Q-2848

Two Component Signal Transduction in *Desulfovibrio* Species

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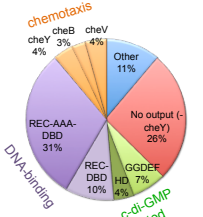
INTRODUCTION

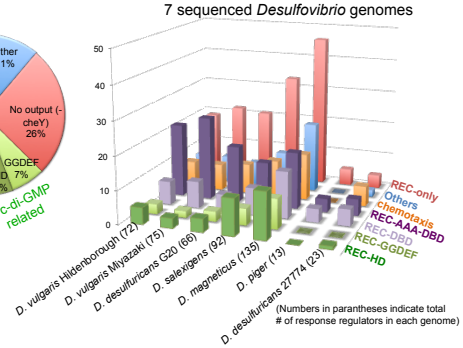
The environmentally relevant *Desulfovibrio* species are sulfate-reducing bacteria that are of interest in the bioremediation of heavy metal contaminated water. Among these, the genome of *D. vulgaris* Hildenborough encodes a large number of two component systems consisting of 72 putative response regulators (RR) and 64 putative histidine kinases (HK), the majority of which are uncharacterized. We classified the *D. vulgaris* Hildenborough RRs based on their output domains and compared the distribution of RRs in other sequenced *Desulfovibrio* species. We have successfully purified most RRs and several HKs as His-tagged proteins. We performed phospho-transfer experiments to verify relationships between cognate pairs of HK and RR, and we have also mapped a few non-cognate HK-RR pairs. Presented here are our discoveries from the *Desulfovibrio* RR categorization and results from the in vitro studies using purified His tagged *D. vulgaris* HKs and RRs.

Distribution of response regulators across sequenced *Desulfovibrio*

D. vulgaris Hildenborough - 72 RRs



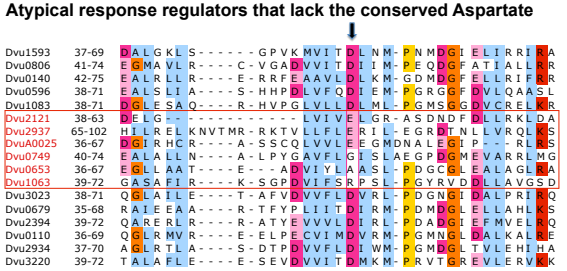
7 sequenced *Desulfovibrio* genomes



(Numbers in parantheses indicate total # of response regulators in each genome)

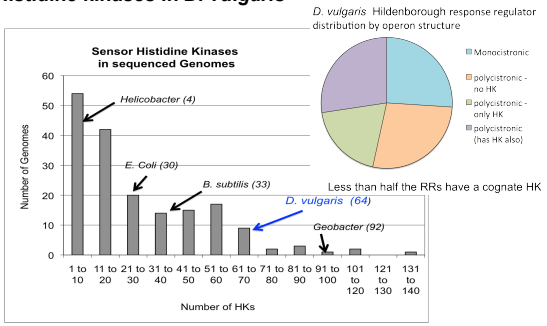
With the exception of the animal isolates *D. piger*, and *D. desulfuricans 27774*, *Desulfovibrios* have a large number of response regulators. Surprisingly, there are only two RRs that are shared among all 7 genomes: Dvu1083 (PhoB) and Dvu3220. There are 25 RRs conserved among the 5 environmental isolates.

Atypical response regulators that lack the conserved Aspartate



These 6 response regulators of *D. vulgaris* Hildenborough also lack some of the other conserved residues of the phosphorylation pocket. Their orthologs in other *Desulfovibrio* also have an atypical active site. They also lack a cognate HK, and they may not be activated by phosphorylation.

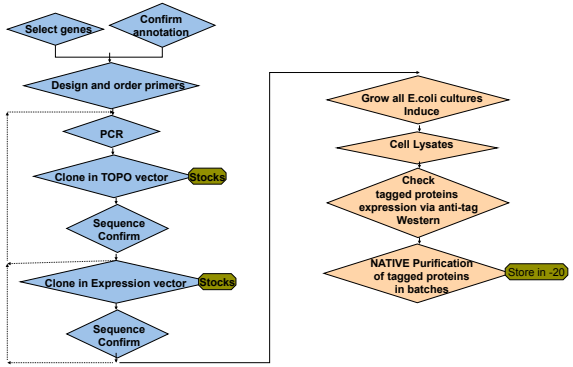
Histidine kinases in *D. vulgaris*



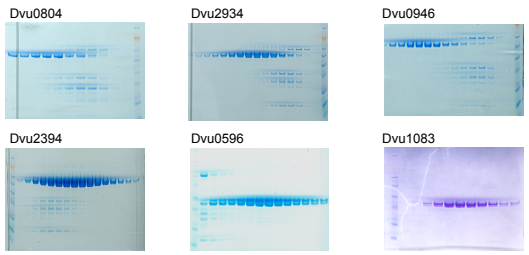
Less than half the RRs have a cognate HK

D. vulgaris contains a large number of two component systems, but for most individual histidine kinases and response regulators, the cognate partners cannot be predicted. In order to map these systems, soluble portions of the sensor HKs and the response regulators were cloned, expressed in *E. coli* and purified as described below.

Cloning and purification scheme for the HKs and RRs



Examples of purified response regulators



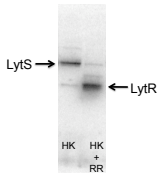
Phosphorylation and phospho-transfer assays to experimentally validate predicted cognate HK / RR pairs

In the case of the Sensor HK LytS (DVU0597) , the predicted response regulator is encoded in the same operon.

The ~57Kd LytS HK has an N terminal 123aa TM region. The cytoplasmic region is ~ 45 Kd. We purified this cytoplasmic portion of LytS for the ATP phosphorylation assay and coupled it with its 25 KD RR partner, LytR.

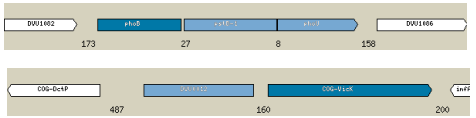
LytS, is successfully phosphorylated using 32P-ATP in 15mins. Less that 5 minutes of incubation with the purified RR, LytR causes reduction in the HK~P and increase in the RR~P.

LytS/ LytR systems in other bacteria are shown to regulate Autolysis. We also have a knockout mutant in the *lytS* gene and are testing for this function in *D. vulgaris*



14% TG SDS PAGE gel

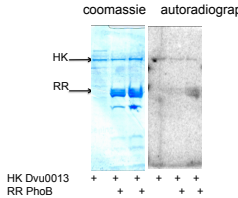
In the case of the Sensor HK VicK(DVU0013) , the predicted response regulator is not encoded in the same operon.



Regulon predictions from Microbes Online (Dehal et al 2009) suggest DVU0013 to be in the same regulon as DVU1083 (phoB).

Prediction tools developed by from Burger and Nimwegen (2008) suggest DVU0013 to be the cognate partner for DVU1083.

Phosphorylation assays experimentally prove DVU0013/ DVU1083 to be a two component system.



- Purified Histidine Kinase and response regulator proteins are being used to confirm and identify the two component systems in the soil bacterium, *D. vulgaris* Hildenborough
- Purified response regulators are being used to conduct Chip based assays that map their regulatory networks (see Poster #880)
- In the case of sensor histidine kinases only the soluble portion of the protein are being used. The soluble portions were delineated by Morgan Price at the Lawrence Berkeley National Laboratory.

ACKNOWLEDGEMENTS

ENIGMA is a Scientific Focus Area Program supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Foundational Science through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.