PROJECT REPORT

Basaltic ocean crust is formed at the axis of mid-ocean spreading ridges. As new ocean floor is formed and moves away from the spreading center, it is cooled by the interaction with seawater. The accumulation of overlying sediments on the ridge flanks and ocean basins prevents continued advective heat loss and results in strong hydrothermal gradients, which drive the largely lateral flow of low temperature fluids (Fig. 1). Aging crustal porewaters remain isolated within buried upper oceanic basement, subjected to increasing temperatures and pressures as plates move away from spreading ridges. There is increasing evidence that supports the presence of actively growing microbial communities within basaltic porewaters. Strong physical gradients acting at different spatial and temporal scales however, may influence the potential metabolic processes active in these habitats. Moreover, long-term changes in fluid temperature may lead to a turnover in the SRM community composition, such that numerically minor members of a SRM population that are more tolerant to higher temperatures become dominant given sufficient time, and their metabolic capabilities could then dictate the response of the net community and the stability of microbial metabolic processes.

The current work intends to specifically characterize the microbial community present in basaltic porewater samples. We hypothesize that the microbial physiological responses to ambient temperatures may be used to characterize the nature, in terms of the geographical origin, of the microorganisms present in deeply buried habitats.

Figure 1. Summary from selected results from ODP leg 168 and related experiments. A. Interpreted composite cross section (red dotted line) from active spreading center to the west. B. Summary of thermal data and chemistry of basement fluids (Shipboard Scientific Party, 2004.).
During the past cruise to the Juan de Fuca Ridge flank (June, 2011) on board R/V Atlantis (Fig. 2), the correlation between active microbial metabolic processes in fluids from ocean crust and the physical parameters that characterize these habitats was explored.

**Figure 2.** A) R/V Atlantis B) We used recently developed seafloor sampling systems to collect large volumes of high integrity basaltic fluids from Circulation Obviation Retrofit Kit (CORK) observatories inserted into crustal boreholes 1025C (47°88.7’N, 128°64.8’W) and U1301 (47°45.2’N, 127°45.8’W) see figure 1B.

The specific goals in this proposed study were: 1) Characterization of temperature and pressure as physiological variables by a quantitatively analysis of the metabolic capacities of microorganisms 2) Exploration of the functional dynamics of physiological bacterial groups in deep subsurface basaltic fluids.

The proposed project has made substantial progress towards the characterization of microbial respiration activity in deep subseafloor habitats and the development of a high pressure thermal gradient system to study the pressure and temperature response of microbial metabolism resulting in the following manuscripts:

1. **Co-evolution of hydrothermal seawater and microorganisms in deep subseafloor basaltic crust (in prep.)**

   Alberto Robador, Sean P. Jungbluth, Douglas E. LaRowe, Robert M. Bowers, Huei-Ting Lin, Michael S. Rappé, Jan P. Amend, and James P. Cowen
Hydrothermal fluids sampled from the upper oceanic basaltic crust of Juan de Fuca Ridge (JFR) flank show evidence of being highly altered over a prolonged time period. However, it is unclear to what extent these fluids have been compositionally modified by microbial activity or how the evolution of such fluids influence the activity and dispersal of microorganisms. In order to address these issues, the rates of microbial sulfate reduction have been quantified and the distribution of sulfate reducing microorganisms (SRM) along the temperature and chemical gradients that occur in fluid circulation pathways in the JFR system have been determined. The rates of microbial sulfate reduction decrease along the JFR flank constrained by the changes in fluid temperature and chemical composition, likely due to decreasing energy availability. Moreover, analysis of the sequence diversity of dissimilatory (bi)sulfite reductase (dsr) genes and small subunit ribosomal rRNA (SSU rRNA) genes of putative SRM across these physicochemical changes reveal important differences in community composition of SRM. Eight novel monophyletic lineages of dsrB genes with no closely related cultivated sequences are described from both the bacterial and archaeal domains in older and more reacted fluids, representing ~74% of the total gene clones described. The data presented in this study can promote understanding of the activity and extent of microbial life in the deep subseafloor biosphere.

2. An incubation system to determine the temperature and pressure as microbial physiological variables in low-energy crustal fluids (in prep.)

Alberto Robador and James P. Cowen

High pressure thermal gradient systems are commonly used to study the pressure and temperature response of microbial metabolism. Current designs, however, have several practical limitations related to the maximum operational pressures and, most important, to the monitoring of multiple samples under constant pressure during long-term incubation experiments. Here we introduce an advanced instrument for the incubation of liquid samples at ultra high pressures (1000 Bar) and temperature gradients (up to 150 °C) that allows continuous sampling during time-course experiments and quantification of real-time changes on manganese, iron and sulfur species of intermediate oxidation state. Current prototype has already been used on board R/V Atlantis during past cruise to the Juan de Fuca Ridge Flanks 2011) and it is on a final developmental stage.