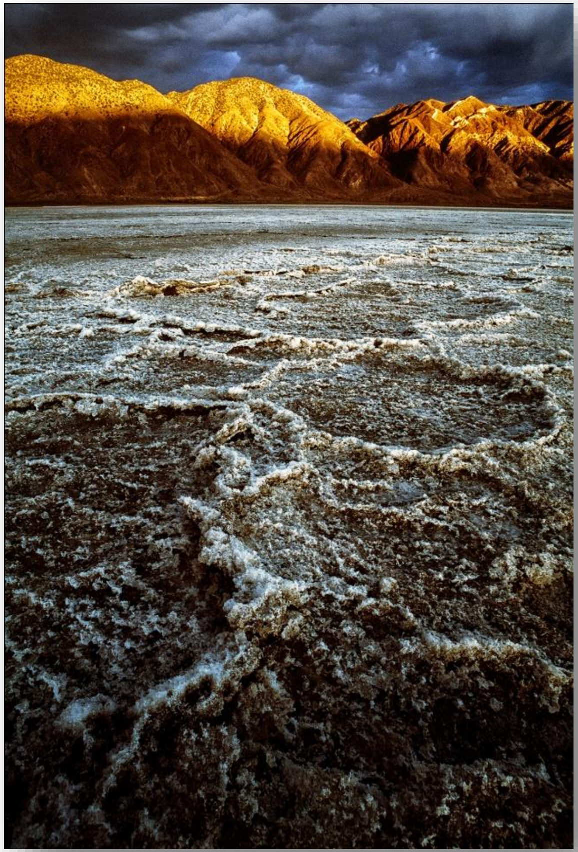
- More “wet“ compared to the Atacama
- Playas and lakes with diverse composition of evaporites

Owens Lake

Searles Lake

Deep Springs Lake





Performance of miniaturized Raman

Native samples from the desert environments

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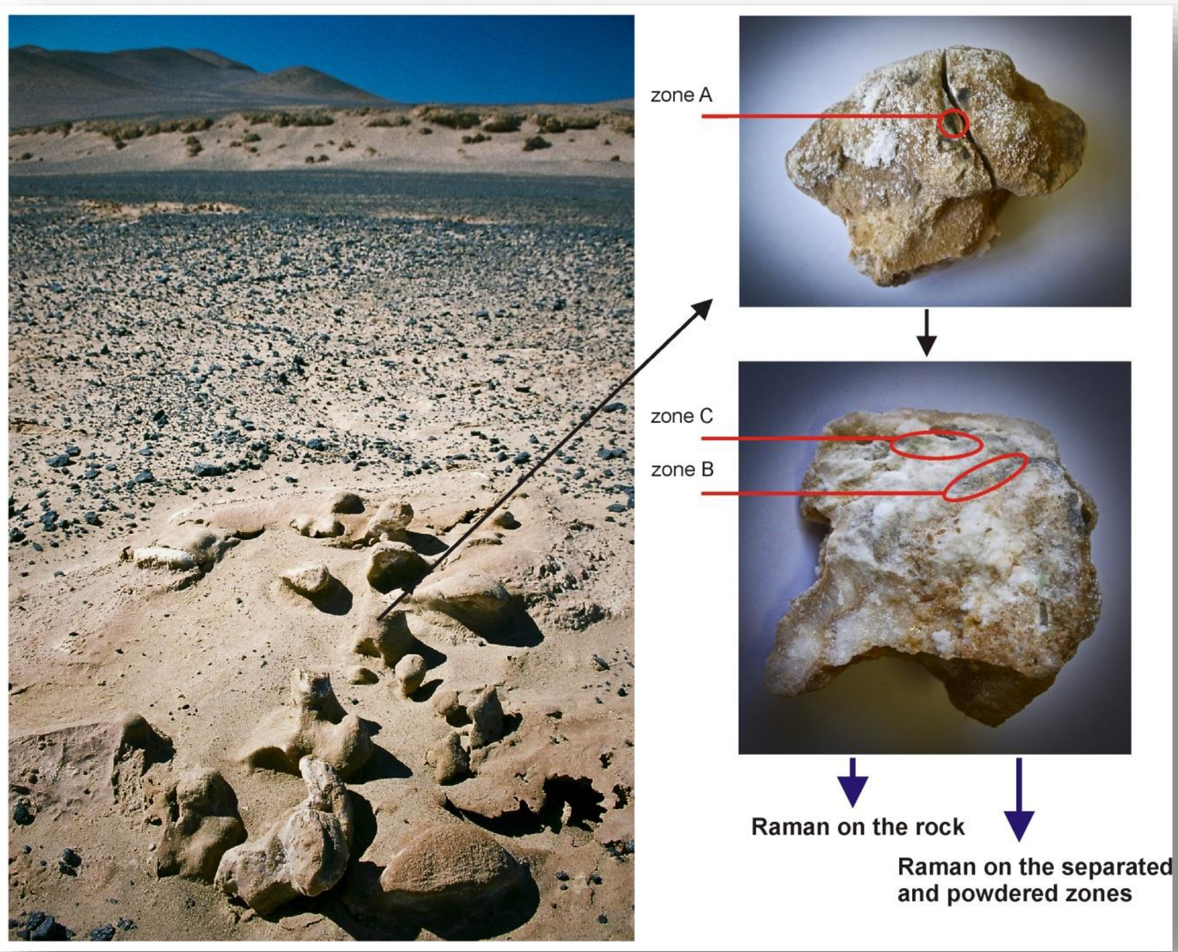
Performance of miniaturized Raman

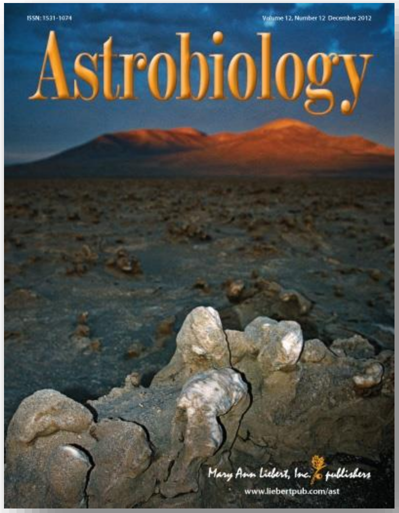
Atacama Desert – halite (NaCl)



ZONE A:
surface, black stripes

ZONE B:
inside the rock, gray bands

ZONE C:
inside the rock, green bands



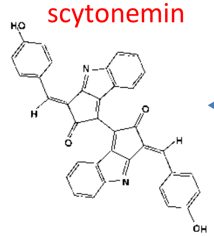
Vítek et al., 2012, Astrobiology

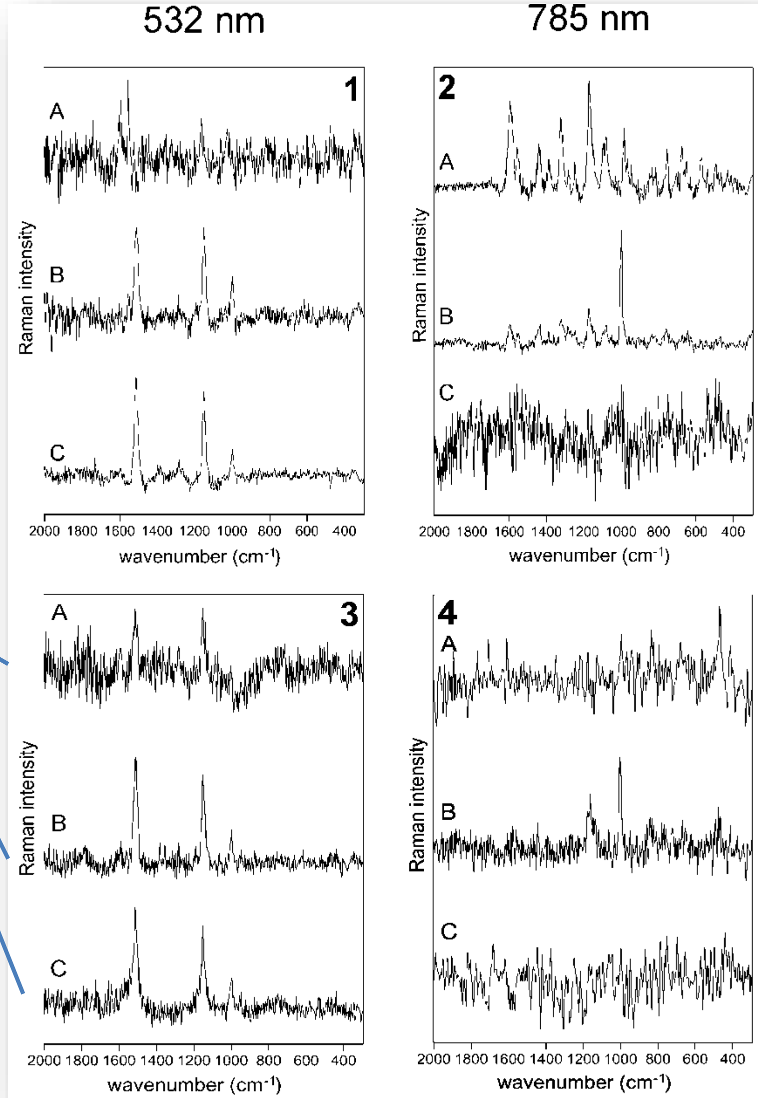
Performance of miniaturized Raman

Atacama Desert – halite (NaCl)

