ESRF	**Experiment title:** Search for bacterial life forms at the surface of the Tatahouine micro-meteorite	**Experiment number**: ME-590
Beamline: ID21	**Date of experiment**: from: 17 April 2003 to: 22 April 2003	**Date of report**: 1 March 2004
Shifts: 12	**Local contact(s)**: Murielle SALOME	*Received at ESRF:*

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Report: This work is in press. The full reference of the two corresponding papers are:

- Lemelle L., Salomé M., Fialin M., Simionovici A. and Gillet Ph. (2004) X-ray imaging of microorganisms distribution on the Tatahouine meteorite. Spectrochimica Acta, in press
- Garcia B.,Salomé M., Lemelle L., Bridot J-L, Gillet Ph., Perriat P., Roux S., Tillement O. (soumis à JACS) Sulfur K-edge XANES study of dihydrolipoic acid capping gold nanoparticles.

Abstract of the first paper

Microorganisms were searched for among the complex microstructures observed on the surface of a fragment of the Tatahouine meteorite inherited from the Tunisian soil in which they were buried. In this view, the chemical compositions, particularly the nitrogen and sulphur compositions, including the sulphur speciation, were investigated using scanning electron microscopy (SEM), electron probe micro-analysis (EPMA) mapping, and scanning X-ray microscopy (SXM). A few 2□m-thick filaments, partly covered by patches of calcite ensuring they were not deposited by a laboratory contamination, were observed by SEM. The EPMA maps show that the portions free of calcite of the filaments have low but constant contents of nitrogen, sulphur and phosphorus. The SXM maps were recorded at 2473.5 eV, 2478 eV and 2482.2 eV, which are respectively characteristic for amino-acid linked sulphur, sulphite (SO_3^{2-}) and sulphate (SO_4^{2-}). The portions of the filaments detected by EPMA are also those that are enriched in amino-acid linked sulphur. The calculated (N / S) elemental ratio is consistent with the one of the dehydrated *E. coli* matter, contrary to the much lower (P / S) elemental ratio. In living cells, the bulk N and S elements are mainly kept in large polymers by covalent bonds, whereas a significant amount of P belongs to small and reactive molecules. We thus can propose that the observed microstructures are dehydrated microorganism in which most of the elements that were composing the polymers were retained, whereas the small electrolytes and molecules were removed.

Sample: one fragment of the Tatahouine meteorite.

Some fragments of this meteorite present complex microflora and surface mineralogy at the micrometer scale inherited from the Tunisian soil in which they were buried. Bacterial DNA (communication mentionned elswhere), terrestrial carbonates and chemical alteration were previously reported. Sub-micrometer structures observed on these surfaces were also extensively studied, and if some of them are proved to be calcite crystals, then others could be nanobacteria.

Results of the ID21 Experiments for the first paper: STEP 1

The XANES spectra of the meteorite were recorded. The energy was scanned between 2450 eV and 2500 eV in 0.125 eV energy steps and 2s dwell time. Micro-XANES spectra in focused mode were also collected on selected positions of the sample. In this case, the zone plate was moved along the beam axis during energy scans to compensate for the change of focal length, which is energy dependent, and keep the sample in focus. The energy was scanned between 2450 eV and 2500 eV with 0.125 eV energy steps and 2s dwell time. These spectra allowed selecting the energies corresponding to characteristic peaks, at which 2D mappings were performed.

Results of the ID21 Experiments for the second paper: STEP 2

We verified by XANES on *E. coli* colonies that sulphur species are mainly thiols (R-S-H) and disulfides (R-S-S-R) in cells. We worked on a possible chemical procedure to increase the Z contrast of bacteria, in order to facilitate their X-ray detection on mineral surfaces. The XANES spectrum of the E. coli marked with a gold colloid suggests that sulphur atoms reacts with gold particles (Figure b).

A XANES study was thus focused on the interaction of a thiolated molecule (DHLA: a dithiol) on gold colloid. In the case of a solid powder of Au nanoparticles capped by the thiolated molecules (Au@DHLA) or of the capped colloid (liquid solution) , one peak is observed at 2473.6 eV. This peak is shifted by 0.8 eV towards higher energies with respect to the white line position in free DHLA (Figure c), thus confirming that sulfur adsorption on gold nanoparticles occurred.

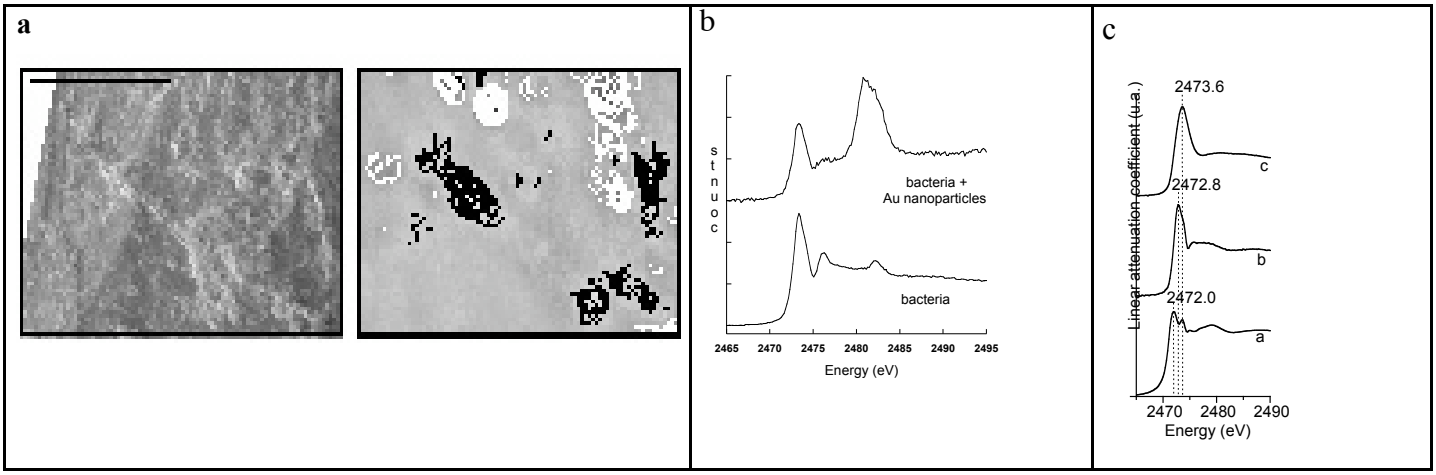

Figure a: SEM image of the studied surface showing filaments, and the map calculated by substracting the map of the S-XRF excited at 2.473 keV to the one at 2.482 keV. Black contrast shows an enrichment in S linked to Amino Acids. The scale bar is 10 microns. Figure b: XANES spectra of dried E. coli, non-marked or marked by gold colloid. Figure c: Sulfur K-edge XANES spectra of (a) thioctic acid (b) dihydrolipoic acid (DHLA) and (c) Au@DHLA (solid state).