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Raman Spectroscopic Analysis of Cyanobacteria on Mars Analogue Material

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Abstract

One of the outstanding scientific questions of our time is to establish whether life ever existed, or is still active on Mars today. In the scope of the ExoMars mission Raman measurements will be performed with the RLS Spectrometer to identify organic compounds and mineral products as indicators of biological activity [1]. In this context it is investigated if and how the Raman spectrum of cyanobacteria is influenced by the Raman signal of the mineral background.

1. Introduction

Raman spectra of cyanobacteria (*Nostoc commune)* on iron (III) oxide and on mineral mixtures assigned to early acidic and late basic Mars have been measured using two excitation wavelengths of 532nm and 633nm. Cyanobacteria were chosen because it is assumed that photosynthesizing cyanobacteria or anoxigenic photosynthesizing microorganisms as direct ancestors of cyanobacteria appeared for the first time on early Earth 3.8 to 3.5 billion years ago. During this time Mars is supposed to have a more temperate climate with a wet phase, where water was supposed to be liquid on the surface and life might have evolved under similar conditions as on Earth. Thus the potential was given on both of the terrestrial planets that ancestors of the cyanobacteria or the cyanobacteria themselves might have evolved in this short time period because of the quiet similar conditions. Some well known pigments of the photosynthesis apparatus of cyanobacteria such as carotene are detectable by Raman spectroscopy. It is of interest to determine these specific pigments in Mars analogue mineral mixtures. In this context it is important to evaluate how the Raman spectrum of the bacteria is influenced by the Raman signal of the minerals on which they rest.

2. Sample Preparation and Raman Measurements

In order to find the spectra that characterize cyanobacteria Raman measurements were performed on pure cyanobacteria, on two different mineral mixtures free of bacteria, and on cyanobacteria on these mixtures.

The mineral mixtures assigned to early acidic and late basic Mars (see table 1a, b) [2] consist of powders of grain size between 25 µm and 1000 µm. The powder is pressed with 4.5 MPa to pellets to retrieve a smooth surface. Raman scattering on smooth surfaces show less multiple scattering on the sample and the spectra show less noise compared to unpressed powder.

Sample preparation of pure cyanobacteria was performed by streaking colonies of a culture of *Nostoc commune* Strain 231-06 (Fraunhofer IBMT Potsdam) on the Mars analogue minerals reaching different stages of single cell and cluster distribution to be close to natural biofilm conditions.

The Raman measurements were performed with a confocal Raman microscope Witec alpha300 R [3] at room temperature under air at ambient pressure. The Raman laser excitation wavelengths were 532 nm and 633 nm. The spectral resolution of the spectrometer was 4-5 cm^{-1}. A Nikon 10x objective was used. The spot size on the sample was in focus less than 1.4 µm. The laser power was 1 mW on the sample. This value is similar to the one expected for the RLS instrument on ExoMars. Values of the integration time were between 1 s and 100 s per measurement and 1 to 100 measurements of one point on the sample were taken.

First, measurements were taken on each mineral mixture sample without bacteria and on pure

cyanobacteria. Second, cyanobacteria were streaked over the surface of the pellets. Third, measurements and scans were performed on these pellets with varying parameters, integration time and number of accumulations. This set of measurements was carried out to distinguish the bacteria from the pellet background and to investigate the influence of each of the involved material and bacteria on the measurement. If cyanobacteria are present beta-carotene is the dominant feature in the spectrum. Only short integration times have been used to avoid saturation. If cyanobacteria are not present, a longer intergration time is necessary in order to identify the different mineral constituents of the sample.

3. Tables

Table 1a: Mineralogical composition of early acidic Martian regolith simulant

Mineral phase	Weight percent
Fe_2O_3	5
Montmorillonite	45
Chamosite	20
Kaolinite	5
Siderite	5
Hydromagnesite	5
Quartz	10
Gabbro	3
Dunite	2

Table 1b: Mineralogical composition of late basic Martian regolith simulant

Mineral phase	Weight percent
Hematite	13
Goethite	7
Gypsum	30
Quartz	3
Gabbro	32
Dunite	15

4. Results and Conclusions

The spectrum of beta-carotene is identified to describe the presence of cyanobacteria, which is in good agreement with literature [4], [5], [6]. The strong fluorescence of the cyanobacteria above 620nm favours an excitation wavelength of 532nm over 633 nm to avoid the fluorescence disturbing the spectrum between 500 cm^{-1} and 2000 cm^{-1}. Scanning a sample with a mineral/bacteria mixture provides the capability to distinguish between mineral areas with and without bacteria. To get optimal spectra of the mineral mixtures and of the cyanobacteria the mesurement parameters need to be adjusted for each of them separately.

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