48. Uncovering Uranium Resistance Mechanisms in *Caulobacter crescentus*

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Project Goals: Depleted uranium is a widespread environmental contaminant that poses a major threat to human health. In contrast to humans and animals where a trace amount of uranium can cause damage to kidneys, liver and heart, it is well known that some bacteria can tolerate high levels of uranium and influence its mobility and bioavailability in the environment. As a non-pathogenic bacterium, *Caulobacter crescentus* is an attractive bioremediation candidate due to its high tolerance to heavy metals, as well as its ubiquitous presence in fresh water lakes and streams, including those contaminated with heavy metals. Our goal is to decipher the physiological basis for U tolerance and elucidate the U biomineralization pathways in *C. crescentus.* Our end goal is to provide insight into the effect of aerobic bacteria on U biogeochemistry and to understand whether aerobic biomineralization could be used in bioremediation applications.

We observed that U (VI) caused a temporary growth arrest in *C. crescentus* and three other bacterial species, although the duration of growth arrest was significantly shorter for *C. crescentus*, which may provide a competitive advantage in the environment.[1] We found that growth recovery was not due to a decrease in U solubility, a common detoxification strategy employed by other microorganisms. Through functional reporter assays, we discovered that *C. crescentus* is able to reduce U bioavailability through the secretion of an unknown, heat-stable metabolite(s). To the best of our knowledge, this represents a unique U detoxification strategy and has important environmental implications for how aerobic bacteria affect U biogeochemistry. Furthermore, our findings provide insight into how microbes cope with (metal) stress under non-growing conditions, a metabolic state that is under-studied but prevalent in the natural environment.

Upon recovery from growth arrest, *C. crescentus* proliferated with normal growth kinetics, during which active U biomineralization occurs. We found that phosphate metabolism facilitated U-P precipitation when organic phosphate (*e.g.,* glycerol-2- phosphate) was provided. Electron microscopic and spectroscopic analyses indicated that microbe-assisted U precipitates were distinct from their abiotic counterparts in both morphology and composition. In particular, we observed cell-surface-bound U-P minerals, indicating a biological assisted process. In addition, we found that a predicted extra-cytoplasmic alkaline phosphatase (CCNA_02545) was responsible for formation of these U-P precipitates; deletion of this gene abolished the formation of the precipitates.

Furthermore, the activity of this enzyme facilitated cell survival in a whole-cell assay under U treatment as well as during growth in minimal medium supplemented with U and glycerol-2-phosphate as the sole phosphate source. Overall we demonstrated that *C. crescentus*-facilitated U biomineralization occurs during active growth and may play an important role in cell persistence in contaminated environments.

To identify the genetic basis for U tolerance in *C. crescentus* on the genome level, we took two independent approaches: proteomic profiling and Tn-seq. We performed a label-free shotgun proteomics study of *C. crescentus* under U, Cr, or Cd exposure.[2] The goal was to identify proteins differentially expressed under heavy metal stresses, and to compare the proteomic results with the

already available whole genome transcriptional data. Under U exposure, a phytase enzyme and an ABC transporter were up-regulated. Heat shock and outer membrane responses were found associated with Cr, while efflux pumps and oxidative stress proteins were up-regulated with Cd. Experimental validations indicated that the phytase plays a role in U and Cr resistance and detoxification, and a Cd-specific transporter confers Cd resistance. Interestingly, analysis of promoter regions in genes associated with differentially expressed proteins suggests that U exposure affects cell cycle progression. The results of this study not only broaden our understanding of the fundamental aspects of metal stress response, but also provide insight into the roles of specific proteins in metal detoxification.

In collaboration with JGI, we employed a Tn-seq approach to identify the essential genomic elements that specifically confer U resistance in *C. crescentus.* The specific steps of our method included: 1) performing ultrahigh-resolution transposon mutagenesis; 2) selecting for mutants that can grow in the presence of U; 3) amplifying genomic regions adjacent to the transposon insertion sites from the pooled mutants; 4) performing high-throughput DNA sequencing to obtain genomic sequence information; and 5) mapping the transposon insertion sites onto the genome of *C. crescentus*. Genomic areas that accumulate transposon insertions under normal growth conditions but tolerate fewer insertions under U selective pressure contain genes that are specifically required for growth under U. Using this approach, we identified 18 genes with significantly lower Tn insertion frequencies under U exposure compared to the no stress control. Through subsequent mutation analyses, we confirmed 15 genes were involved in U tolerance, including those that partake in type-I secretion, flagella assembly, stationary phase response, and general stress response, many of which are also involved in cell cycle progression. Together, we are starting to gain a basic understanding of strategies employed by *C. cresecentus* for coping with U toxicity at the physiological and molecular levels.

References

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