Coupling identity and metabolic function of single cells in environmental microbiology with NanoSIMS

H.U. Ehrke[1], F. Horréard[2], F. Hillion[2]

1. CAMECA GmbH, Carl-von-Linde-Str. 42, D-85716 Unterschleissheim, Munich, Germany.
2. CAMECA, 29 Quai des Grésillons, 92622 Gennevilliers Cedex - France.

horreard@cameca.com
Keywords: nanosims, spectrometry, FISH, microbiology, environment

We describe an instrumental and methodology development aiming at coupling microorganism's phylogenetic identity and function in soil, water, sediment or other ecosystems. By combining a pulse-chase experiment with in situ hybridization using a halogen labeled oligonucleotide probe, metabolically active, uncultured cells can at the same time be phylogenetically identified.

Method: A NanoSIMS (Secondary Ion Mass Spectrometer) instrument with 50nm lateral resolution has been used in order to obtain in one single measurement:

1) the phylogenetic identity of single cells through the visualization of oligonucleotide probe hybridization signal (similar to FISH method but replacing fluorescent oligonucleotide by an isotopically or elementally labeled probe),

2) the quantitative measurement of the metabolic activity of these individual cells by using stable isotope labeling in pulse-chase experiment.

SIMS is based on the sputtering and ionization of surface atoms by a focused beam of primary ions scanned across a sample. Ejected secondary ions are here mass filtered in a magnetic sector and detected in a multicollection (up to seven masses in parallel). Samples must be dehydrated before analysis as the instrument works under high vacuum.

For marine microbiology, cells can be collected on gold-coated polycarbonate filters and simply dried. For soil experiments, resin embedding followed by microtoming can be used as in Electron Microscopy.

Results: we show application of the technique allowing quantitative metabolism activity measurement (using 13C and 15N labeling experiments) of individual phylogenetically identified bacteria (using F or I labeling). The capability of measurement on individual uncultured cells from their natural environment reveals heterogeneities inside bacteria populations. It allows measurement of transfers of labeled species and interactions between different individual cells. It can be correlated with TEM images of same samples.

Coupling a stable isotope labeling experiment with sequential harvesting can also give temporal information.

Finally we introduce two newly developed modes of operation:

1) Low energy (< 100eV) reactive ion pre-sputtering before analysis. This allows to enhance the ionization yield by orders of magnitude with limited sputtering of the sample. This is interesting for very surfacic measurements (ex: membranes).

2) Faster, higher current isotope analysis of whole cells. In this mode the primary ion spot originally of 50nm size is enlarged up to a few µm (the whole targeted cell size). Each individual cell isotopic ratio is automatically chained in so-called “grain mode”. The instrument's throughput is then dramatically increased, at the cost of the loss of intracellular resolution.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-239, © Verlag der TU Graz 2009