

Metaproteomics Reveals a Dynamic Metabolically Diverse Subsurface Microbial Community in the Antrim Shale

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The deep subsurface, one of the poorest understood segments of the biosphere, is an important part of global biogeochemical cycling and may provide clues to the evolution of life under a low-oxygen, primitive Earth. The different temperature/pressure/pO₂ conditions and substrates available in the deep subsurface, as compared to surface environments, necessitate a wide range of specialized microbial cellular functions and community interactions required for life to persist. These novel biological solutions may have potential bioenergy and other biotechnology applications. Recent advances in subsurface metagenomics (entire complement of gene sequences in a bulk sample) have opened the door to more in-depth investigation of these systems. However, metaproteomic (entire complement of all proteins in a bulk sample) analysis of the deep subsurface has not been widely utilized due in part to methodological challenges associated with complex environmental samples. Metaproteomic studies stand to provide greater understanding of subsurface microbial communities as proteins represent active function in biological systems and not just potential function represented by genes.

In this study, we performed metaproteomic analysis of water from a natural gas well producing from the Antrim Shale at a depth of 675 to 792 feet below the surface in order to characterize the active cellular functions and metabolisms of the resident microbial community. Our results showed diverse prokaryotic and eukaryotic metabolism and biosynthesis pathways, indicating a dynamic, active microbial community capable of many different modes of obtaining energy from the environment. For example, methanogenesis, sulfate reduction, diverse carbon utilization metabolisms, amino acid synthesis and other biosynthesis pathways were present in the sampled microbial community. The distribution of different metabolisms indicates that subsurface shale environments can support a wide range of microbial taxa and energy utilization pathways. The presence of methanogenesis reveals that biogenic methane production contributed to natural gas in this reservoir. Cobalamin and heme biosynthesis pathways were found, indicating active cobalt and iron utilization. RNA polymerases and ribosomal proteins for Bacteria, Archaea, and Eukarya were present in the microbial metaproteome as well. These results demonstrate the power of combined genetic and proteomic analyses to characterize subsurface microbial activity.