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Imaging of microbial cells in niches of marine sediment using in situ DNA and electron staining

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Ubiquitous presence of microbes and their inherent activity make them extremely important for a better understanding of physical, chemical, and biological processes in the subsurface environments. In situ detection of submicron microbial cells in the marine sediments is initial steps towards the detailed understanding of microbial life in subseafloor biosphere. However, there are almost no adequate techniques to observe microbes in their in situ condition. Here, we developed a technique that enables the observation of in situ microbes within subseafloor sediments. The technique includes resin-embedding followed by thin sectioning of marine sediments to fix the microbes as well as microstructures in the sediments. Microbial cells in the sediment are visualized under a fluorescent microscopy by staining with SYBR Green I. Then, cells are also visualized with a scanning electron microscopy/energy dispersive spectrometry (SEM-EDS) by applying electron staining of microbial cells. Under SEM-EDS observation, we could match the points where heavy elements used for electron staining are concentrated with the points where SYBR Green I-stained cells were observed in fluorescent microscopy, indicating the microbial cells are selectively recognized in SEM-EDS observation. The coupled SYBR Green staining and electron staining method facilitate the observation of in situ microbes and their interaction with sedimentary minerals even in soft sediment samples with high spatial resolution.

Keywords: electron staining, microbe, SYBR Green staining