

## Exploring life at its limits in the seafloor biosphere through scientific ocean drilling

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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 surface ecosystems on our planet. Previous studies of the seafloor sedimentary biosphere have demonstrated that activity of microbial communities is generally extremely low, mainly because of the limit of nutrient and energy supply. However, the limits and habitability in deep seafloor sediments have still remained largely unknown; seafloor microbial biomass, activity and functional/taxonomic/physiological diversity are possibly constrained significantly by each or the combination of multiple geophysical and geochemical factors, such as temperature, pH, pressure, salinity, porosity, and availability of nutrient and energy. In addition, understanding of the limits and habitability of life in the deep seafloor biosphere may hold the clue to the issue regarding the origins and evolution of seafloor life and its ecological consequences in Earth's history.

The Integrated Ocean Drilling Program (IODP) Expeditions 329 and 337 were the microbiology and biogeochemistry-dedicated scientific ocean drilling, targeting on very distinct oceanographic settings; in 2010, using the *JOIDES Resolution*, Expedition 329 explored deep-water sediments (water depth: 3,740~5,695m, sediment depth: ~131m) down to the basement at 7 sites in the ultra-oligotrophic South Pacific Gyre. During Expedition 337 in 2012, the *Chikyu* explored ultra-deep sediments (water depth: 1,180m, sediment depth: ~2,466m) associated with deeply buried coalbeds in the northwestern Pacific coast. The samples and data collected during these drilling expeditions, as well as rapid technological developments, provided unprecedented opportunities to study the limits and habitability of life in the seafloor sedimentary biosphere.

The number of microbial cells is measured by newly developed cell separation and fluorescent image-based cell count techniques, revealing that cell abundances in both ultra-oligotrophic and deep coalbed-associated sediment samples are several orders of magnitude lower than those previously observed in shallow sediments of the ocean margins. The finding of very small microbial populations under two distinct extreme seafloor conditions leads to subsequent questions: What are the environmental constraints for habitability of seafloor life? What are ecological roles of seafloor microbial activity in biogeochemical carbon and other elemental cycles? What are genetic and metabolic functions and its diversity of microbial communities? How and why can they live in such deep and extremely energy-limited conditions?

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