Integrated Approaches to Explore the Biosphere Inhabiting the Deep Subsurface of the Illinois Basin, USA

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The majority of microbial life inhabits formation water and mineral surfaces within the subsurface of our planet. The subsurface microbial biosphere therefore plays a dynamic role in a variety of biogeochemical processes. The cross-disciplinary studies have been performed on the indigenous microbial communities inhabiting the Cambrian Mt. Simon Sandstone of the Illinois Basin. This is a high porosity and permeability hydrocarbon-free geological formation that is analogous to many oil and gas reservoirs around the world. This work combined subsurface microbiology (omics-based and culture-based), geochemistry, and mineralogy to quantitatively track subsurface microbial ecology, microbe-water-rock interactions, and their associated metabolic potentials. The Mt. Simon therefore serves as a uniquely well-suited and accessible natural subsurface environment to understand biogeochemical processes that range from the molecular-to-basin scale, with results directly applicable to analogous oil fields.

In the course of this work, we have successfully isolated and characterized novel iron-reducing organisms, *Orenia metallireducens* and *Tepidibacillus* decaturensis from the 1.7-2.01 km deep formation water of the Illinois Basin. Combined genomic, physiological, thermodynamic and metabolomic analyses has demonstrated that the distinct phenotypic and genotypic traits of these deep-subsurface organisms compared to the well-studied model iron-reducing bacteria (e.g., *Shewanella* and *Geobacter spp.*). Further studies on *Orenia metallireducens* suggested a new mechanism for facilitated fermentation by microbial iron reduction. In contrast to the ferric iron acting solely as an electron sink to promote fermentation, we found that iron reduction benefited this bacterium by neutralizing the acid generated ("souring") due to fermentation and maintaining stable pH conditions for microbial metabolism. In addition, using amplicon- and community-based metagenomics, our work has identified Mt. Simon microbial communities that are spatially heterogeneous (by depth) in both structure and diversity. Metagenomic analyses at the 1.78 km deep interval detected a low-diversity community dominated by *Halomonas sulfidaeris*. In this 98% *Halomonas*-dominated community sequence analysis of identified genes suggested that this population could utilize iron and nitrogen, recycle indigenous nutrients and can potentially degrade aromatic organic compounds associated with hydrocarbons.