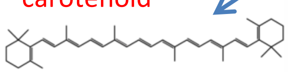
Vítek et al., 2012, Astrobiology

scytonemin

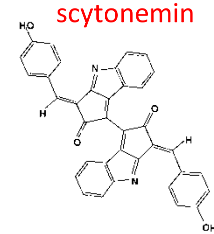




carotenoid



scytonemin



directly on the rock

no organic signal

on the powdered samples

Performance of miniaturized Raman

Vítek et al., 2012, Astrobiology

halite zones of interrest	**532nm**	**785nm**
A - rock	weak **S**, *after positioning*	**S,** *after positioning*
B - rock	**C** + very weak **S**, *after positioning*	**S,** *after positioning*
C - rock	**C**, *stable*	N/A
A - powder	**C**, *stable*	N/A
B - powder	**C**, *stable*	N/A
C - powder	**C**, *stable*	N/A

Raman signal obtained on different zones within halite from the Atacama Desert: **S = scytonemin, C = carotenoid, N/A = no signal obtained**

Performance of miniaturized Raman

Mojave Desert

Halite	$NaCl$
Thenardite	Na_2SO_4
Trona	$Na_3(CO_3)(HCO_3) \cdot 2H_2O$
Burkeite	$Na_6(CO_3)(SO_4)_2$

Atacama Desert

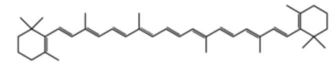
Gypsum	$CaSO_4 \cdot 2H_2O$

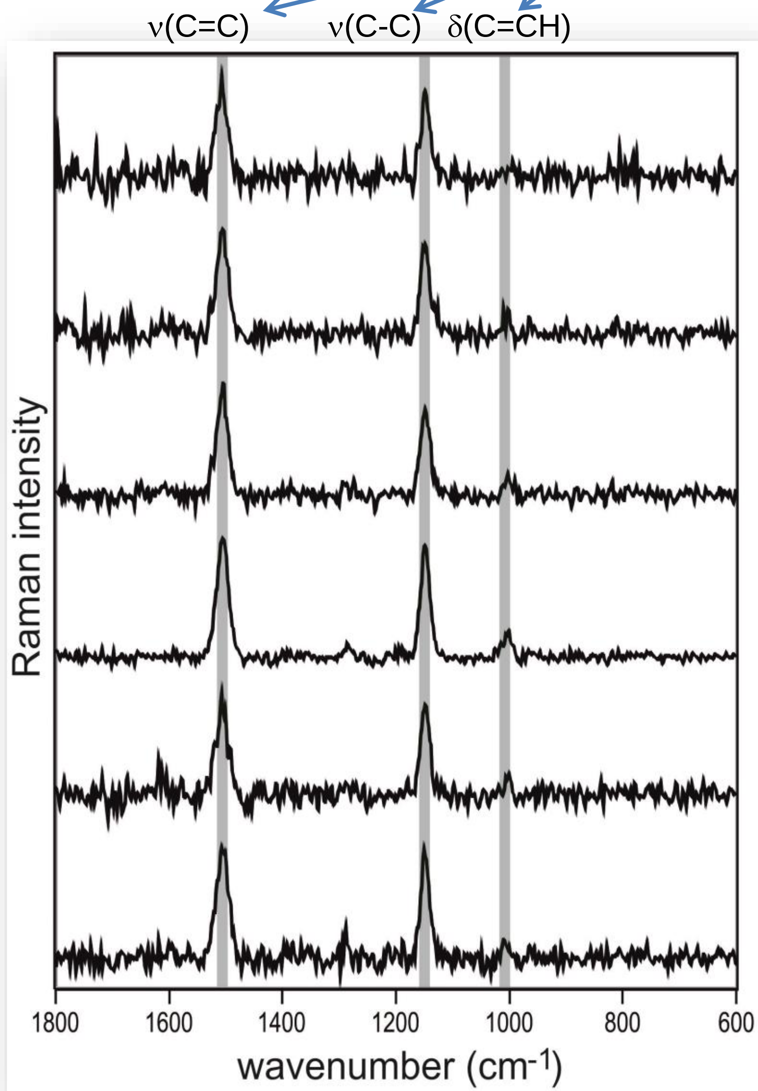
Performance of miniaturized Raman

Searles Lake

Halite NaCl

carotenoid





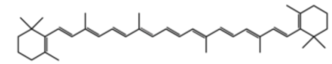
on the powdered samples

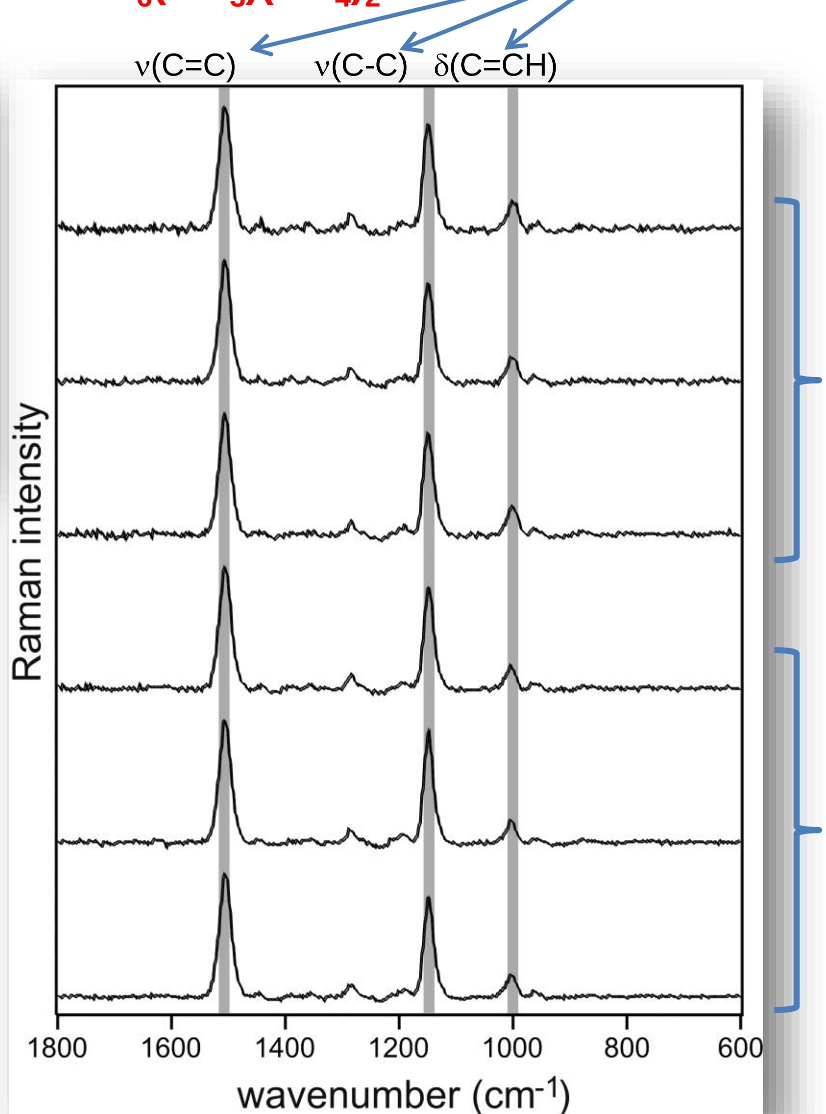
directly on the rock

Vítek et al., 2015, Philosophical Transactions of the Royal Society A (in press)

Performance of miniaturized Raman

Deep Springs Lake **Burkeyite $Na_6(CO_3)(SO_4)_2$**





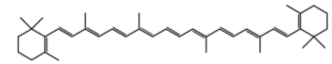
Vítek et al., 2015, Philosophical Transactions of the Royal Society A (in press)

