

97. Altered Biofilm Formation in *Desulfovibrio vulgaris* Hildenborough by Laboratory-Driven Evolution

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Project Goals: The goal of Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to push the frontier of knowledge on the impact of microbial communities on ecosystems. As part of this, we must link genetic capacities to ecological function. Heavy-metal- and radionuclide-contaminated groundwater is common in DOE sites of interest. In these sites, the microbial communities are often predominantly attached to sediment particles as a biofilm. To better understand these communities, we must consider the biofilm growth strategy in a genetic context. Our goal is to determine the mechanisms and genetic requirements of biofilm formation in a common heavy-metal reducing bacterium, *Desulfovibrio vulgaris* Hildenborough (DvH). In pursuing this goal, inter-laboratory collaboration facilitated by ENIGMA has led to the discovery that two wild-type DvH strains (from MO and MT), both originally from ATCC 29579, have diverged in biofilm formation due to laboratory-driven evolution.

Desulfovibrio vulgaris Hildenborough (DvH) is a sulfate-reducing bacterium present in heavy-metal and radionuclide contaminated sites, often as a biofilm. Yet, the genetic requirements of DvH biofilm formation have not been determined. A biofilm reactor system has recently been established to address this lack of information. After optimization, it became apparent that the DvH-MO is partially deficient in biofilm formation as compared to data published for what should have been the same strain used by our ENIGMA collaborators at Montana State University (DvH-MT). In our reactors, DvH-MT performs as it does in Montana, forming a steady-state biofilm within 96h. DvH-MO biofilm lags for ~120h; however, upon reaching steady-state, the biofilms are compositionally similar. While both strains originated from ATCC 29579, laboratory-specific culturing resulted in a divergence in biofilm formation without direct selection under biofilm growth conditions. The genomes were re-sequenced from planktonic cultures of DvH-MT and DvH-MO, and DvH-MO steady-state biofilm. Twelve single nucleotide polymorphisms (SNPs) were ubiquitous in planktonic DvH-MO but were absent in DvH-MT. In contrast, no SNPs occurred at a frequency >50% in DvH-MT that were absent in DvH-MO. Of note in DvH-MO, a SNP in the ABC transporter (DVU1017) of a type I secretion system (T1SS) has resulted in an Ala to Pro change in a conserved α helix near the ATP-binding site. We hypothesize that this Ala to Pro change inhibits protein transport by the T1SS. DVU1012 and DVU1545 both contain T1SS export protein motifs and have been shown to be abundant in the DvH biofilm matrix. An inhibition of DVU1012 and/or DVU1545 transport may have caused biofilm deficiency in DvH-MO. In-frame deletion mutants of DVU1017, DVU1012, and DVU1545 are being constructed in DvH-MT. These mutants are being screened for biofilm phenotypes. In future work the Pro codon change will be introduced into DvH-MT and restored to Ala in DvH-MO. These results will clarify what has caused this difference in two supposedly identical cultures and determine genes required for biofilm formation in DvH. This discovery emphasizes the importance of monitoring laboratory-driven evolution, especially between collaborating laboratories, and provides insight into the genotype-to-phenotype relationship.

This material by ENIGMA- Ecosystems and Networks Integrated with Genes and Molecular Assemblies

(<http://enigma.lbl.gov>), a Scientific Focus Area Program at Lawrence Berkeley National Laboratory is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological & Environmental Research under contract number DE-AC02- 05CH11231.