Title
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**Q-2848 Two Component Signal Transduction in *Desulfovibrio* Species**

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**INTRODUCTION**

The environmentally relevant *Desulfovibrio* species are sulfide-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 domain outputs 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 also have mapped a few non-cognate HK-RR pairs. Presented here are our discoveries from the *Desulfovibrio* Hildenborough 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

With the exception of the animal isolates *D. piger* and *D. desulfuricans* 27774, *Desulfovibrio* 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 five environmental isolates.

**Atypical response regulators that lack the conserved Aspartate**

- **Dvu2937**: 60-102
- **Dvu2025**: 32-97
- **Dvu487**: 32-97
- **Dvu2397**: 32-97
- **Dvu2398**: 32-97
- **Dvu110**: 32-97
- **Dvu2394**: 37-70
- **Dvu2328**: 37-70

These 8 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.

**Cloning and purification scheme for the HKs and RRs**

**Examples of purified response regulators**

- Dvu0804
- Dvu2034
- Dvu0946

**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 ~57kDa 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 phosphorlyation assay and coupled it with its 25 Kd RR partner LyR. LyS, is successfully phosphorylated using 32P-ATP in 15mins. Less than 5 minutes of incubation with the purified RR, LyR causes reduction in the HK~P and increase in the RR~P.

LytS / LyR 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*.

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

**Regulator predictions from Microbes Online (Dehal et al 2009)**

- **DVU0013** to be in the same region as DVU1083 (PhoB)

**Prediction tools developed by from Burger and Nimwegen (2006)**

- **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.**

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