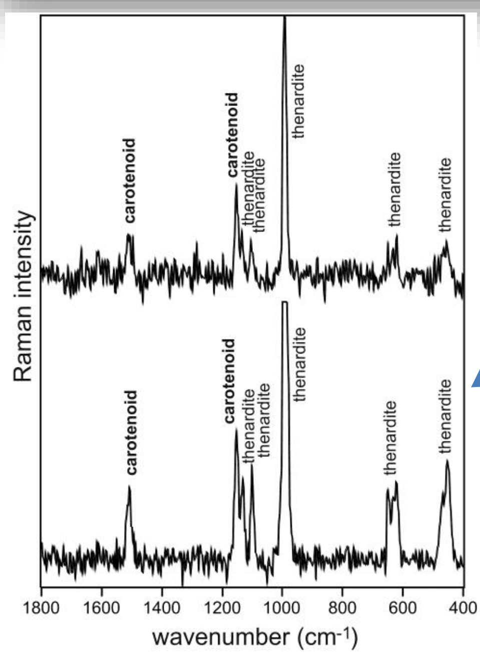
Performance of miniaturized Raman

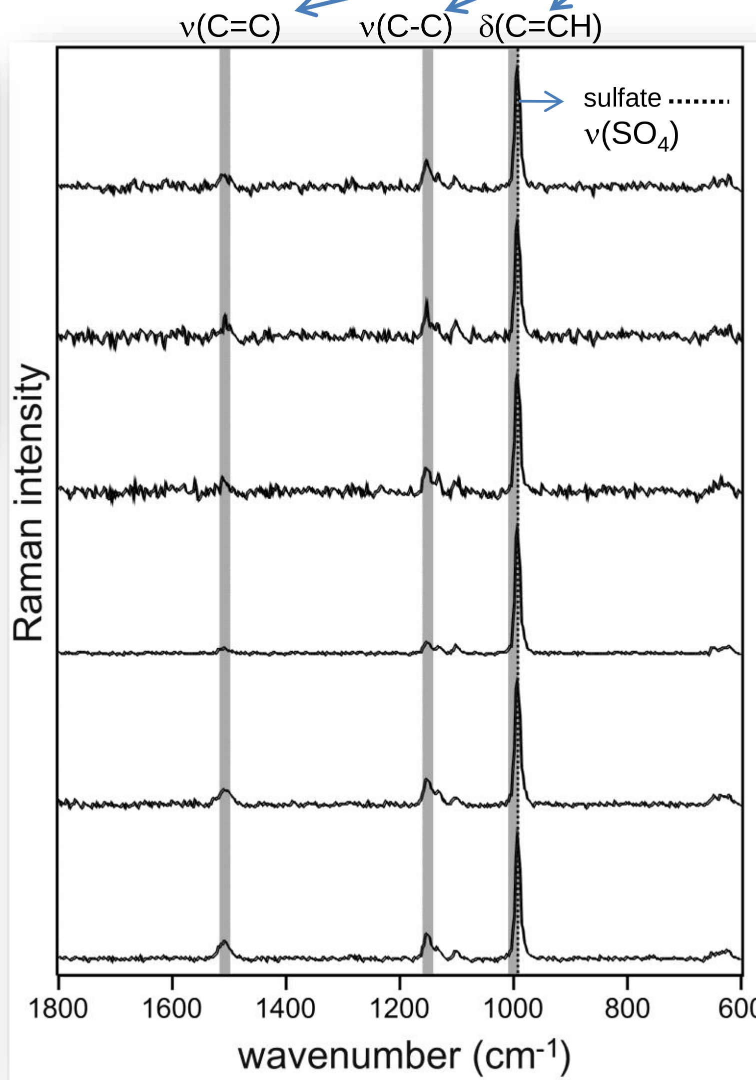
Deep Springs Lake

thenardite Na_2SO_4
~30 cm subsurface crust

carotenoid







on the powdered samples

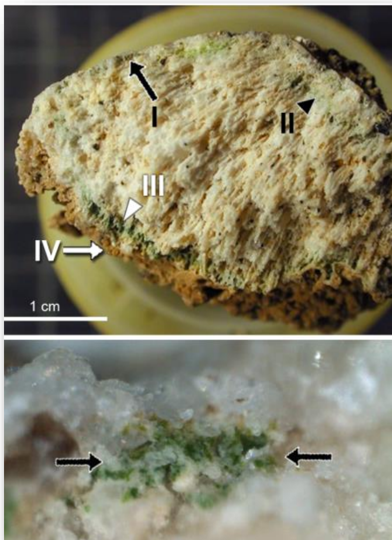
directly on the rock

Vítek et al., 2015, Philosophical Transactions of the Royal Society A (in press)

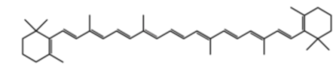
Performance of miniaturized Raman

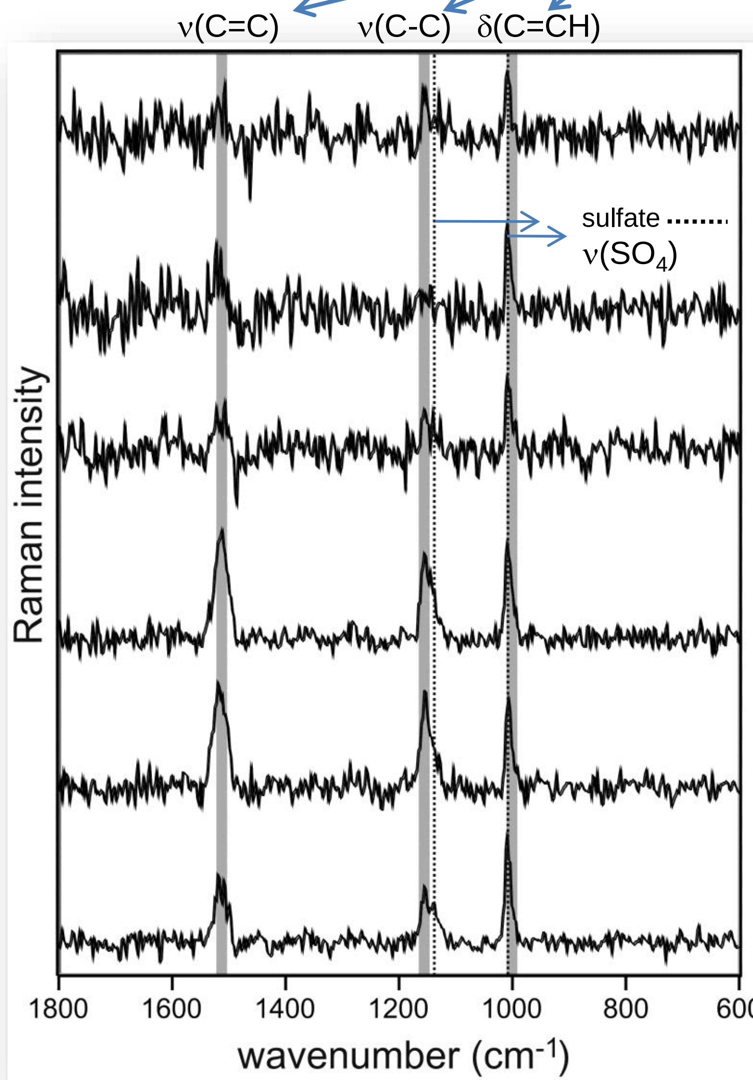
rock vs. powder

Atacama Desert - gypsum



carotenoid





on the powdered samples

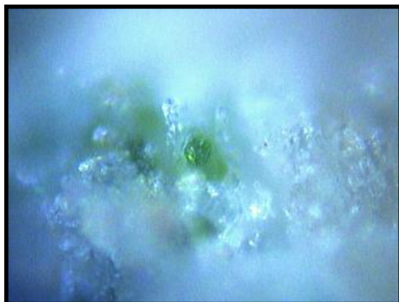
directly on the rock

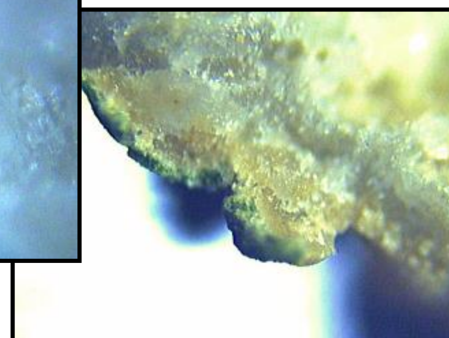
Vítek et al., 2015, Philosophical Transactions of the Royal Society A (in press)

