

## Detection of microbial life in the oceanic crust aged 13-100 million years

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Microbial life is widely distributed in extremely oligotrophic habitats. However, the extent and microbiological nature of such oligotrophic habitats are poorly constrained due to the lack of scientific knowledge of the oceanic crust, which has been estimated to be the largest but least accessible microbial habitat. Several lines of evidence previously suggest that seafloor microbial life exists within young ocean crusts on the flanks of mid-ocean ridge systems where fluid circulation is thermally driven. As the oceanic crust is aged, the deposition of sediment cover and the heat loss appear to dramatically alter the physicochemical properties of the oceanic crust after 10–15 Myr. Despite the fact that the oceanic crust older than 10 Ma covers >50% of Earth's lithosphere, microbial life in the vast crustal habitat has been poorly explored mainly due to microbial contamination from drilling fluid made from surface seawater. 13.5–100 Ma basaltic rocks in the oceanic crust distributed with South Pacific Gyre were explored through Integrated Ocean Drilling Project Expedition 329, where primary productivity in the surface seawater is exceedingly low. By undertaking the routine evaluation of microbiological contamination with fluorescent microspheres added to drilling fluid, 11 out of 15 core samples were found to be undetectable for fluorescent microspheres from the core interior. 16S rRNA-based molecular phylogenetic analysis was conducted by pyrosequencing of the 15 core samples, drilling fluid samples and a negative control from the laboratory manipulation was conducted. As a result, pyrosequencing was successful for five core samples undetected for microsphere contamination, one contaminated core sample and one drilling fluid sample and the laboratory control. After carefully excluding contamination sequences, 92–370 sequences and 18–134 operational taxonomic units (OTUs) based on >97% similarity were obtained from the five core samples and subjected to further phylogenetic affiliation. Microbial community structures were shifted in shallow pillow lavas thinly covered with oxygenated sediments from the  $\epsilon$ -proteobacterial dominance at 13.5 Ma and the  $\beta$ -proteobacterial dominance at 33.5-Ma, which is also represented by the inferred metabolic shift from chemoautotrophy to heterotrophy. In the ~100-Ma lava flows, methanotrophy in basaltic rocks with fractures filled with secondary minerals was indicated by the phylogenetic relationship to known methane-oxidizing bacteria and archaea. Although more research is needed to clarify the biomass, biodiversity and metabolic activities of microbial life in the upper oceanic crust, microbial communities revealed in this study might be widely distributed and playing important roles in the global carbon cycling in previously unrecognized pathways.