

Deciphering the origin and metabolic potential of deeply buried sediment bacteria in the deep biosphere

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Rivers transport large amounts of terrigenous sediment into the oceans, and terrigenous microorganisms may hitch a ride on the sediment particles. Very little is known about whether and how these terrestrial microbes were buried in marine sediment and participate in degradation of marine organic matter in the deep biosphere. We obtained three sediment samples, 6R-3, 19R-1~25R-3, and 29R-7 at 1,498, 1,951~1,999, and 2,406 meters below the seafloor (mbsf) from the Shimokita coalbed from IODP 337. A number of piezophilic, gram-positive spore-forming bacteria were isolated from the sediment samples (Fang et al., 2017). In this study, we test two bacterial species, *Virgibacillus* sp. 6R3-15 and 19R1-5, which were isolated from the buried coal-bearing sediment at 1498 and 1951 mbsf. Complete genome sequencing confirmed 6R3-15 and 19R1-5 are the same strain. Intriguingly, the two species shared 100% 16S rRNA gene and identities 80.26% bidirectional top-hit genes with *V. pantothenicus* DSM26^T, which was isolated from soil. Genomic study showed that compared to all 34 *Virgibacillus* genomes (re)classified based on GTDB taxonomy, 6R3-15 (19R1-5) retained several strain-specific genes in degradation of phenolic compounds (including catechol, 4-methylcatechol and 4-hydroxyphenylacetate), which are apparently enriched in the coalbed. Physiological experiments confirmed that 6R3-15 (and 19R1-5), but not *V. pantothenicus* DSM26^T, can utilize catechol as the sole carbon source at both *in situ* and atmospheric pressure conditions. Importantly, our phylogenetic studies showed those phenol degrading genes were also derived from terrestrial environments, possibly via horizontal gene transfer. Taken together, our results indicate that *Virgibacillus* sp. 6R3-15 and 19R1-5 were probably derived from a terrestrial source via sediment transport to the ocean, and then buried in the forearc basin (off the Shimokita Peninsula). These microbes serve as good model organisms to investigate