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

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Several lines of evidence previously suggest that subseafloor 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 sediment deposition and the heat loss dramatically alter the physicochemical properties of the oceanic crust after 10–15 Ma. 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. 13.5–100 Ma basement basaltic rocks distributed within South Pacific Gyre were explored through Integrated Ocean Drilling Project Expedition 329. 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. As a result, pyrosequencing was successful for five core samples undetected for microsphere contamination associated with 92–370 sequences and 18–134 operational taxonomic units (OTUs) based on >97% similarity. In 13.5 Ma and 33.5 Ma pillow lavas thinly covered with oxygenated sediments, microbial community structures were shifted from the ε-proteobacterial dominance at 30.0 meters below seafloor (mbsf) and the β-proteobacterial dominance at 36.6 meters below seafloor, which is also represented by the inferred metabolic shift from chemoautotrophy to heterotrophy. In the ~100-Ma lava flows at depths of 109.6 and 121.8 mbsf, methanotrophy in basaltic rocks with fractures filled with secondary minerals was indicated by the dominance of bacteria related to methane-oxidizing *Methyloprofundus sedimenti* with >97% similarity and the detection of archaea affiliated within ANME-1. As microbial cells were microscopically observed in the interior of mineral-filled fractures without fluorescent microspheres, it is likely that microbial communities populated by potential methanotrophs are metabolically active.