

Characterization of a Nitrate-Respiring, Multi-Metal-Resistant *Bacillus* Species Highly Abundant in Heavily Contaminated ORR FRC Subsurface

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Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) is using a systems biology approach to understand the interactions between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field and computational methods.

A significant goal of microbial ecology is the development of models that accurately predict how environmental perturbations will impact the structure and function of microbial communities. Identification of representative isolates is essential for the high-resolution physiological studies required for proper parameterization of these models. Frequently, pure-culture laboratory studies fail to select appropriate isolates that are relevant to an environment either in terms of taxonomic abundance or metabolic function. Here, we present the successful isolation and characterization of a *Bacillus cereus* strain (designated CPTF) that is extremely abundant in the subsurface of the Oak Ridge Reservation Field Research Center (ORR FRC) that is immediately adjacent to the highly-contaminated S-3 ponds (Area 3). The S-3 ponds contain high levels of legacy uranium, nitric acid, and other mixed metals waste that has leached into the surrounding environment. The CPTF 16S rRNA gene has 100% sequence identity to the most abundant ASV observed across an ORR FRC Area 3 subsurface metagenome survey. The CPTF-matching ASV is present in all 32 subsurface samples collected from Area 3. Its abundance across individual samples ranges from 0.02 – 40.5% with a median abundance of 3.8%.

The ORR FRC Area 3 subsurface poses a significant physiological challenge to microorganisms living at the site—with high concentrations of nitrate, mixed metals waste, and low pH. The porewaters of the soils contain nitrate at concentrations up to 19,600 ppm and pH values as low as 3.0. Notably, the CPTF-matching ASV is observed across the full span of measured porewater nitrate concentrations. Furthermore, as the CPTF-matching ASV is ubiquitous across the sampled Area 3 subsurface, it also exists concurrent with elevated levels of

uranium (up to 1130 ppm) and other metals measured in the same survey. On-going work seeks to understand the persistence of CPTF at the ORR FRC despite these significant environmental stressors. Experimental work has shown that CPTF grows robustly with high site-relevant levels of nitrate and high concentrations of nitrite. CPTF respire nitrate with nitrite as the major end-product. Phenotypic observations and genome analysis suggest that nitrite can support fermentative ammonification by this strain. CPTF also has high tolerance to multiple metals at site-relevant concentrations both individually and in combination. The metals tested include U, Al, Ni, Cd, Mn, Fe, Cu, and Co. Genome analysis of CPTF revealed metal efflux pumps, metal-responsive transcriptional regulators, and numerous other metal resistance determinants. High-throughput proteomic analysis is underway to understand the cellular response to ORR groundwater-relevant metals under nitrate-respiring conditions to explore how mixed waste contamination impacts the physiology of this abundant contaminated site isolate. The results of this work will facilitate our understanding of the persistence of dominant taxa at highly-contaminated DOE sites.

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