

106. Understanding the Role of Pelosinus In Uranium-Contaminated Environments

Andrew Lancaster¹, Michael P. Thorgersen¹, Alexey E. Kazakov⁴, Farris L. Poole¹, Kara De Leon², Sagar Utturkar³, Pavel S. Novichkov⁴, Judy D. Wall², Steven D. Brown³, Kelly S. Bender⁵*(bender@micro.siu.edu), Michael W. W. Adams¹, Adam P. Arkin⁴, and Paul D. Adams⁴

¹University of Georgia, Athens, GA; ²University of Missouri, Columbia MO; ³Oak Ridge National Laboratory, Oak Ridge, TN; ⁴Lawrence Berkeley National Laboratory, Berkeley, CA; ⁵Southern Illinois University, Carbondale, IL

<http://enigma.lbl.gov>

Project Goals: The overarching goal of Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to understand environmentally relevant microbial communities and their environmental interactions. With many environments being impacted by varying concentrations of a range of metals, it is critical to elucidate the mechanisms that microbial communities employ to tolerate, to assimilate, and to metabolize metals. Our goal in this interdisciplinary project is to understand the genomics, regulation, metabolism, and uranium assimilation of *Pelosinus*, a dynamic genus that includes U(VI)- and Cr(VI)-reducing strains isolated from DOE sites of interest.

Pelosinus is a genus of the strictly anaerobic phylum Firmicutes possessing strains with U(VI)- and Cr(VI)-reducing capabilities that have been isolated from the Hanford Nuclear Reservation (HNR) 100-H and Oak Ridge Field Research Center (ORFRC)^{1,2,3}. To further understand the environmental significance and metabolic capabilities of *Pelosinus*, our group has selected two of these strains, UFO1 and JBW45, for further genomic and phenotypic analysis. These were isolated from the HNR and ORFC, respectively^{2,3}. Comparative analysis of the complete UFO14 and JBW45 genome sequences² indicated genome sizes of 5.1-5.3 MB containing a range of genes that suggest diverse metabolic capabilities. Phenotypic analyses indicated that both strains could be cultured in the laboratory on R2 media utilizing fumarate as a substrate.

Because previous U(VI)-reducing studies on *Pelosinus* sp. strain UFO1 have shown both extracellular binding of U and intracellular deposition of U precipitates³, we performed metal analyses to further understand U assimilation in strain UFO1. While this analysis indicated that strain UFO1 is inhibited by 200 μ M U, it also resulted in the isolation of U-binding membrane proteins. These proteins were identified as UFO_4202-4203, which contain S-layer domains and combine to form a major protein found both in the membrane and secreted into the growth medium. Subsequent metal analysis of strain JBW45 also identified an extracellular U-binding protein containing an S-layer domain with 78% and 42% similarity to UFO_4202 and UFO_4203, respectively. Based on the metabolic potential and unique metal biochemistry of UFO1 and JBW45, regulatory analysis was also performed. A comparative study of transcription regulatory genes from the families of the metal sensing regulators revealed a substantial difference between UFO1 and JBW45 strains that may contribute to phenotypic diversity of these strains. Only four of ten MerR family regulators from UFO1 are conserved in JBW45, and only three of seven ArsR family regulators from JBW45 are present in UFO1. We also applied a conservative propagation procedure to build 26 draft regulons in UFO1 using a collection of manually curated regulons from 11 Bacillales genomes from the RegPrecise⁵ database. Genome analysis of UFO1 also identified 52 potential riboswitch elements. These elements include switches with ykkC/yxkD and yybp/ykoy motifs that have been shown to control efflux pumps/multi-drug resistance and manganese homeostasis/tellurium resistance in other bacteria, respectively. To further understand the biology and regulation of strains UFO1 and JBW45, genetic system development is currently underway with mutant construction by conjugation showing promise. Future collaborative work to elucidate the unique U metabolism of UFO1

and JBW45 includes further characterization of U-binding proteins, RNA-Seq to identify U-responsive genes, regulons and sRNA analyses, as well as isolation of new strains from wells containing high levels of U from the ORFRC.

References

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