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Diversity and Ecological Niche Preferences of Subsurface-Adapted Methanotrophic Bacteria from the Deep Biosphere of Homestake Mine

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Methane is one of the most common sources of energy and carbon in the deep biosphere, and methanotrophy is hypothesized to sustain diverse microbial communities alongside chemolithoautotrophy in subsurface systems. However, the extent of the contribution of methanotrophy to primary production and C-cycling remains unknown, due to our lack of knowledge about the distribution, diversity, abundance and functional features of methanotrophs in subsurface ecosystems. To fill the gap, the ecology of methanotrophic communities was characterized from borehole fluids and mineshaft materials in the former Homestake gold mine.

DNA was extracted from subsurface samples taken at the 1700 feet and 4850 feet levels and used as a template for amplifying the 16S rRNA and pmoA genes. Amplicon and metagenomic libraries were sequenced using the Illumina platforms. A high diversity of aerobic methanotrophic bacteria within a broad range of clades (some have not previously been reported in the subsurface biosphere) were observed. Type-Ib methanotrophs were enriched in biofilms, type-IIa dominated in groundwater and several uncultured lineages exclusively occurred in mineshaft sediments. It is therefore likely that subsurface methanotrophs occupy different ecological niches based on their specific geochemical preferences. Bacterial communities inhabiting mineshaft biofilms shared moderately similar community composition with those in nearby groundwater, indicating methanotrophs trapped/concentrated (˜27% in bacteria) in biofilms likely originated from the seeping groundwater. Such recolonization and enrichment could change the local C-flow, as methanotrophs are likely to be the primary producers in biofilms based on network analysis of communities.

Closest-related sequences to several mine OTUs were exclusively observed in other subsurface habitats, suggesting these obligate subsurface-colonizing methanotrophs were indigenous inhabitants and potentially adapted to subterranean life. Ten methanotrophic genomes with a range of degrees of novelty were recovered, and some genomic characteristics, present in Homestake mine genomes but absent in closest surface neighbors (e.g, possession of stress-response and metal-tolerance genes) could help them cope with extreme conditions. This in-depth survey revealed that a remarkably diverse, abundant and novel subsurface-adapted indigenous aerobic methanotrophic community were present in the deep subsurface biosphere.

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