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Extremophiles under pressure: Integrating experimental and field studies at deep-sea hydrothermal vent sites

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In the great depths of the Earth's interior, forces capable of moving continents sculpt the seafloor and form volcanoes transferring materials and energy to the ocean. Seawater circulation within these magmatic provinces results in the development of deep-sea hydrothermal vents that are often considered to resemble the conditions on early Earth. It is under these extreme conditions that, by a synergy of chemistry and biology, life thrives in the absence of light and sparse nutrients. Notably, anaerobic chemolithoautotropic bacteria that inhabit these extreme environments tend to branch deeply in the tree of life, probably retaining relics of early metabolic pathways.

Here, we present data from shipboard continuous-culturing incubations of hydrothermal vent fluids conducted during an R/V Atlantis-ROV Jason/Medea expedition to the deep-sea vents sites (2500 m) at 9^{o}50'N East Pacific Rise (January of 2014). This was accomplished through a multi-disciplinary and multi-institutional collaborative effort to collect; transfer and culture vent fluids from the diffuse flow sites, onboard the vessel under seafloor pressure conditions (250 atm). Experiments were designed to study the cycling of N through the metabolic processes of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) under *in-situ* deep-sea vent temperature and pressure conditions.

In detail, we studied the growth/metabolism of nitrate-reducing microorganisms at mesophilic (30 ℃) and thermophilic (60 ℃) conditions at pressures ranging from 5 to 250 atm. A high pressure bioreactor was employed onboard the ship to allow direct study of the biological functions of microorganisms collected from diffuse-flow vent fluids (Fig. 1). This experimental approach facilitates continuous culture of microorganisms at temperatures ranging from 25 to 120 ℃ and pressures up to 680 atm. The system allows incubating microbial communities in medium enriched with dissolved gases, under aerobic or anaerobic conditions, while permitting periodic sampling of the incubated organisms with minimal physical/chemical disturbance inside the reactor.

Experiments conducted simulate the subsurface biosphere environment and the continuous mixing of seawater and hydrothermal fluids in the oceanic crust. Vent fluids were delivered to the bioreactor under high pressure and homogeneously mixed with aqueous media solution enriched in dissolved nitrate, hydrogen, and ^{13}C-labeled bicarbonate to facilitate the growth of nitrate-reducing chemolithoautotrophic bacteria. Two distinct sets of experiments were carried out for 356 and 100 hours. During the course of the experiments we monitored the growth of deep-sea microbial communities by measuring cell density and the concentrations of dissolved aqueous species directly involved in nitrate based metabolism, such as NO_3^-, NH_4^+, $H_{2(aq)}$ and $H_2S_{(aq)}$. Subsamples were also collected for a number of shore based analyses to determine: i) the $^{15}N/^{14}N$ isotope composition of NO_3^-/NH_4^+ and constrain kinetic isotope effects associated with denitrification/DNRA; ii) to study the rates of autotrophic carbon fixation by NanoSIMS; iii) to perform single cell genomics on the microbial populations grown in the bioreactor and (iv) to isolate and characterize novel microorganisms from the communities established in these experiments. In short, experimental results constrain the function and metabolic rates of the native denitrifying microbial communities residing at moderate temperature conditions (30 ℃), while DNRA metabolic pathways were identified for the populations residing at higher temperature diffuse flow fluids (60 ℃).

キーワード: extremophiles, deep-sea hydrothermal vents, chemolithoautotropic bacteria, high pressure, continuous culture, nitrate-based metabolism
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