Piezophiles isolated from deep marine subsurface sediment are all Gram-positive bacteria

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Marine sediments cover more than two-thirds of the Earth's surface, and represent a major part of the deep biosphere. Owing to its vast size and intimate connection with the surface biosphere, particularly the oceans, the deep biosphere has enormous potential for influencing global-scale biogeochemical processes, including energy, climate, carbon and nutrient cycles.

IODP Expedition 337 drilled to the Shimokita coalbed off the coast of Shimokita Peninsula, Japan in 2012. We obtained sediment samples from the coalbed at depths of 1498, 1951~1999, and 2406 meters below seafloor (mbsf). The core samples were mixed with MB2216 growth medium and cultivated under anaerobic conditions at in situ tempeature and pressure. The culture mix was then plated on MB agar plate and incubated at anaerobic conditions. We isolated 10 different bacteria, including *Virgibacillus* sp., *Bacillus* sp., *Staphylococcus* sp., and *Robinsoniella* sp. Bacterial piezophily was confirmed in high pressure growth. The isolates were tested at in situ temperature (35ºC and 45ºC) and pressures of 0.1, 33, and 52 MPa (Megapascal). The 16S rRNA gene sequences of bacterial isolates were determined and hylogenetic tree was constructed. Bacterial membrane phospholipid fatty acids were also analyzed. It is interesting to note that all of the isolates from the deep marine subsurface sediment were Gram-positive, spore-forming, anaerobic piezophilic bacteria. Phylogenetic analysis shows that the bacterial isolates are clustered within the phylum Firmicutes, genera Bacilli and Clostridia. The bacterial isolates contain predominantly C_{15} and C_{17} methly-branched fatty acids, consistent with the Gram nature of the isolates. Our results suggest that microbial communities in the deep biosphere are probably dominated by spore-forming, Gram-positive piezophilic bacteria.