# VIMSS ESPP: Deciphering the roles of two-component systems in *Desulfovibrio vulgaris* Hildenborough

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ESPP2 Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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## INTRODUCTION

One of the primary goals of the Environmental Stress Pathway Project (ESPP) is to map the response of the anaerobic sulfate reducing soil bacterium, *Desulfovibrio vulgaris* Hildenborough to its environment. Two component systems, comprised of Histidine Kinase and Response regulator proteins, present the primary and ubiquitous mechanism in bacteria for initiating cellular response towards a wide variety of environmental conditions. In *D. vulgaris* Hildenborough, more than 70 such systems have been predicted, but remain mostly uncharacterized. The ability of *D. vulgaris* to survive in its environment is no doubt linked with the activity of genes modulated by these two component signal transduction systems. To map the two component systems to the genes they modulate, the availability of deletion mutants provides an important tool. Here we present an overview of the predicted histidine kinases in *D. vulgaris* and describe a strategy to create library of histidine kinase knock out mutants in *D. vulgaris*. We use the OmniLog® workflow to conduct a wide phenotypic characterization of the knock out mutants generated. To illustrate our strategy we present results from our study of the histidine kinase in the predicted *kdp* operon of *D. vulgaris*. The high-affinity potassium uptake Kdp complex is well characterized in other bacteria where it facilitates K+ uptake in low K+ or high Na+ conditions. Typically, the activity of the Kdp system is modulated by the KdpD/E two-component signal transduction system, where KdpD is the sensor histidine kinase and KdpE is the response regulator. The *D. vulgaris kdp* operon contains a gene with predicted response regulator function and two separate genes annotated for the sensor kinase function (*kdpD* and DVU3335). Interestingly, only one of these two, DVU3335, contains a conserved histidine kinase domain which is absent the *D. vulgaris kdpD* candidate. However, DVU3335 does not encode the well-conserved motifs associated with KdpD. We created a knock out mutant in the DVU3335 gene. The DVU3335 knock out strain showed a growth deficiency in low K+ conditions and when exposed to low K+ conditions was unable to upregulate genes in the *kdp* operon. Phenotypic microarrays were used to obtain a broader comparison of the mutant and wild type strains. Our results show that the major differences between the wild type and the mutant are in response to salt stress and support the role of DVU3335 in modulating K+ uptake during low K+ and high Na+ conditions.

## METHODS

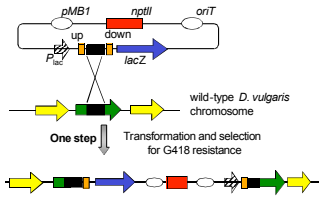


**Figure.1 Schematic representation of the proposed high-throughput method to inactivate and barcode genes in *D. vulgaris*.** ~ 750 bp internal sequence is amplified for the target Histidine Kinase gene from the *D. vulgaris* genomic DNA. Unique UP and DOWN barcodes are introduced during amplification and the resulting fragment is cloned into a suicide vector (e.g. TOPO vector). The suicide vector is transformed into *D. vulgaris* followed by selection on the antibiotic reporter results in the incorporation of the entire plasmid at the locus of homologous recombination causing gene deletion via gene disruption. Using this strategy a library of knockout mutants have been generated for the Histidine Kinase genes in *D. vulgaris*.