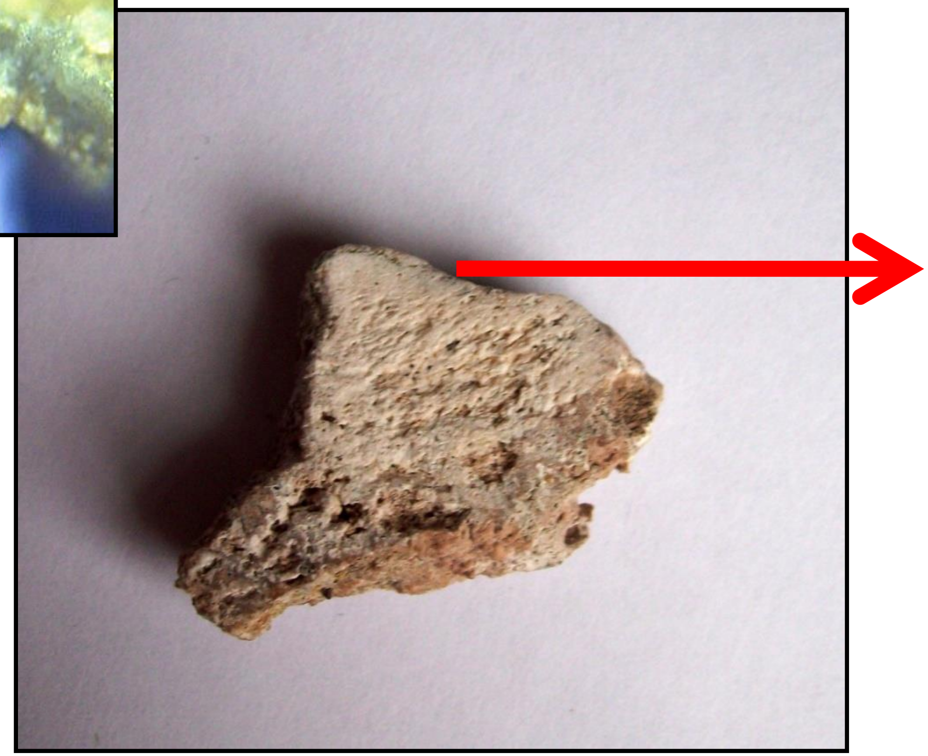
Endolithic phototrophs in gypsum

→

Gypsum crust – endolithic algae

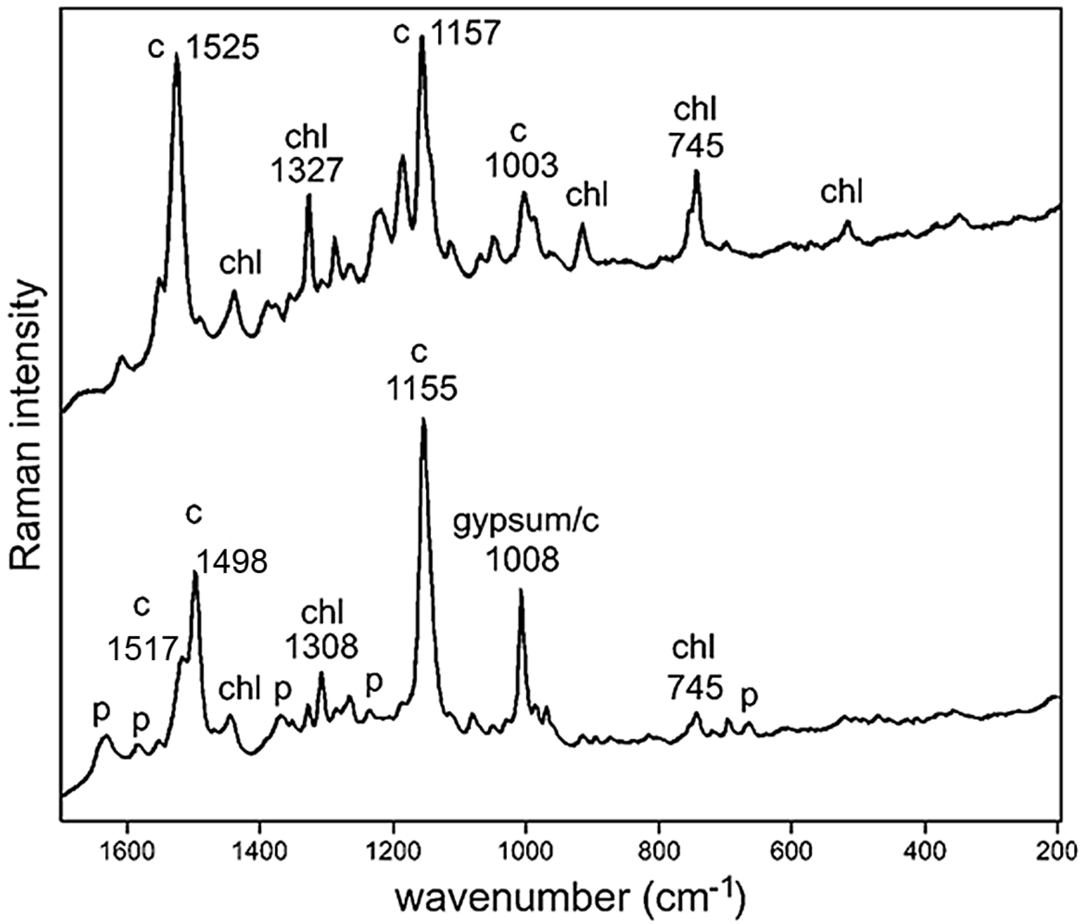






algae
+ cyanobacteria

Atacama Desert – gypsum crust



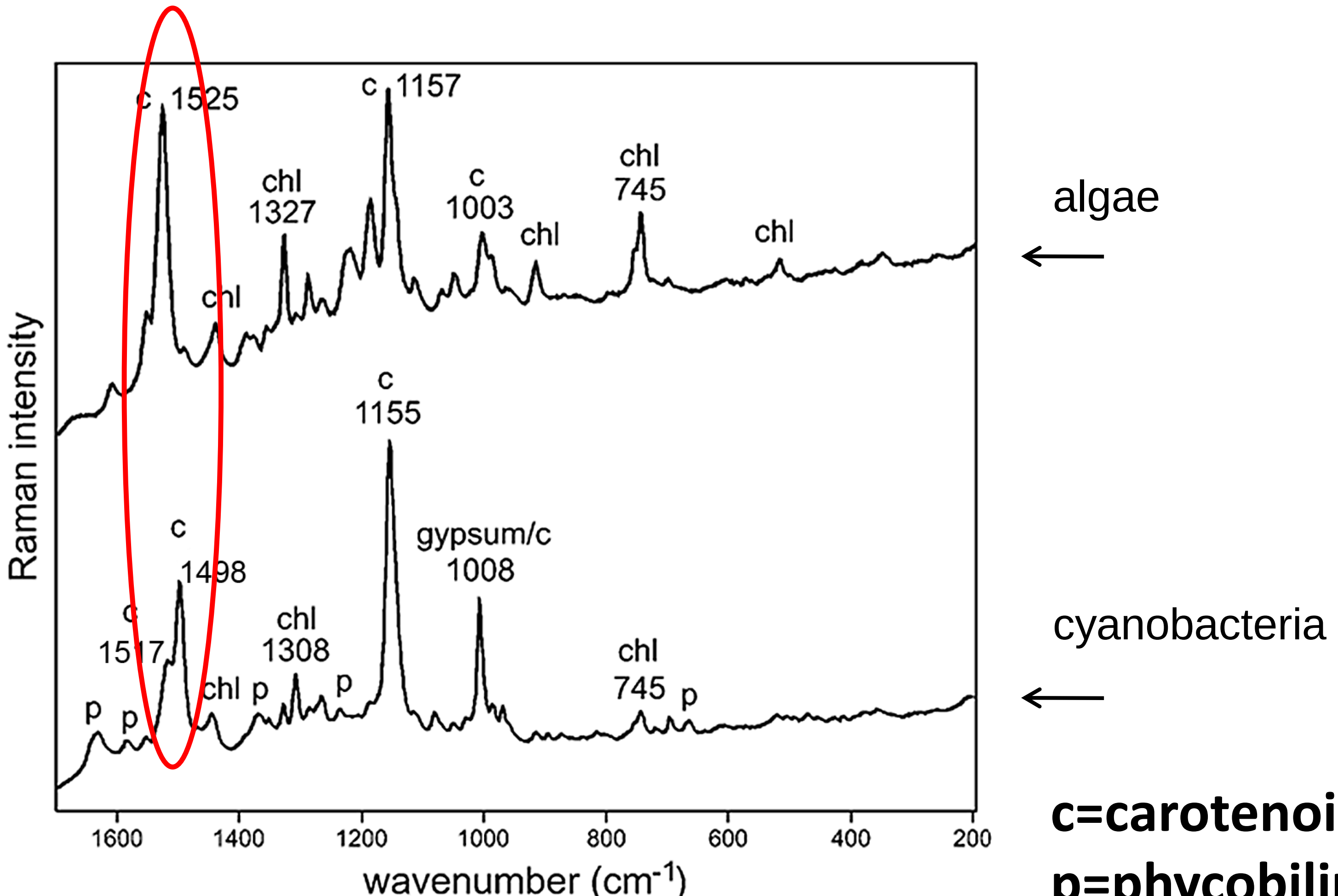
algae

cyanobacteria

c=carotenoids
p=phycobiliprotein
chl=chlorophyll

Vítek et al., 2013, Geomicrobiol. J.

