High-Throughput Genetic Analysis of Rhodanobacter Reveals Genes Important for Metal Tolerance

Li-Wen Wang (Audrey_LiWenWang@lbl.gov)[1]*, Trenton K. Owens[1], Hans K. Carlson[1], Alexey E. Kazakov[1], Morgan N. Price[1], A.M. Deutschbauer[1,2], A.P. Arkin[1,2], and **P.D. Adams[1,2]**

[1]Lawrence Berkeley National Lab, Berkeley; [2] University of California at Berkeley

http://enigma.lbl.gov

Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction 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.

Abstract:
Rhodanobacter species are highly abundant at the Oak Ridge Reservation site, which is contaminated with heavy metals, high nitrate, and low pH [1]. To uncover the molecular mechanisms underlying their survival in this extreme environment, we constructed a whole-genome barcoded transposon mutant library in *Rhodanobacter* sp. FW104-10B01 (10B01), through which the fitness of nearly all non-essential genes can be evaluated in parallel through competitive growth assays and DNA barcode sequencing. Our early attempts to mutagenize 10B01 were inefficient, which we hypothesized was due to the native restriction modification systems in this strain. To overcome this inefficiency, we used PacBio sequencing of the 10B01 genome to identify methylated motifs and then constructed a custom transposon delivery vector minus these motifs. This new vector was much more efficient (100-fold) in making mutants in 10B01, and we subsequently used this vector to construct a final library of over 460K uniquely barcoded 10B01 mutants. Using this library, we conducted over 100 mutant fitness assays in the presence of elevated metal concentrations, including for many of the major selective stressors at the site, such as U, Mn, Al, Cd, Zn, Co, and Ni [1]. From these data, we identified heavy-metal efflux pumps that were important for fitness in the presence of a number of metals, and in many instances these efflux pumps were specific for only one or a handful of metals. To enable single-gene follow-up investigation, we archived a collection of 192 plates of individual mutants in monoculture (>18K single mutants). By tracking transposon insertions using barcode sequencing, we are able to accurately locate mutants in the archived library and thereby connect specific Rhodanobacter genotypes to phenotypes through analysis of mutants [2, 3, 4]. Overall, our new genetic tools and resources for *Rhodanobacter* will facilitate research on the mechanisms by which these bacteria survive in heavily contaminated environments.

References

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