



Multi-drilling of Sedimentary Rock Reveals Deep Terrestrial Subsurface Bacterial Distribution Constrained by Geological Setting

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While our knowledge of the deep marine subsurface biosphere has developed rapidly, our understanding of the microbiology of the deep terrestrial subsurface environment is still limited to geochemical considerations of functional bacterial distribution within a given geological setting. Some studies on bacterial distribution conducted using molecular techniques have been undertaken, but these were restricted to igneous hard rock environments. The impetus for studying terrestrial subsurface environments arises in response to social interest which has increased from considerations of aquifer pollution to include site selection for depositing nuclear waste. An understanding of deep terrestrial subsurface microbiology in both igneous and sedimentary rock environments is thus important. For this purpose we obtained deep-water samples at depths of up to 482 m from three boreholes drilled in sedimentary rock in two formations in Hokkaido, Japan. Bacterial counts of 4.61×10^4 to 5.06×10^6 cells/mL were obtained by total direct count (TDC), which is similar to that encountered at the marine subsurface. However, the vertical distribution of bacteria did not simply reveal a decrease in abundance with increased depth from the surface. Considerable activity of representatives of the domain *Bacteria* was revealed using in situ fluorescence hybridization (FISH) at the transition zone of the two sedimentary rock formations. Horizontal heterogeneity of microbial distribution in subsurface environment was also shown by a relatively high abundance observed in members of the Archaea from another borehole

drilled only 1.5 km northeast in the same formation. Detailed gene analysis of both *Bacteria* and *Archaea* suggests diversity in site specific activity exists within a given geological settings of sedimentary rock.