Global census of microbial life in marine subsurface sediments.

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Introduction: Over the past decade, it has been demonstrated that nemerous microbial life are distributed in global marine subsurface sediments, and its total biomass is estimated to be 2.8 x 10^{28} cells, corresponding ~1% of biomass carbon on Earth [1]. To date, culture-independent molecular techniques have been dramatically improved, enabling us to provide detailed views of naturally occurring microbial communities, even for low biomass and cultivation-resistant microbial communities in a variety of geological habitats [2]. For example, using a newly developed hot-alkaline DNA extraction method [3] together with an improved cell separation technique [4], it is now possible to detect, enumerate and identify the deep sedimentary microbes more accurately and sensitively than before. Regarding quantification microbial genes in environmental DNA, the conventionally used real-time PCR assay is significantly hampered by the PCR inhibitors such as humic acids and polysaccharides, especially for organic-rich sedimentary habitats on ocean margins. However, a recently developed digital PCR using microfluidic devices is less affected by such inhibitory substances, providing absolute quantification of the target genes [5]. Using these technical advances on quantitative and qualitative molecular ecological approaches, one of the major scientific objectives on the deep subseafloor biosphere research is to understand the global census of subseafloor microbial population and its diversity.

Materials and Methods:

Sediment Samples.

Over 200 sediment samples were collected in 15 drilling sites; e.g., the eastern equatorial Pacific and Peru Margin (ODP Leg 201), Juan de Fuca ridge flank (IODP Exp. 301), South Pacific Gyre (IODP Exp. 329), Nankai Trough (IODP Exps. 315 and 316), off Shimokita of Japan (CK06-06, IODP Exp. 337), Gulf of Mexico (IODP 308), and Porcupine carbonate mound (IODP Exp. 307).

DNA extraction.

Five to ten grams of frozen sediments were used for the DNA extraction using a hot-alkaline method. Briefly, microbial cells were lysed in a warmed alkaline solution. After neutralization and centrifugation, DNA was extracted from supernatant using the phenol chloroform-isoamyl alcohol treatment. The bulk DNA extracts were purified by silica membrane columns.

Quantification of 16S rRNA gene by digital PCR.

The absolute number of bacterial and archaeal 16S rRNA gene in the extracted DNA was measured by digital PCR using BioMark™ HD system and pdPCR 37K IFC (Fluidigm, Tokyo, Japan).

Results and Discussions: Using less-biased digital PCR technique allowed us to evaluate the abundance of bacteria and archaea at various depths and oceanographic locations. The abundance of 16S rRNA genes was logarithmically decreased with increasing depth, which is in good agreement with previous observations. The number of bacterial 16S rRNA genes was generally higher than those of archaeal one. The current estimation of average ratio between archaeal and bacterial 16S rRNA genes was ~0.37. Since most bacteria have multiple copies of the 16S rRNA gene, our result indicates that archaeal population in the subseafloor environments is almost comparable to bacteria and significantly contributes to the global subseafloor microbial biomass. The ratio was found to be independent from the depth, whereas slightly different from site by site, potentially affected by some environmental factors such as geophysical, energetical, hydrological characteristics.

Our on-going effort of the global census of deep microbial life through the digital PCR has provided a new information of absolute quantify of the specific genes in the deep subseafloor biosphere. The next step will be to study the geographical distribution of microbial diversity and community structure by deep-sequencing of the genes on a global scale.

References:

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