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TOF-SIMS IMAGING OF BIOMARKERS IN MICROSCOPIC SECTIONS OF MICROBIAL MATS

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Time of Flight - Secondary Ion Mass Spectrometry (ToF-SIMS) is a technique that allows to simultaneously analyse inorganic and organic molecules on solid surfaces (Benninghoven et al., 1994, Belu et al., 2003). A most advantageous property of ToF-SIMS is its ability to generate images displaying the intensities of any detected ion in a given area of interest while achieving spatial resolution on a very small scale (sub-µm, e.g. Hagenhoff, 2000). To date, this is not possible with any of the extract-based techniques routinely used in biomarker studies, namely GC/MS and LC/MS (coupled gas chromatography/mass spectrometry, coupled liquid chromatography/mass spectrometry). In organic ToF-SIMS analyses, compound identification can be achieved through the precise mass determination of the molecular species, ideally in combination with GC/MS and/or LC/MS data.

We used ToF-SIMS with a Bi_3^+ cluster primary ion source for the study of lipid biomarkers in native microbial mats from methane seeps in the Black Sea (Michaelis et al., 2002), and from the subsurface biosphere of the Äspö tunnel in Sweden (Pedersen, 1997). On 10µm-thick microscopic cryosections of a Black Sea microbial mat, for instance, we simultaneously analysed archaeal isopranyl core lipids, together with their intact diglycoside (gentiobiosyl-) derivatives. Utilizing the imaging capability of ToF-SIMS, the spatial distributions of these biomarkers were mapped at a lateral resolution of < 5µm in 500 x 500μm^2 areas on sections. Different biomarker 'provenances' within this area were distinguished by individual patterns of the isopranyl glycerol diethers archaeol and hydroxyarchaeol, glycerol dialkyl glycerol tetraethers (GDGT), and gentiobiosyl-GDGT (Fig. 1).

Because ToF-SIMS is quasi-non-destructive it was possible to examine the studied areas 'post-measurement' using conventional microscopy, which enabled to associate the individual lipid patterns with particular morphological traits. Hydroxyarchaeol was directly associated with the precipitation of irregular, methane-derived $CaCO_3$ crystallites, whereas

GDGT-rich regions of the section revealed fluorescent, filamentous microorganisms showing the morphology of known methanotrophic ANME-1 archaea.

ToF-SIMS molecular imaging, in conjunction with other techniques, reveals interesting perspectives for a wide range of geological, ecological, biological, and medical applications, which share the need to detect and localize organic and inorganic compounds at high resolution. Potential geobiological applications include the elucidation of microbially assisted mineral precipitation, the analysis of organic compounds in unculturable microorganisms, and the clear-cut assignment of biomarkers to their biological source.

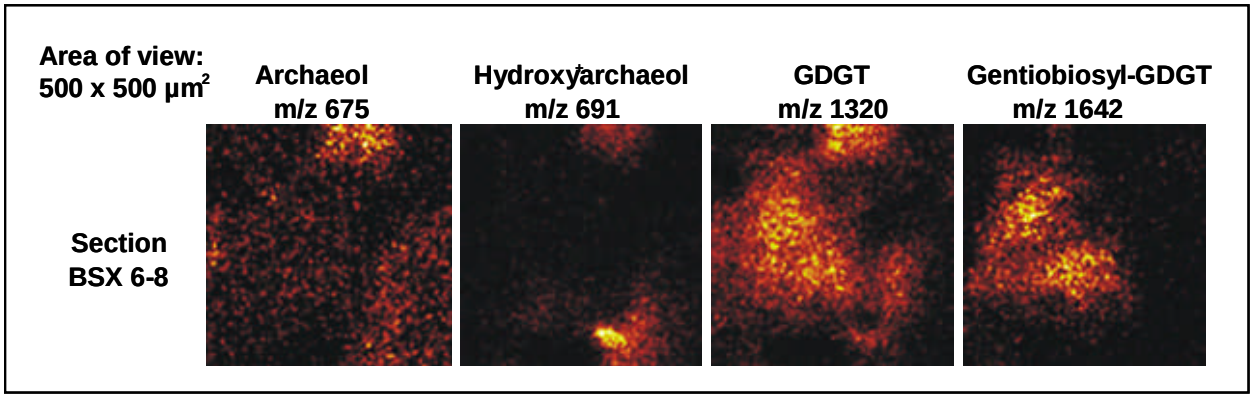

Figure 1. ToF-SIMS images of archaeal lipid biomarkers (as sodium adducts $[M+Na]^+$) on a microscopic section of a methanotrophic microbial mat from the Black Sea, revealing the lipid patterns of individual microbial colonies. Brightness corresponds to relative signal yield.

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