

# Viruses, Vesicles, and other Biological Nanoparticles: The Sub-cellular Biosphere of a Deeply Buried 2km-Deep, 20-Million-Year-Old Coalbed Community

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## Abstract

Horizontal gene transfer is an important driver of adaptation and evolution in microorganisms. Transducing biological nanoparticles such as viral particles are believed to be key facilitators of horizontal gene transfer. In deep seafloor sediments, energy can be highly limiting, supporting only extremely slow metabolisms. In such low-energy, isolated environments where communities may subsist for millions of years, the mechanisms of subsurface microbial adaptation and evolution remain a mystery. Virus particles have been found everywhere that life has been found, including deep subsurface environments. Although microorganisms are abundant and active in the Earth's subsurface, the role of viruses in shaping and influencing these slow-growing communities is only recently starting to be explored. Here, we analyzed the deeply buried microbial community from a lignite coalbed layer 2km below the seafloor offshore Shimokita, Japan (IODP Expedition 337) that had been buried for 20 million years. We harvested cells ( $>0.2\mu\text{m}$ ) and biological nanoparticles ( $<0.2\mu\text{m}$ ) from a bioreactor enrichment seeded by lignite core samples. We sequenced DNA from the cells and nanoparticles and subsequently analyzed the metagenomes. Within the nanoparticle metagenome, numerous complete novel virus genomes were reconstructed. Comparison of the virus genomes to the prokaryotic MAGs (metagenome assembled genomes) revealed that many of the virus genomes had been integrated prophage within bacterial genomes, suggesting the potential for virus-host interactions to occur in the deep seafloor. Additionally, lysogeny may be an important survival mechanism for viruses in deeply buried, low-energy environments. Host genes were found to be packaged by viral particles, demonstrating the potential for specialized and general transduction by viruses. Not only viral particles, but there was also evidence that membrane vesicles and

gene transfer agents may participate in transduction in this deep subsurface community. Horizontal gene transfer mediated by biological nanoparticles may be an important mechanism of adaptation for deep subsurface microbial communities and may provide insight into possible evolutionary processes shaping microbial communities in the deep subsurface. These results may also shed some light onto the nature of viral infection in the subsurface, potentially revealing insights about the long-term persistence of life under extreme energy limitation and how viruses may survive this over geological timescales.

## **Keywords**

subsurface, horizontal gene transfer, transduction, bacteriophage, prophage

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## **Conflicts of interest**

The authors have declared that no competing interests exist.