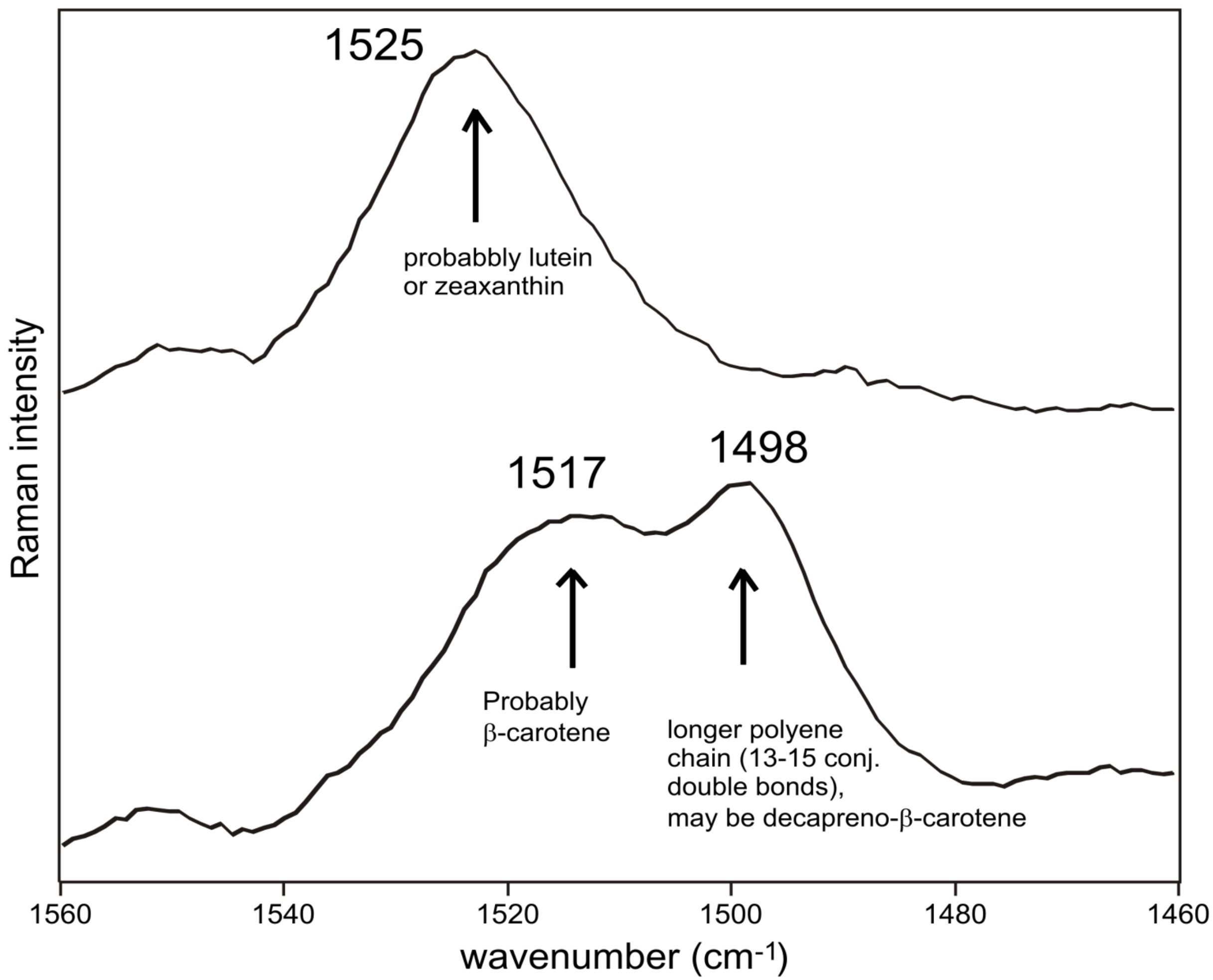
Atacama Desert – gypsum crust



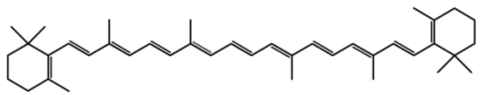
c=carotenoids
p=phycobiliprotein
chl=chlorophyll

Vítek et al., 2013, Geomicrobiol. J.

Atacama Desert – gypsum crust

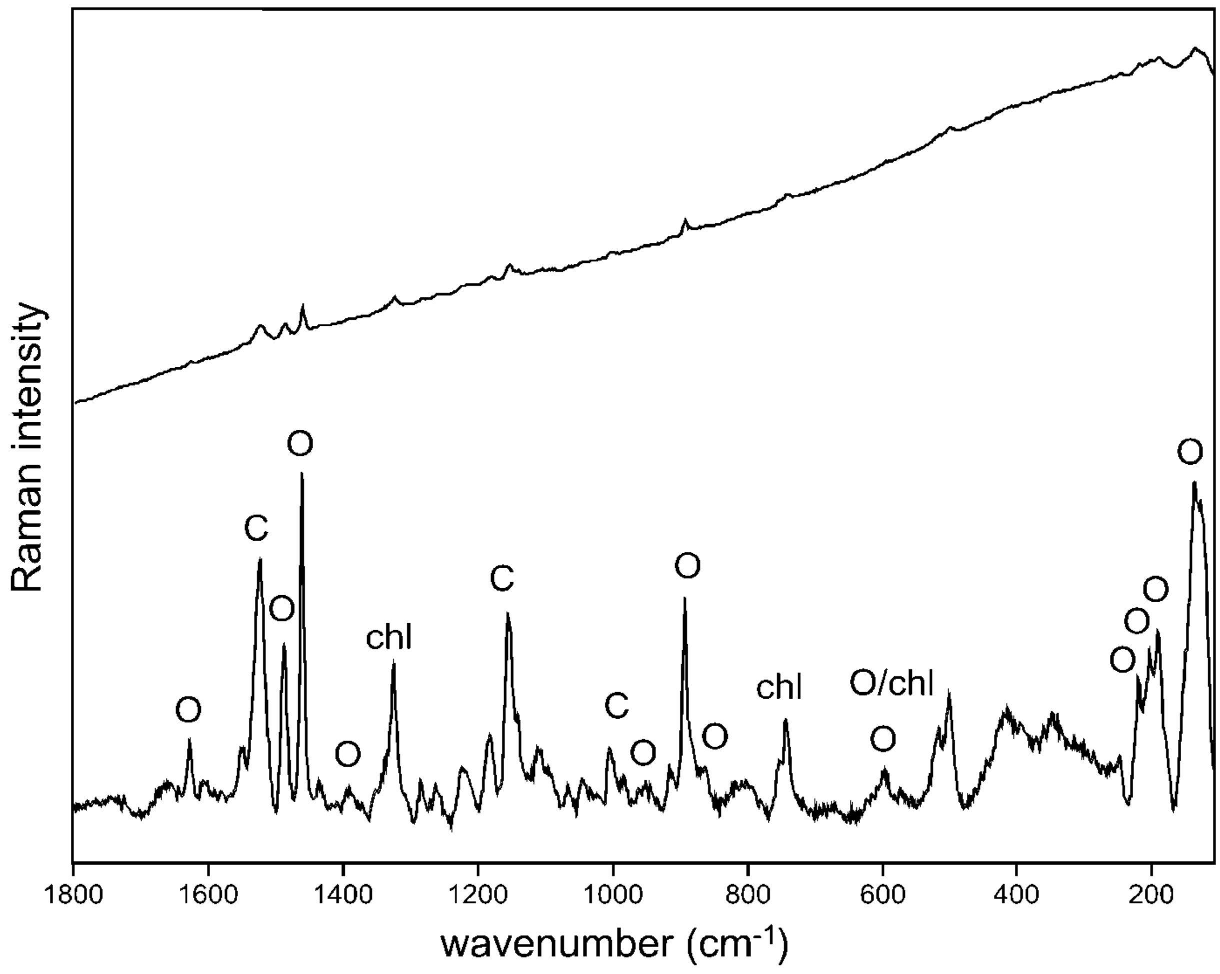


Carotenoid ν_1 (C=C) band



Vítek et al., 2013, Geomicrobiol. J.

Atacama Desert – gypsum crust



c=carotenoids
chl=chlorophyll
O=oxalate

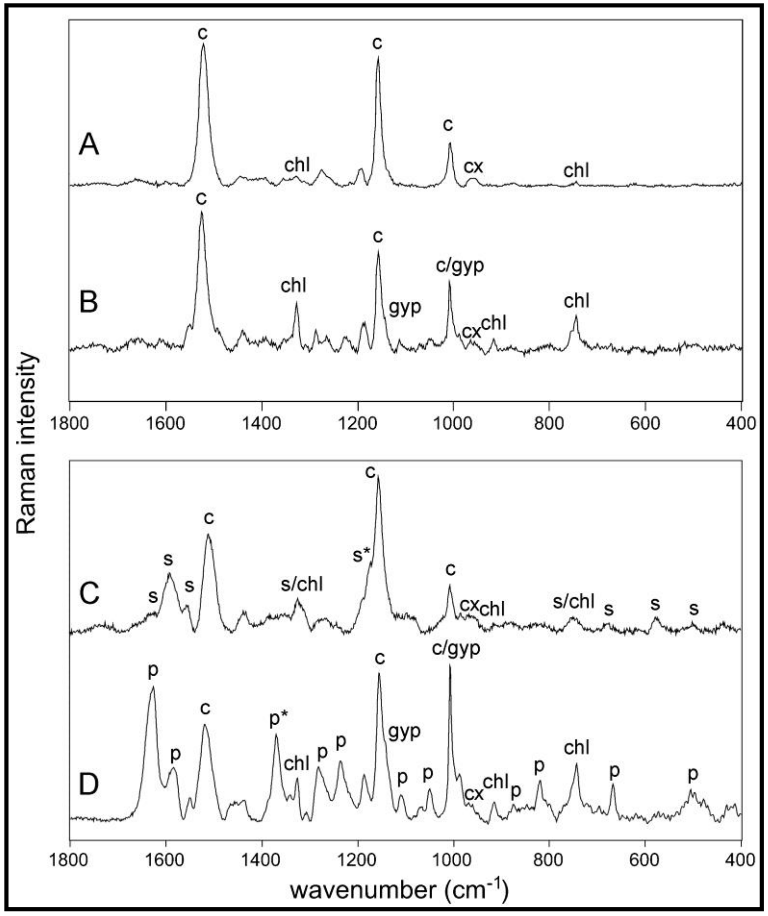
Vítek et al., 2013, Geomicrobiol. J.

