Limits of Life in the Deep Subseafloor Biosphere: New Insights from IODP Expedition 337

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Introduction: Deep drilling of marine subsurface offers unique opportunities to explore how life persists and evolves in the Earth's interior ecosystems. There are very few natural environments on Earth's surface where life is absent; however, the limits to life are expected in the subsurface world. Processes that mediate genetic and functional evolutions of the deep life may be very different to those in the Earth's surface ecosystems. Previous studies of the subseafloor sediment have demonstrated that activity of microbial communities is generally extremely low, mainly because of the limit of nutrient and energy supply [1,2]. However, the limits to habitability in deep subseafloor sediments have still remained to be determined; which constraints involve a variety of geophysical and geochemical properties, such as temperature, pH, pressure, salinity, porosity, and availability of nutrient and energy. Understanding of these fundamental issues on Earth's deep biosphere may hold the clue to the mysteries of primordial microbial ecosystems on our planet or the life's habitability on other planetary bodies.

Integrated Ocean Drilling Program (IODP) Expedition 337: Expedition 337 was the first expedition dedicated to subseafloor microbiology that used riser drilling technology of the deep-sea drilling vessel *Chikyu* [3]. IODP drill Site C0020 is located in a forearc basin formed by the subduction of the Pacific plate off the Shimokita Peninsula, Japan, in the northwestern Pacific at a water depth of 1,180 m. Seismic profiles suggested the presence of deep, coal-bearing horizons at ~2 km subseafloor depth. Our primary objectives during Expedition 337 were to study the relationship between the deep microbial biosphere and the subseafloor coalbed and to explore the limits of life in horizons deeper than ever probed before by scientific ocean drilling. Among the questions that guided our research strategy was: Do deeply buried hydrocarbon reservoirs such as coalbeds act as geobiological reactors that sustain subsurface life by releasing nutrients and carbon substrates? To address this question and other objectives, we penetrated a 2,466 m deep sedimentary sequence with a series of coal layers at ~2 km below the seafloor. Hole C0020A is currently the deepest hole in the history of scientific ocean drilling, and hence provided an unprecedented opportunity to study the limits of life in the subseafloor biosphere.

Preliminary results and perspective: During Expedition 337, over 1,700 microbiological and biogeochemical samples have successfully been obtained, for which rigorous contamination controls enable differentiation of contaminants from indigenous microbial communities. The estimated temperatures in 2 km-coalbed layers are ~50ºC and thus provide confortable conditions for microbial life. We conducted gas chemistry and isotopic analyses using a new real-time mud-gas monitoring during riser-drilling operation, which provided the first indication of biologically mediated CO_2 reduction to methane at the 2 km-deep coalbed layers. The numbers of microbial cells are generally notably lower than those expected based on the global regression line of sedimentary microbial biomass on the Pacific margins [4]. What are the constraints for the very low biomass in the deep sedimentary habitats? Interestingly, increase of microbial biomass was observed at the coal layers. This finding suggests possible contribution of microbial activity to the diagenetic process of organic matter, subsequently providing nutrient and energy substrates to the living biomass.

On-going efforts on the correlation between cell abundance and various physical properties, which were characterized by over 900 discreet samples and wireline logging, as well as cultivation-dependent and –independent molecular ecological and biogeochemical studies, will provide us some new insights into the limits of life and habitability in the deep subseafloor biosphere.

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