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Detection and phylogenetic identification of labelled prokaryotic cells on mineral surfaces using electronic microscopy and X-Ray microimaging

B. Ménez (1), C. Rommevaux-Jestin (1), M. Salomé (2), Y. Wang (1), P. Philippot (1) and E. Gérard (1,3)

(1) IPGP, CNRS-UMR 7154/ Centre de Recherches sur le Stockage Géologique du CO_2 (IPGP/TOTAL/SCHLUMBERGER), Paris, France, (2) European Synchrotron Radiation Facility, Grenoble, France, (3) ESE, CNRS UMR 8079, Université Paris-Sud, Orsay, France (menez@ipgp.jussieu.fr / Phone : 33-1 44 27 77 23)

The implication of intraterrestrial microbes in geochemical cycles is now well recognized. However, owing to the few number of appropriate methodologies allowing to probe these ecosystems, the exploration of their metabolic diversity, energy sources, and biogeochemical transformations is limited. Here, we demonstrated the ability of electronic microscopy and X-ray imaging using synchrotron radiation to localize and investigate the phylogenetic affiliation of individual prokaryotic cells on mineral surfaces when combined with a newly developed protocol based on fluorescence *in situ* hybridization coupled to ultra-small immunogold. For this purpose, we hybridised universal and specific fluorescein-labelled oligonucleotide probes to the ribosomal RNA of prokaryotic microorganisms in heterogeneous cell mixtures. We then used antibodies against fluorescein coupled to subnanometer gold particles to label the hybridised probes in the ribosome. After increasing the diameter of the metal particles by silver enhancement, the specific gold–silver signal was visualised on various substrates (e. g. carbonates, quartz, basaltic glass) by optical microscopy, transmission electron microscopy (TEM), scanning electron and X-ray microscopy (SEM and SXM, respectively). The possibility of associating simultaneously with the phylogenetic identification of microorganisms, the chemical and structural characterization of associated mineral phases (i. e. inorganic substrate and biomineralizations), offers great interest for assessing the geochemical impact of subsurface microbial communities and unraveling microbe and mineral interactions in the deep biosphere.