Gypsum crust – endolithic algae and cyanobacteria

MTQ site in the Atacama Desert

Algae

Cyanobacteria



c=carotenoid
cx=xanthophyll
p=phycobiliprotein
chl=chlorophyll
s=scytonemin

Wierzchos et al., in prep., Environ. Microbiol.

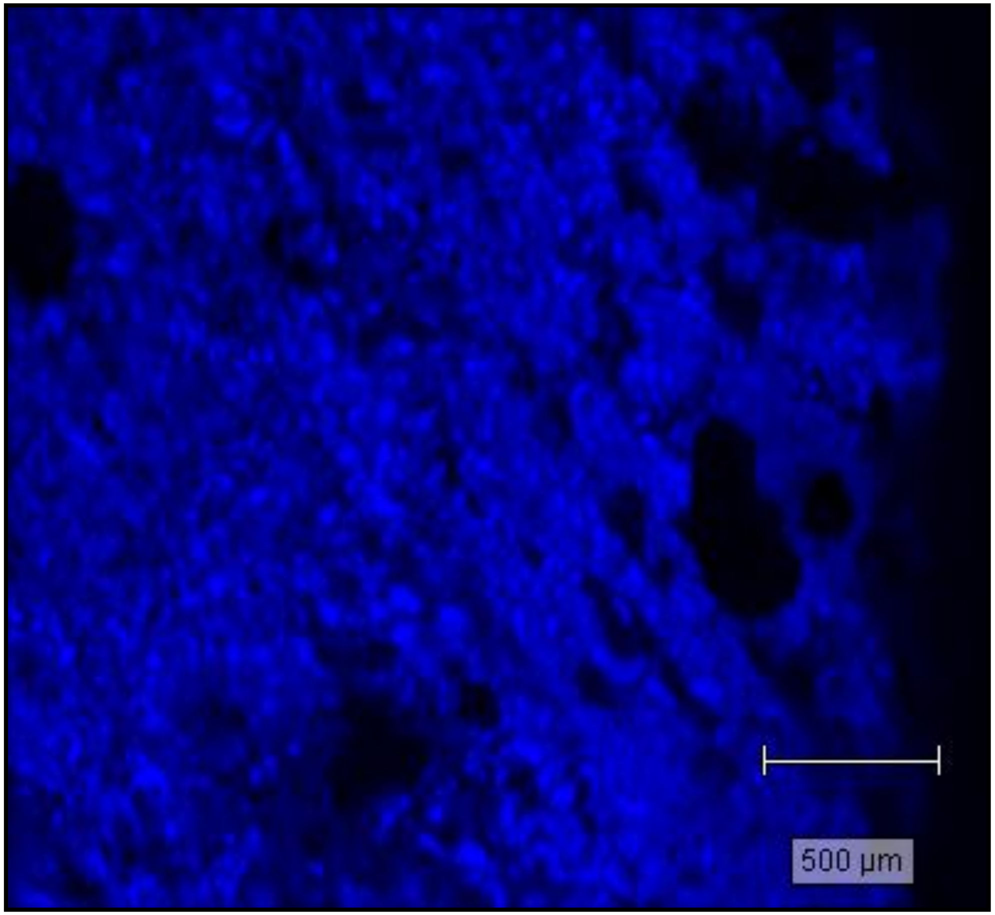
Gypsum crust – endolithic algae

Streamline™ Renishaw system

~ 36000 spectra, 0,343 s each
~ 3 hours acquisition

● gypsum ν_1 →

signal to baseline
between 1004 – 1012 cm^{-1}



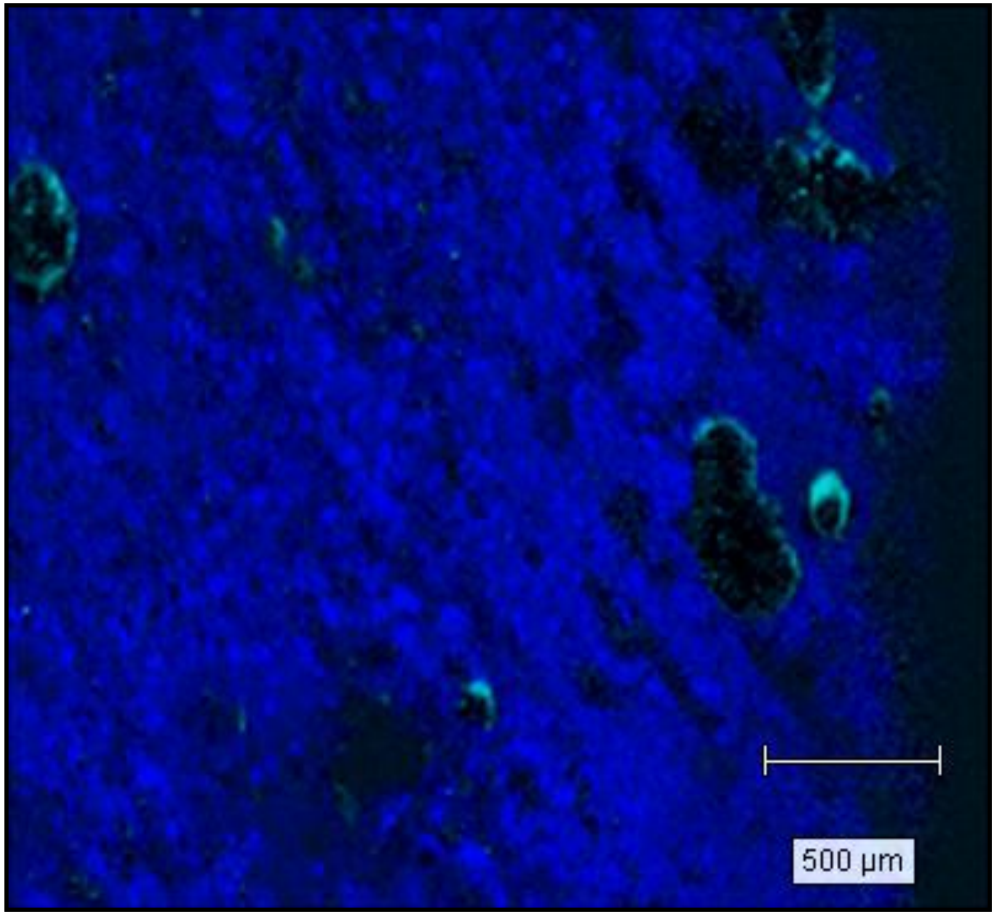
Gypsum crust – endolithic algae

Streamline™ Renishaw system

~ 36000 spectra, 0,343 s each
~ 3 hours acquisition

● anhydrite ν_1

signal to baseline
between 1020 – 1030 cm^{-1}



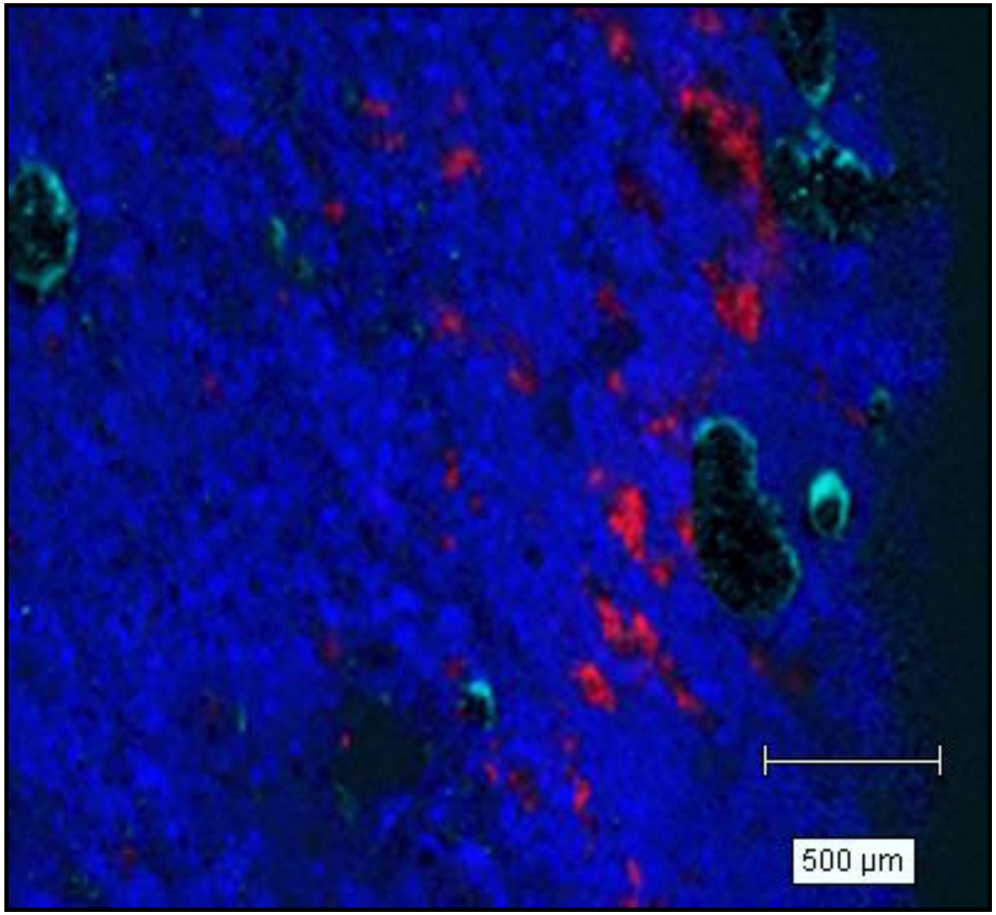
Gypsum crust – endolithic algae

Streamline™ Renishaw system

~ 36000 spectra, 0,343 s each