## RESULTS Phenotypic analysis of a knock out mutant

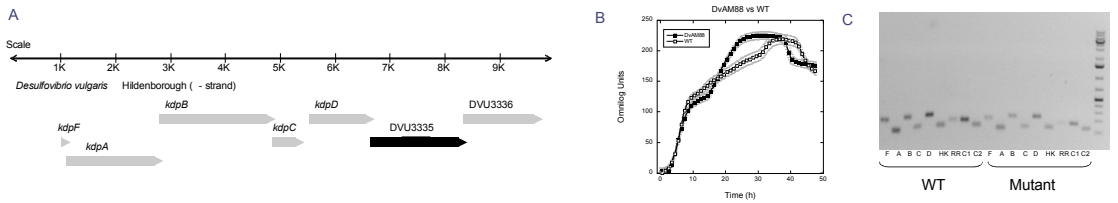


**Figure 2: (A)** The Kdp operon structure. In the *D. vulgaris* there are two sensor proteins in the Kdp operon, *kdpD* ; which contains all the $K^+$ and $N^+$ sensing domains delineated in *E. coli*, and a gene down stream, DVU3335, that contains the conserved histidine kinase domains. The hashed block represents the locus used to create insertional mutant using the single cross-over strategy. **(B)** Growth curves of the *D. vulgaris* wild type and DvAM88 in defined Lactate sulfate medium show no difference between the two strains. **(C)** mRNA expression of genes from the *kdp* operon were measured in the two strains under these conditions show that all genes in the *kdp* operon upstream of the gene disruption locus have similar expression levels. This is are consistent with equivalent growth in the two strains.

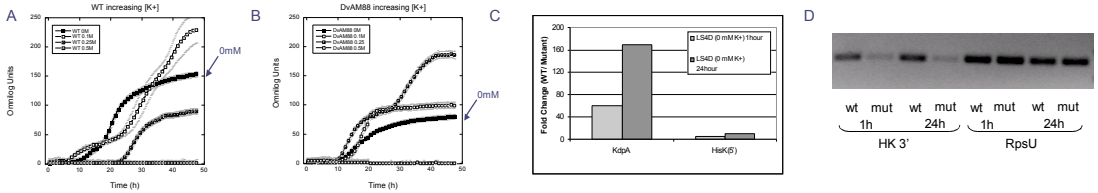


**Figure 3: (A, B)** Growth curves of *D. vulgaris* wild type and DvAM88 with varying concentrations of $K^+$ showed that the mutant strain required a higher level of $K^+$ concentration in the defined LS medium for optimal growth. **(C)** Consistent with this growth defect, Q-PCR data showed that while *kdp* genes are highly upregulated in *D. vulgaris* WT within an hour of growth in low K+ medium, such an upregulation was not observed in DvAM88. Very little expression was observed for the genes downstream of the insertional locus. *rpsU* mRNA levels was constant across all conditions and time points and was used to normalize the data.

## Strategies to characterize knockout mutants and confirm Histidine Kinase / Response regulator pairs

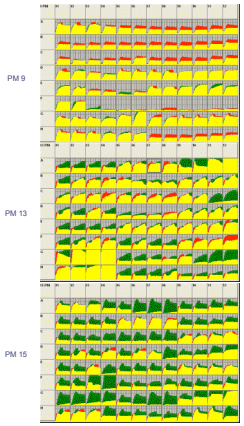


**Figure 5.** Use of Omnilog™ strategy to obtain high throughput phenotypic data: Prefabricated Biolog Phenotype MicroAarray™ plates can be used to assess the phenotypic response of the WT and mutant strain to multiple conditions. Each 100-µL well of a 96-well PM plate contains a single substrate, such that the 96-well plate can be used to examine the growth of an organism on 96 different substrates simultaneously or to test the phenotypic response of a given selection of chemicals.

Shown here are PM plates 9, 13 and 15 used to survey two *D. vulgaris* strains. Complete list of conditions tested is available at the manufacturer's website, (biolog.com/pmMicrobialCells.html). Kinetic plots are generated by the OmniLog® instrument, an incubated chamber which measures growth as a factor of light transmittance of the culture every 15min for up to 150 hours. OmniLog® (OL) units is as defined by the manufacturer. (biolog.com/PM_FAQ.html).

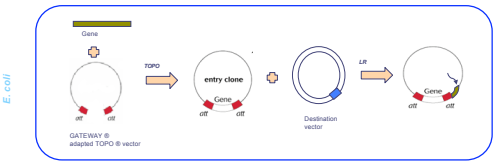


**Figure 5** The LR strategy is being used to create an expression library of *D. vulgaris* Histidine Kinases and Response regulators in *E. coli*. Purified proteins will be used in phospho-transfer assays to confirm histidine Kinase / response regulator pairs.

## ACKNOWLEDGEMENT

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