海底下生命圏の探究における技術的ブレークスルー

Technological breakthroughs in search of the deep subseafloor biosphere

*諸野 祐樹[1,2]、寺田 武志[3]、伊藤 元雄[1,2]、稲垣 史生[1,2]
*Yuki Morono[1,2], Takeshi Terada[3], Motoo Ito[1,2], Fumio Inagaki[1,2]

1.海洋研究開発機構高知コア研究所、2.海洋研究開発機構海底資源研究開発センター、3.マリン・ワーク・ジャパン
1.Kochi Institute for Core Sample Research, JAMSTEC, 2.R&D Center for Submarine Resources, JAMSTEC, 3.Marine Works Japan

During the first microbiology-dedicated scientific ocean drilling, the Ocean Drilling Program (ODP) Leg 201 off Peru and Eastern Equatorial Pacific in 2002, the number of microbial cells was evaluated by direct counting of acridine orange-stained cells under fluorescent microscopy, and the minimum quantification limit (MQL) of cell number was approximately 10^5 cells/cm^3 of sediment. Althought this technique is still applicable to high-biomass sedimentary habitats such as shallow organic-rich sediments near the seafloor, some innovative technological breakthroughs have been long required in order to explore low-biomass habitats close to the limit of biosphere. A decade later since Leg 201, we developed a computer image-based cell detection and enumeration method for deep sedimentary microbes. It enabled discriminable cell recognition based on the difference of fluorescence color between intracellular DNA and non-biological mineral particles after DNA stain with SYBR Green I, and resulted in objective and statistically mean cell numbers with higher reproducibility. In addition, we standardized a new protocol for effective cell separation from sedimentary mineral grains using a multi-layer density centrifugation. The combined use of this cell separation technique with flow cytometry or cell sorter opened the way to more fast, sensitive, and precise cell counting than before, even for very low-biomass sediment samples. For example, under the strictly controlled ultra-clean lab condition, our current minimum quantification limit approaches to less than 10 cells/cm^3 of sediment, at least 4 orders of magnitude lower than that during Leg 201. The sorted cells in each well are applicable for single cell-genomic study using the genome amplification techniques. Moreover, the separated cells can be concentrated and placed at one place on the membrane filter, and then isotopic ratios (i.e., $^{13}C/^{12}C$, $^{15}N/^{14}N$) and elemental abundances of each single cell can be analyzed on rastered ion imaging with nano-scale secondary ion mass spectrometry (NanoSIMS). To date, based on these technological breakthroughs, we are finally ready for exploring the limits of subseafloor life and the biosphere through scientific ocean drilling.

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