~ 3 hours acquisition

● carotenoid ν_1 →

signal to baseline
between 1500 – 1535 cm^{-1}

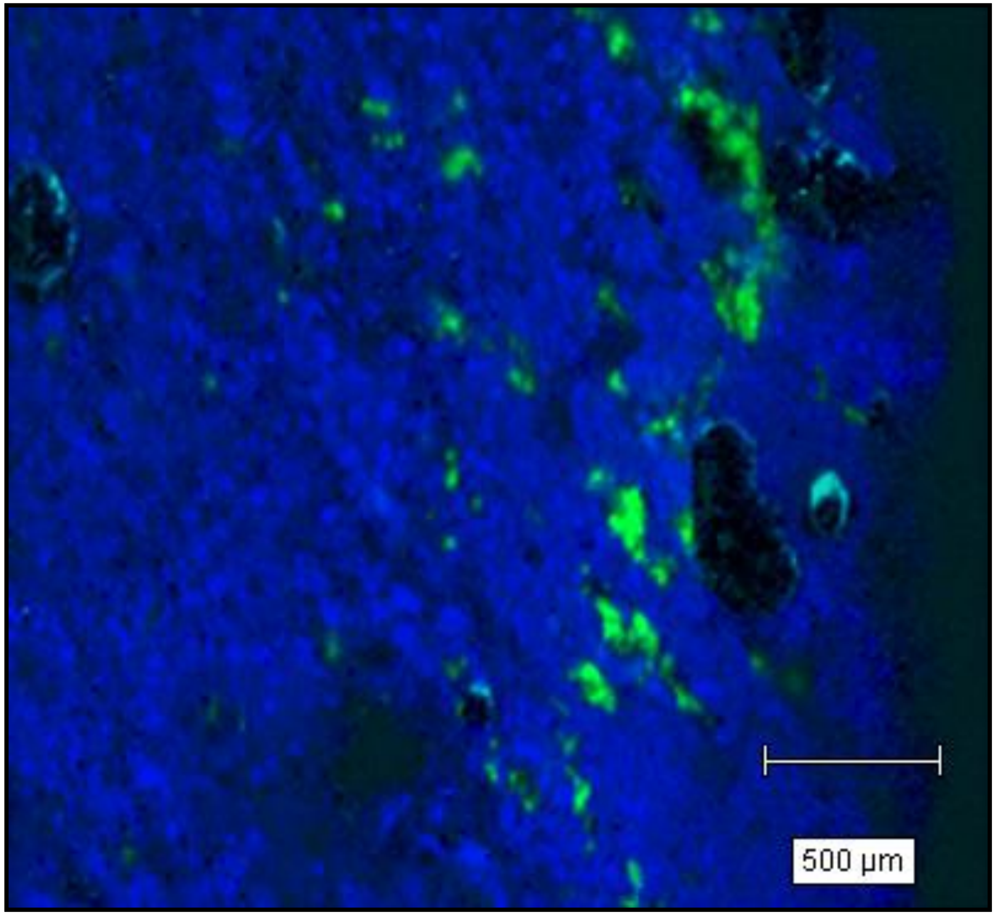


Gypsum crust – endolithic algae

Streamline™ Renishaw system

~ 36000 spectra, 0,343 s each
~ 3 hours acquisition

● chlorophyll



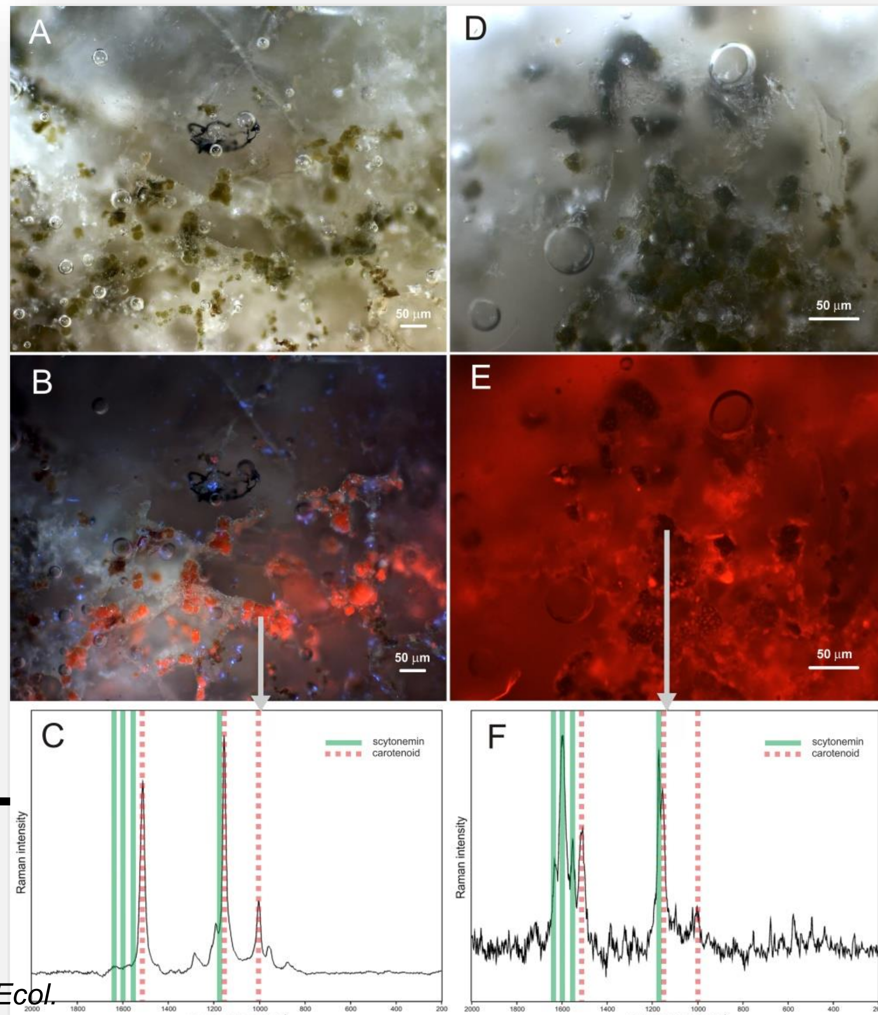
signal to baseline
between 1315 – 1330 cm^{-1}

Halite crust – endolithic cyanobacteria

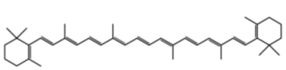
Halite crust – endolithic cyanobacteria

Green cell aggregates

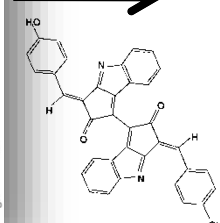
Black cell aggregates

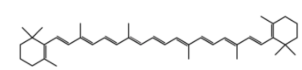


carotenoid



scytonemin
+ carotenoid



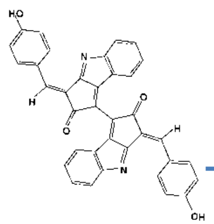


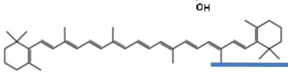
Vítek et al., 2014, FEMS Microbiol. Ecol. (in press)

