Hydrothermal fluids from the oceanic crust stimulate metabolic activities of deep-biosphere populations (IODP Leg 301)

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Microbiological studies on sediment cores collected during DSDP, ODP and IODP have consistently demonstrated the presence of microbial communities in deep marine sediments down to several hundreds of meters below the seafloor (Parkes et al., 1994; 2000). Furthermore, recent investigations indicated the extension of the deep biosphere into the upper layers of the oceanic crust (Cowen et al., 2003; Cowen, 2004, Huber et al., 2006, Nakagawa et al., 2006). These porous volcanic layers are characterised by the circulation of seawater, forming the largest aquifer on Earth.

Due to their geochemical composition, circulating fluids are supposed to fuel the deep biosphere by intrusion of oxidized compounds from below (DeLong, 2004). To test this hypothesis by measuring microbial activities, we have participated in IODP Leg 301 to the Juan de Fuca Ridge, in the northeast Pacific Ocean. This location is one of the most intensively studied areas in terms of fluid flow hydrology and impact on sedimentological settings (Wheat et al., 2000; Fisher et al. 2005). At IODP Site 1301 (water depth: 2650 m, sediment coverage: 265 m) the diffusion of sulfate into the sediment column from both sides, the seafloor and the basement, is resulting in the formation of two sulfate-methane interfaces (Fig.1). Such transition zones are possible hot spots of microbial activity.

Our investigations focussed on the quantification and characterisation of indigenous microbial populations, metabolic activity measurements, and the isolation of abundant deep-biosphere inhabitants with special emphasis on sulfate-reducing bacteria. During Leg 301 high quality, non-contaminated sediment samples were obtained, as indicated by PFT measurements (Lever et al., 2006). Direct cell counting was performed using Sybr Green I as a fluorescing dye. Total cell counts followed a similar trend with depth as found at other ODP sites (Parkes et al., 1994). They decreased from $5.2 \cdot 10^8$ in the upper sediment samples to $1.7 \cdot 10^7$ cells per cm^3 in deeper layers, while a slight increase was observed towards the basement. This increasing microbial abundance near the bedrock was confirmed by molecular biological methods (Fig.1). Elevated cell numbers at the sediment-basement interface gave the first evidence for a microbial community stimulated by crustal fluids.

As a general measure for metabolic active microorganisms we determined exoenzyme activities in sediment samples, using substrate analoga (MUF). Potential phosphatase activity was low in the phosphate-rich upper fifty meters of the sediment column (about 30 µmol of MUF-phosphate$\cdot g^{-1} \cdot h^{-1}$). The activity increased slightly in phosphate-poor layers down to 166 mbsf and tenfold within the next twenty meters and had its maximum in the phosphate-depleted sediments directly above the basement (Fig. 1). This finding indicates, that near the bedrock not only the numerical abundance of the microbial community is influenced by crustal fluids but also their general metabolic activity.

The diffusion of sulfate from the oceanic crust into the methanogenic zone stimulated sulfate reduction and anaerobic oxidation of methane (AOM). Sulfate reduction rates (SRR) decreased from 8 nmol·cm^{-3}·d^{-1} at the seafloor by a factor of 450 within the upper ten meters and dropped strongly to about 0.1 pmol·cm^{-3} d^{-1}. A tenfold increase of SRR was detected at the lower sulfate-methane interface and remained elevated within fluid-influenced layers. AOM rates were generally low, but measurable along the entire sediment column. A maximum of 4.5 pmol·cm^{-3}·d^{-1} was observed around the lower sulfate-methane transition zone exceeding the sulfate reduction rates within these layers (Fig.1).

