Single-Base Change by Laboratory-Driven Evolution Eliminates Biofilm Formation in *Desulfovibrio vulgaris* Hildenborough

Kara B. De León[1]* (deleonkb@missouri.edu), Grant M. Zane[1], Gregory P. Krantz[2], Matthew W. Fields[2], Judy D. Wall[1], Adam P. Arkin[3,4] and **Paul D. Adams**[3,4]

[1]University of Missouri, Columbia; [2]Montana State University, Bozeman; [3]Lawrence Berkeley National Laboratory, Berkeley, CA; [4]University of California, Berkeley

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Project Goals: The goal of Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to advance fundamental knowledge on the impact of microbial communities on ecosystems. Subsurface microbial communities, including those in heavy-metal and radionuclide sites of interest to the DOE, are commonly attached to sediment particles as a biofilm. To understand these communities, we must consider biofilm growth strategy from a genetic context. *Desulfovibrio* are important heavy-metal reducers that contribute to the overall redox of the environment. Our goal is to determine the genetic requirements for biofilm formation in *Desulfovibrio vulgaris* Hildenborough (DvH). In pursuing this goal, an inter-laboratory collaboration through ENIGMA has led to the discovery that two wild-type DvH strains have diverged in biofilm formation ability likely due to laboratory-driven evolution and has revealed a possible mechanism for biofilm formation in DvH.

(A) DvH on glass slide from
(B) CDC Biofilm Reactor

Desulfovibrio vulgaris Hildenborough (DvH) is a sulfate-reducing bacterium present in heavy- metal and radionuclide contaminated sites that is capable of heavy-metal reduction and contributes to the overall redox state of the environment. Though predominantly found attached to sediment particles as a biofilm, the genetic requirements of DvH biofilm formation have not been determined. Our goal is to determine the mechanisms and genetic requirements of biofilm formation in DvH. In pursuing this goal, inter-laboratory collaboration as part of ENIGMA has led to the discovery that two wild-type DvH strains, both originally from ATCC 29579, have diverged in biofilm formation capacity due to laboratory-driven evolution. The wild-type DvH used at the University of Missouri (DvH-MO) is partially deficient in biofilm formation as compared to data published for what should have been the same strain used in Matthew Fields' lab at Montana State University (DvH-MT). The genomes were re-sequenced from

planktonic cultures of DvH-MT and DvH-MO, and DvH-MO steady-state biofilm. In DvH-MO, a single nucleotide polymorphism (SNP) in the ABC transporter (DVU1017) of a type I secretion system (T1SS) has resulted in an Ala635 to Pro change in a conserved α helix near the ATP-binding site. However, after DvH-MO forms a biofilm, a secondary SNP predominates and results in Leu635. We hypothesized that the Ala to Pro change inhibited protein transport by the T1SS and the secondary SNP resulting in a Leu restored transport. These predictions were confirmed by introduction of the SNPs into DvH-MT by site-directed mutagenesis. Therefore, protein transport via the T1SS is required for biofilm formation in DvH and a single nucleotide change due to laboratory-driven evolution is sufficient to stop biofilm formation. Proteins encoded in DVU1012 and DVU1545 both contain T1SS export motifs and are abundant in the DvH biofilm matrix. Inhibited transport of these proteins may have caused biofilm deficiency in DvH-MO. The double deletion mutant is deficient in biofilm formation similar to ΔDVU1017. As either DVU1012 or DVU1545 is sufficient to form biofilm, these biofilm structure proteins likely require DVU1017 for export. These findings have led to a proposed mechanism for biofilm formation in DvH and emphasize the importance of monitoring laboratory-driven evolution, especially between collaborating laboratories.

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