Halite crust – endolithic cyanobacteria

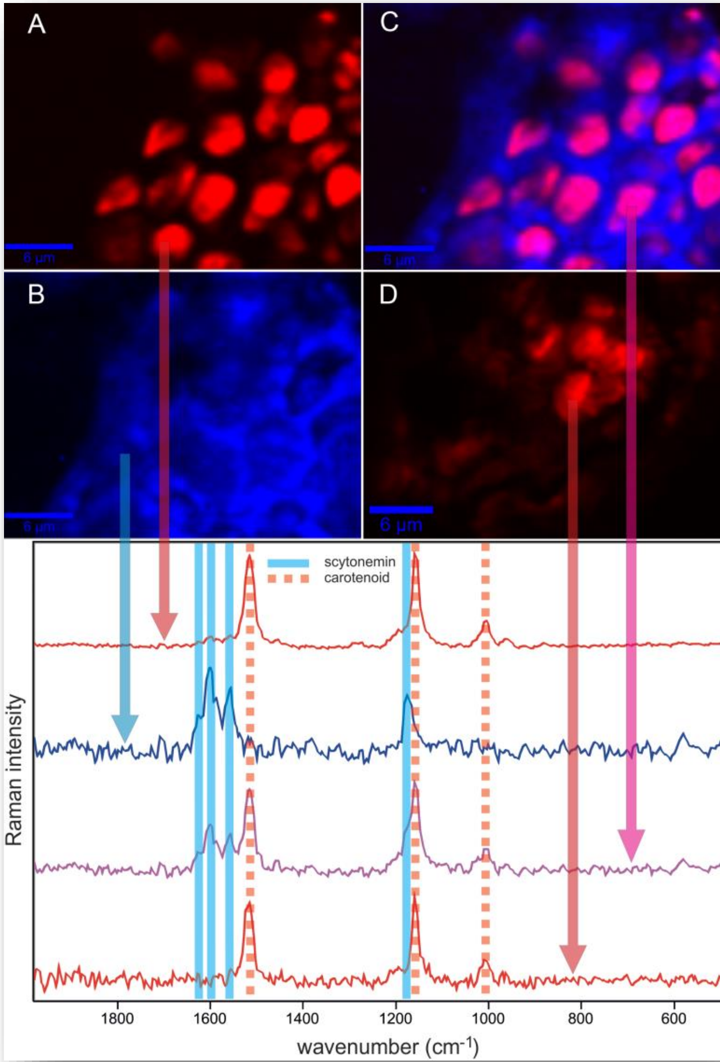
Raman imaging

A, B, C – black aggregates
D – green aggregates





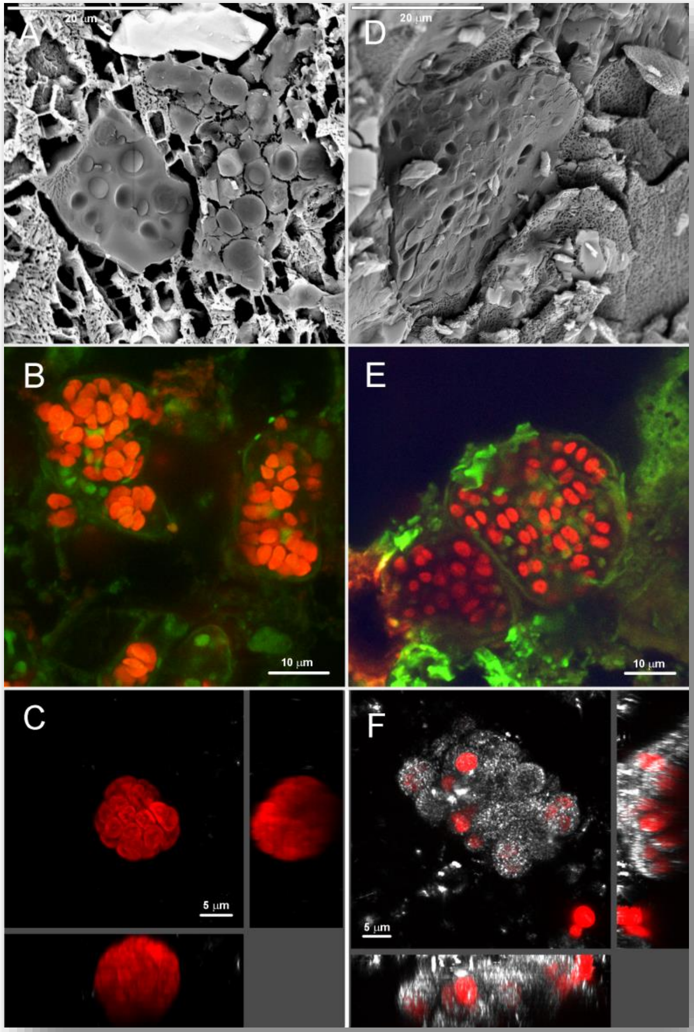
- scytonemin
- carotenoid
- mixture of both



Vítek et al., 2014, FEMS Microbiol. Ecol. (in press)

Halite crust – endolithic cyanobacteria

Green cell aggregates →

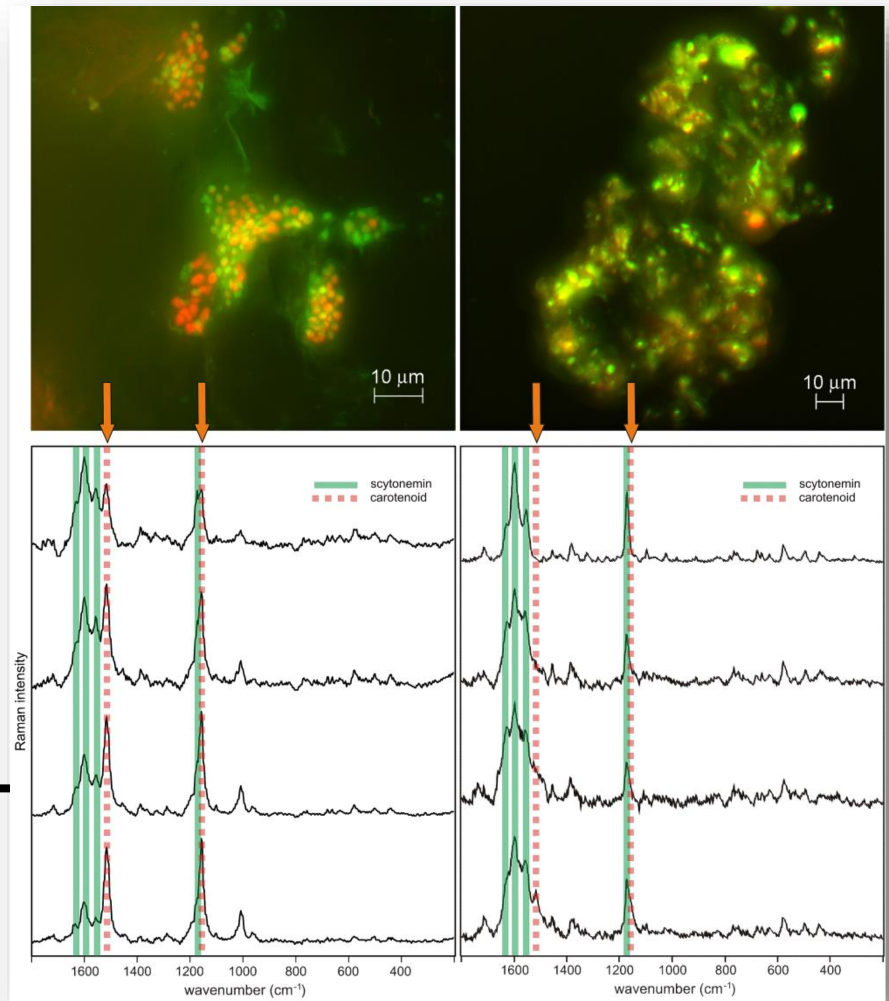


← **Black** cell aggregates

Vítek et al., 2014, FEMS Microbiol. Ecol. (in press)

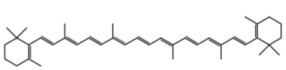
Halite crust – endolithic cyanobacteria

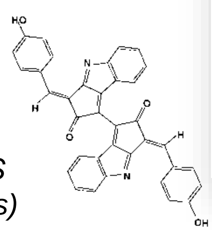
Black cell aggregates
Inside the rock



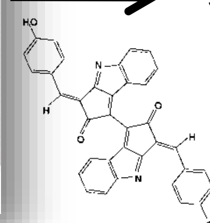
Black cell aggregates
Rock suface

scytonemin
+ carotenoid





scytonemin



Vítek et al., 2014, FEMS Microbiol. Ecol. (in press)

Conclusions

• Raman spectroscopy proved to be an excellent tool for examination of survival strategies of phototrophs in extreme environments – through study of biomolecular response to various stress factors

• Miniaturized Raman system with 532 nm laser was successfull in detection of microbial carotenoids in Mars-analog rocks

• Raman imaging as an excellent tool for mapping of the spatial distribution of biomolecules

• Very strong method in combination with different imaging techniques (Fluorescence microscopy, SEM, etc.)

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