

The Metal Resistome Of An Environmental Bacterium

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Project Goals: We aim to develop versatile high throughput methods for microbial functional genomics studies. In this project, we will map the metal resistome of an ENIGMA isolate across various metals, and expand this approach to other environmental microorganisms, which will identify common and unique mechanisms bacteria have evolved to deal with elevated metal stress.

Understanding how microbes survive and grow under metal-stressed conditions is a fundamental challenge in environmental microbiology. Though many individual studies have been done in microorganisms with well-known metals including zinc, copper, and chromium, there is little information about global metal-resistance genetic determinants across many different kinds of metals. To address this, we developed a high-throughput culturing system for systematic determination of inhibitory concentrations of metals under aerobic and anaerobic conditions. Using this system, we determined the half maximal inhibitory concentration (IC50) for 24 metals against *Pseudomonas fluorescens* N2E2, an environmental isolate from the Oak Ridge field research site. To globally identify the gene(s) responsible for resistance to these 24 metals, we are using randomly barcoded transposon site sequencing (RB-TnSeq) to identify genes with resistant and hypersensitive phenotypes to elevated metal stress. Here, we present our experimental system and preliminary analysis of metal-resistance determinants in *P. fluorescens* N2E2 with a focus on the similarities and differences in the response of this bacterium to different metals. To facilitate follow-up studies of the functional genomic data, we developed an efficient system to construct in-frame deletion mutants. This genetic system does not just facilitate single gene function studies, but can also be used as a platform for generating double mutants for genetic interaction studies. Taken together, our experimental system will push forward our understanding about the importance of genes as single determinants to metal resistance but also how they interact with each other as part of more complex cellular networks.

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