

## **INTACT POLAR LIPIDS IN THE MARINE DEEP BIOSPHERE – STRUCTURAL DIVERSITY AND QUANTITIES**

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The deep biosphere is thought to contain live biomass that represents up to 10% of the total carbon in live cells of our planet (Parkes et al., 2000). This vast ecosystem has become the research focus of microbiologists and geochemists to address key questions like: What types of microbes thrive in deeply buried sediments? And, what are the processes they are mediating? Recent studies have provided information on metabolic activities and quantities of deeply buried prokaryotic cells (Biddle et al., 2006; Inagaki et al., 2006; Schippers et al., 2005, 2006). However, some fundamental questions remain unresolved or even lead to highly controversial answers. For example, various techniques appear to disagree already at the domain level on WHO actually inhabits this ecosystem. Molecular biological methods like catalyzed reporter deposition - fluorescent in situ hybridization (CARD-FISH) and quantitative polymerase chain reaction (Q-PCR) suggest a predominance of bacterial over archaeal cells (Schippers et al., 2005; Inagaki et al., 2006). On the other hand FISH and intact polar lipids (IPL) suggest a predominance of archaea among live prokaryotes (Biddle et al., 2006).

Intact membrane lipids are considered to be markers for live subsurface cells (Sturt et al., 2004) since the polar headgroup is cleaved off the core lipid after cell death. Careful determination of response factors in calibration series with IPL standards shows no preferential detection of one compound class over the other. However, there are several principal difficulties that have to be overcome while analyzing environmental samples: general low abundance of target IPL compounds and the complex matrix with a high background of degraded material.

A robust analytical protocol based on high performance liquid chromatography coupled to ion trap multistage mass spectrometry (HPLC-IT-MS<sup>n</sup>) was developed and applied to a set of samples from the Peru margin surface (RV Sonne SO147), ODP Legs 201, 204, 207, and IODP Expeditions 301 and 311. The major bacterial IPLs identified comprise phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) diacylglycerides with C<sub>16</sub> and C<sub>18</sub> acyl groups. The diversity in archaeal IPLs is limited to different core lipid structures, e.g. archaeol-based diether lipids vs. glycerol dibiphytanyl

glycerol tetraether (GDGT) lipids with various combinations of sugar headgroups. The composition of archaeal IPLs appears to be linked to the diversity of archaeal phylotypes.

The observed IPL concentrations were converted to cell concentrations for comparison to results from molecular biological approaches. Surface sediments are dominated by bacterial IPLs with possible admixtures of eukaryotic lipids while archaeal lipid concentrations are low. Concentrations of bacterial lipids decline rapidly to levels significantly lower than those of their archaeal counterparts. The analysis of ODP/IODP samples from deeply-buried horizons shows evidence for bacterial lipids in about 20% of samples analyzed to date. On the basis of these observations in combination with results of degradation experiments of archaeal and bacterial IPLs under typical anaerobic sedimentary conditions (Pamela Rossel et al., unpubl. data), we interpret the predominance of archaeal IPLs as evidence for a far more important role than suggested by other techniques. A composite view comprised of ~ 60 samples from both surface and deeply buried sediments on the abundance of total prokaryotic lipids in subsurface environments provides an interesting comparison to corresponding data on intact cells (cf. Parkes et al., 2000): concentrations of IPLs decline more rapidly with depth than the counts of intact cells from a global data set.

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