

# Microbial Osmotolerance Mechanisms in Hydraulically Fractured Shale Elucidated Through Metagenomics Analysis

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## Project Goals:

The process of extracting natural gas from deep shale requires the injection of large volumes of fluid and proppants. These fluids interact with the shale matrix and return to the surface with distinct biogeochemical changes. As the salinity of produced fluids approaches equilibrium with that of the shale formation a low diversity microbial community of halotolerant fermenters and methanogens is established. The goal of this project is to improve our understanding of the growth and adaptations of the microbial community in the hydraulically fractured wells. We have identified three specific objectives to understand the in situ physiologies and kinetic rates governing biogeochemical reactions in these microbial communities: (1) characterize variables influencing growth parameters and membrane features of shale taxa, (2) characterize interactions between shale matrices and microorganisms, and (3) elucidate engineered and environmental processes driving biogeochemical signatures at field scale.

## Abstract

Previous research has shown salinity is a major driver of microbial community composition and function in hydraulically fractured shales, with produced fluids from shale wells increasing to brine level salinities during the first six months of natural gas production. In this study, we investigated the mechanisms for osmotolerance in bacterial metagenomes from hydraulically fractured shale wells in the Appalachian Basin (Marcellus Shale and Utica-Point Pleasant Formation). With increasing salinity and decreased diversity, we identified three osmotolerance adaptations in the microbial community. Genes for Na<sup>+</sup>/K<sup>+</sup> transport increase in relative abundance with time of production as do genes for osmolyte import/biosynthesis. This indicates that the microbial community uses a combination of a salt-in strategy, where cells maintain osmotic balance by K<sup>+</sup> importation coupled to Na<sup>+</sup> export, and a compatible-solute strategy where cells gain osmotolerance via importation/synthesis of organic osmolytes such as glycine, betaine and ectoine for osmotolerance. A second finding in the metagenome sequences is the increase in gene abundance for sodium-dependent respiration with increasing salinity. A higher Na<sup>+</sup> gradient outside the cell results in a sodium-tight membrane, and enables sodium-dependent ATP synthesis in some taxa. This research provides an improved understanding of genomic osmoprotectant capabilities, including membrane associated osmoprotectant proteins in deep shale microbial communities during high salinity gradients, with implications on halotolerant bacterial energetics in this and other hypersaline environments.

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