



## Diversity of endolithic bacteria in seafloor basalt

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In recent years, a number of studies have provided evidence suggesting that chemical energy stored in the oceanic lithosphere can support a substantial microbial community. At mid-ocean ridge systems, fresh basalt is exposed at the seafloor and interacts with cold, oxygenated seawater. The chemical energy associated with the oxidation of reduced components in the basalt during water-rock reactions at the seafloor may contribute to microbial growth in these settings. Yet, these bare-rock habitats remain poorly studied with respect to the physiological and phylogenetic diversity of microbial communities that may be supported by oxidative weathering reactions.

In this study, we have investigated the phylogenetic diversity of microorganisms living within basaltic crust at the seafloor and the changes in these microbial communities with increasing extents of oxidative rock alteration. Seafloor lavas were sampled using the DSV *Alvin* from the East Pacific Rise (EPR) between 9° 28'N and 9° 50'N during two research cruises (AT11-7 and AT11-20) on the R/V *Atlantis* in 2004. Samples for molecular biological analyses were selected to represent a variety of flow morphologies, ages (up to 20 kyrs), and degrees of alteration. Total environmental DNA was extracted from crushed basalts and amplified by polymerase chain reaction (PCR) using 16S rRNA bacteria specific primers. Clone libraries were then constructed and sequenced for phylogenetic analyses. Microscopy techniques such as confocal laser scanning microscopy and scanning electron microscopy were used to assess the overall extent of basalt alteration. These methods were supplemented with catalyzed reporter deposition-fluorescent in-situ hybridization (CARD-FISH) to identify and quantify active bacterial communities on the surfaces of natural, unprocessed samples.

Phylogenetic and microscopic analyses indicate that diverse, yet distinct populations of bacteria inhabit different lavas, and these microbial communities shift with changes

in basalt alteration state. More specifically, an increase in species level diversity is observed with respect to an increase in weathering product accumulation (oxides, clays, etc.). Although our methods do not allow for direct determination of physiology, the close relation of our sequences to sequences of organisms with known metabolisms permits some preliminary assessments about the microbial communities supported by basalt alteration. It can be inferred that metal and sulfur-oxidizing autotrophic communities first colonize the fresh, unaltered rock substrate and provide the basis for more complex, heterotrophic communities as weathering persists. The results from this study provide the type of information necessary to better assess the impact that microorganisms have on ocean crust evolution.