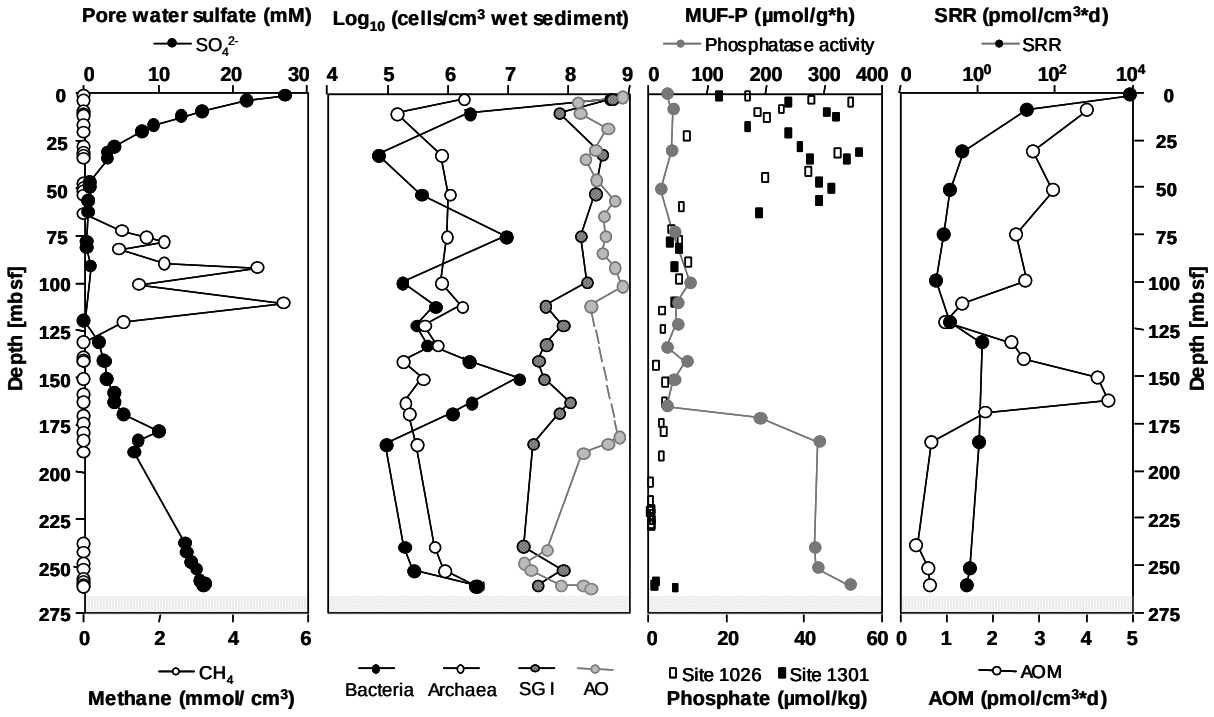

Fig. 1: Depth profiles of geochemical parameters, microbial abundance and metabolic activity.
Total cell counts were determined using Acridine orange (AO) and Sybr Green I (SG I). The number of *Archaea* and *Bacteria* were quantified after using real-time PCR.

The cultivation of indigenous microorganisms was performed in dilution series onboard the Joides Resolution immediately after core recovery. Microscopic analysis showed microbial growth in the majority of cultures regardless the sediment depth. In relation to total cell counts up to 3.6 % of all microorganisms were cultivated. The highest cultivation efficiency was achieved with sediment samples from the transition zones.

Molecular screening was used to overview the diversity of cultivated microorganisms. The first subcultures from representative layers of the entire sediment column exhibited unique colonies (Fig.2). Enrichments contained bacteria that were closely related to *Vibrio* sp., *Desufobacula toluolica* and *Cytophaga sp*. as well as one so far uncultured *Firmicute*, previously detected in a clone-library from the Nankai Trough. The most abundant cultured microorganisms will be quantified molecular biologically to determine their distribution along the sediment column (Süß et al., 2006). The physiological characterisation of isolates will help to understand their adaptation to the deep biosphere (Batzke et al., 2007).

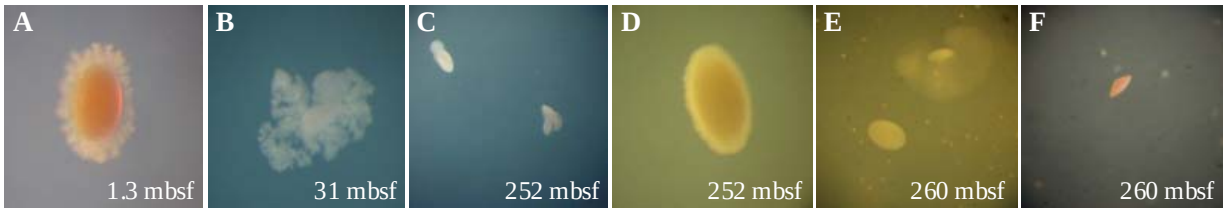

Fig. 2: Deep-agar colonies formed after up to six months of anoxic incubation at 20°C in medium amended with low concentrations of 36 different substrates.

In conclusion, our studies have confirmed the hypothesis that hydrothermal crustal fluids support microbial life in the deep biosphere. We were able to measure microbial activities along the entire sediment column. Both, microbial abundance and metabolic activity were stimulated at the fluid influenced sulfate-methane transition zone and near the sediment-basement interface. Regarding the worldwide expansion of the crustal fluid aquifer, we assume that this impact is a major driving force for deep subsurface populations on a global scale.

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