ESRF	**Experiment title:** Detection of remnants of bacterial activity on micrometeorites	**Experiment number:** ME-402
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Report:

This work is in press. The abstract is pasted bellow. The full reference of the corresponding paper is:

Lemelle L., Simionovici A., Susini J., Oger P., Chukalina M., Rau Ch., Golosio B., Gillet P. (in press) Journal de Physique IV .

Abstract. X-ray imaging techniques at the best spatial resolution and using synchrotron facilities are foreworn as powerful techniques for the search of small life forms in extraterrestrial rocks under quarantine conditions. Absorption and fluorescence X-ray microtomographies on submillimeter silicate assemblages inside a container reveal the mineralogical microenvironments were life should be looked for in priority. Limitations with respect to bacteria detection are due to the difficulties to obtain information about light elements ($Z \leq 28$), major constituents of biological and silicate samples. The X-ray signature of a "present" bacteria on a silicate surface was defined by X-ray mapping, out of a container, as coincident micrometer and oval zones having strong P and S fluorescences (S-fluorescence being slightly lower than P-fluorescence) and an amino-linked sulfur redox speciation. The detection of a single cell along with new procedure to calculate tomographic views will allow considerable improvements of 3D detection of life by X-ray techniques.

1. INTRODUCTION

The search for small life forms in extraterrestrial rocks started with the report of some evidence of bacteria remnants in the martian meteorite ALH84001. The debate has now moved to more general questions. What are the structural, mineralogical and chemical criteria necessary for assessing the presence of remnants of life forms in rocks from the Earth, meteorites and Mars? These criteria must be further refined in the forthcoming years for the characterization of the Martian samples which should be available in 2010. One of the fundamental issue concerning these samples is to verify whether or not life exists and has existed on Mars. Beside this fundamental issue, these exceptional samples, available in only very small quantity, must first be characterized under quarantine conditions to limit the contamination of both the samples and the terrestrial atmosphere. It is also essential for studying rare extraterrestrial samples to describe them using non-destructive techniques. In this respect, it has been emphasized that X-ray diffraction, absorption, fluorescence and associated imaging techniques at the best spatial resolution using synchrotron facilities are among the most powerful tools to be used for these small samples. We applied them to adress one kind of questions:what are the present or past traces of bacteria activities on silicate surfaces detectable with X-rays?

2. SAMPLES

Bacterial suspension of DH5α strain (*E. coli*) producing a green fluorescent protein and K84 strain (*Agrobacterium rhizogenes*) producing siderophores are prepared at different concentration ranging from 0.05 to 1 DO_{600nm}. Silicate surfaces (001) of micas are placed in the bottom of an optical transparent reactor and are inoculated by the bacterial suspensions in aqueous buffer or bacterium-free buffer. The colonization of the surface is followed in situ, in vivo and in real time for duration varying from 1 min to 4 days by transmission and fluorescence optical modes. Samples are then air-dried. Two kinds of bacterial distribution are produced: biofilm or individualized single-cells.

3. X-RAY DETECTION OF BACTERIAL ACTIVITIES ON SILICATE SURFACES.

Traces of life were not detected, nor identified such as, on all the meteorites studied through a quartz capillary. Therefore, we tested the possibilities of the X-ray fluorescence mapping techniques on bacteria/silicate assemblages prepared in the laboratory and directly placed under the beam.

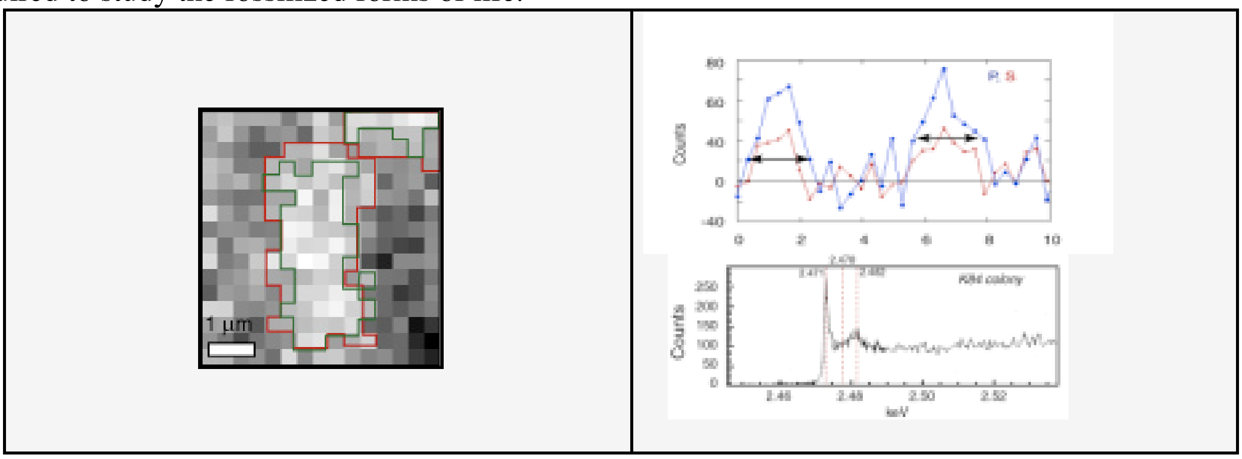
3.1 Experiment

X-ray fluorescence mapping was carried on the scanning X-ray microscope of the ID21 beamline of the ESRF. A monochromatic beam of 2.8 keV was focused with a beamsize of 0.33 x 0.33 μm^2 using a Fresnel zone plate. The fluorescence of the sample was collected under vacuum on a Hp Ge detector. 2D fluorescence images were performed pixel by pixel by scanning the sample in the beam. Some XANES spectra near the sulfur K-edge were recorded by scanning the primary excitation energy using a Si double crystal monochromator.

3.2 Results

X-ray maps show the bacteria deposited on the mica as a rectangular and micrometer zone enriched both in phosphorous (Figure a) and sulfur (Figure b). Xanes spectra near the sulfur edge of the bacteria is as expected, characterized by a strong peak at 2.473 keV attributed to the amino-linked sulfur (Figure b). These results suggest that the minimal thickness of biofilm that can be detected by fluo-microtomography must be smaller than the 30^{th} µm already observed.

The search and diagnostic of any “fresh” bacteria on a silicate surface is possible by X-ray mapping. The X-ray signature of a present bacteria can be therefore defined as coincident micrometer and oval zones having strong P and S fluorescences (S-fluorescence being slightly lower than P-fluorescence), and an amino-linked sulfur redox speciation. Natural silicate surfaces presumably contaminated by bacteria as the Tatahouine surface will be studied. This work is a consistent first step for the definition of past-bacteria, required to study the fossilized forms of life.



a) b)

Figures: a: Map of the normalized intensity of P fluorescence (K_α = 2.0 keV) on a 25 μm^2 mica surface covered by one bacteria. b: Comparison of the variations of the intensities (background free) of the fluorescence of P (K_α = 2.0 keV) and S (K_α = 2.3 keV) along a line scan of 10µm.