

66. Mechanisms leading to the evolution of novel glucose utilization by *Shewanella oneidensis* MR-1

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Project goals: Our broader project goals are to integrate novel experimental techniques and computational approaches with a tractable, model syntrophy to predictably understand the role of spatial structure on the function and evolution of microbial communities that mediate metal reduction. This syntrophic microbial community is composed of an engineered, lactate fermenting *Escherichia coli* and *Shewanella oneidensis* MR-1, a preeminent model for anaerobic metal reduction. A limitation to this effort has been an incomplete characterization of the metabolic capabilities of *S. oneidensis*. Under common laboratory conditions, *S. oneidensis* rapidly evolves to utilize glucose as a carbon and energy source. The goal of this study has been to elucidate the molecular mechanisms underlying this novel phenotype, to refine the existing genome-scale metabolic model for *S. oneidensis*, and to explore options to restrict use of glucose by *S. oneidensis* in future experiments.

Carbon metabolism is historically a defining feature in microbial species identification and characterization. In the past decade, with the rapid increase in microbial genomic information, use of carbon utilization as a species identifier has lost favor in light of molecular-based phylogenetics. However, this same information has fueled the construction of numerous genome-scale metabolic models, and provided an unprecedented look into the role microbial metabolic diversity plays in global carbon and nutrient cycling; changing how we perceive the impact of microbes on the environment. That being said, we often view carbon metabolism as a fixed, intrinsic characteristic of a microbe. What is apparent from meta-genomic data and experimental evolution studies is that the suite of carbon substrates a microorganism consumes is not necessarily a static regimen, but rather a plastic, malleable phenotype. Here, we explore one such metabolic innovation; the molecular mechanisms that lead to the evolution of novel glucose utilization by *Shewanella oneidensis* MR-1 – a preeminent model for diversity in anaerobic respiration.

One of the defining characteristics of *S. oneidensis*, in contrast to many other Shewanellaceae, is its inability to catabolize complex carbon substrates such as glucose, or other carbohydrates. This has led to much speculation regarding the ecological niche of *S. oneidensis*. Under aerobic growth conditions, we and a number of other researchers have observed spontaneous mutations resulting in emergence of glucose catabolism (Glu⁺). To date, the underlying mechanisms driving this change in phenotype, and their consequences on cellular metabolism, have remained unknown. Via whole genome re-sequencing of Glu⁺ mutants, we discovered the recurrent source of the Glu⁺ phenotype was the result of a large deletion causing constitutive expression of genes involved in *N*-acetyl glucosamine (GlcNac) metabolism. We show that these genes are necessary for glucose transport and phosphorylation of glucose to glucose-6-phosphate – allowing for subsequent glycolysis. Through ¹³C-labeling experiments, we found that the resulting glucose-6-phosphate was oxidized through the Entner-Doudoroff Pathway, confirming speculation of an incomplete Embden-Meyerhof-Parnas Pathway in *S. oneidensis*. To explore other possible mutations that may result in a Glu⁺ phenotype, we performed saturating transposon mutagenesis. In addition to mutations in GlcNac metabolism, we identified several insertions proximal to putative carbohydrate transporters or carbohydrate kinases.

These data suggest a common mechanistic theme leading to novel glucose metabolism – coopting divergent carbon transporters and kinases to produce glucose-6-phosphate. Collectively, these results shed light on cryptic carbon metabolism in *S. oneidensis*, providing additional data to further refine genome-scale metabolic models in this important model organism. More broadly, we demonstrate a framework to explore latent metabolisms in other environmentally relevant microbes that may have profound impacts on global carbon and nutrient cycling.