Moore Foundation Data Sharing
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Protocol: construction of a fluorescence reporter system for visualization of gene expression in a marine bacterium in real-time.

Here, I describe the genetic engineering protocol used to construct a 3-color fluorescent reporter system to visually report *dmdA* and *dddW* gene expression in a marine bacterium.

Introduction

- The genes *dmdA* and *dddW* encode the enzymes that are responsible for the two pathways of DMSP (dimethylsulfoniopropionate) degradation (Figure 1).

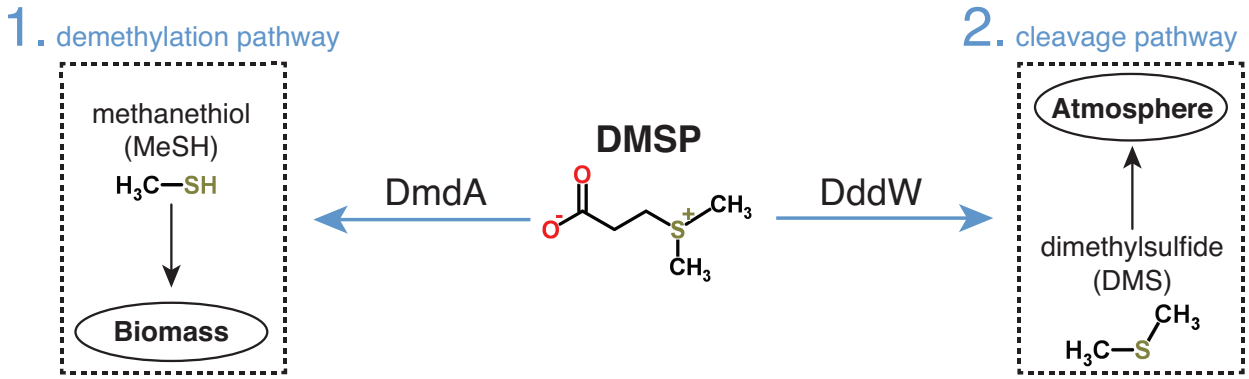

Figure 1: DMSP is degraded by bacteria in the ocean via two competing pathways: demethylation and cleavage pathways.

- *Ruegeria pomeroyi* DSS-3 is our model marine microorganism for DMSP degradation (Gonzalez, 2003). This species can utilize both pathways of DMSP degradation.

- pRK415 is a broad-host-range vector for Gram-negative bacteria (Mather *et al.*, 1995), and is used to express exogenous genes in *R. pomeroyi* DSS-3 (Reisch *et al.*, 2011).

Fluorescent reporter system design rationale

- In order to probe the relative expression levels of the two degradation pathways of DMSP within a single cell, I designed a **three-color fluorescent reporter plasmid** (Figure 2). This design is a modification of the pZS2-123 plasmid (Cox *et al.*, 2010).

- The plasmid contains three promoter fusions: i) the promoter of *dddW* (cleavage pathway) fused to *cfp* (cyan fluorescent protein gene), ii) the promoter of *dmdA* (demethylation

pathway) fused to *rfp* (red fluorescent protein gene), and iii) a weak constitutive promoter (the *lac* promoter) fused to *yfp* (yellow fluorescent protein gene). Including all promoter fusions on a single plasmid allows the comparison of fluorescence intensities among the different colors *within a single cell*.

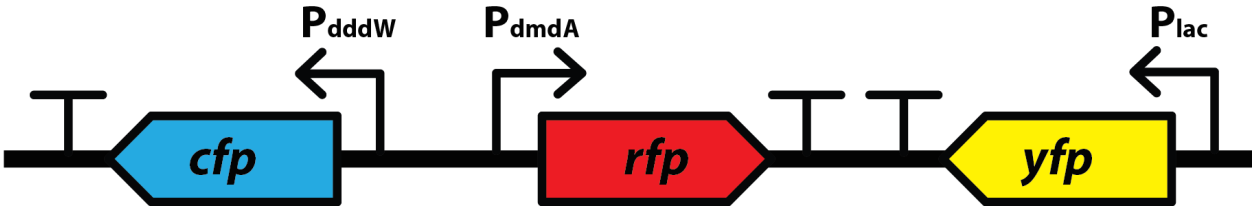

Figure 2: Fluorescent reporter system constructed to visually report *dddW* (CFP) and *dmdA* (RFP) expression. P = promoter; T = terminator; italics = coding DNA sequence; the Synthetic Biology Open Language is used to represent genetic elements.

- YFP expressed under the control of a weak constitutive promoter facilitates the elimination of dead cells from downstream image analyses, whereby a cell with no detectable yellow fluorescence is considered non-viable. It is also used to normalize fluorescence intensity values by plasmid number (for which YFP fluorescence intensity is a proxy), thus enabling fluorescence intensity comparisons *among different cells within a single population*.

- The three fluorescent protein variants (Venus YFP variant with mutations of the Citrine YFP variant incorporated; Cerulean CFP; and mCherry RFP) were chosen for maximal spectral separation, and for their fast maturation times (Cox *et al.*, 2010).

Protocol

- The Gibson assembly protocol (Gibson *et al.*, 2009) was used to clone the three promoter fusions into the pRK415 vector backbone for expression in *R. pomeroyi* DSS-3.

- Promoters of *dddW* and *dmdA* were defined as the 250 bp region upstream of each gene, and were PCR amplified from *R. pomeroyi* DSS-3 genomic DNA.

- Following established protocols for Gibson assembly (New England BioLabs Inc.), fragments were created by PCR amplification using primers with 30 bp overlapping overhangs between fragments. 5 fragments were assembled:
  i. pRK415 vector, cut open at the multicloning site (MCS) with restriction enzymes *HindIII* and *EcoRI*
  ii. *cfp* gene and its terminators, PCR amplified from the pZS2-123 plasmid (Cox *et al.*, 2010)
  iii. 250 bp upstream of gene *dddW* (called P_{dddW}), amplified from *R. pomeroyi* DSS-3 genomic DNA

iv. 250 bp upstream of gene *dmdA* (called P_{dmdA}), amplified from *R. pomeroyi* DSS-3 genomic DNA
v. *rfp* and *yfp* genes and their terminators, amplified from pZS2-123 plasmid (Cox *et al.*, 2010)

- Successful constructs were verified by sequencing, and transformed into *R. pomeroyi* DSS-3.

References

Cox RS, Dunlop MJ, Elowitz MB. (2010). A synthetic three-color scaffold for monitoring genetic regulation and noise. *J Biol Eng* **4**:10.

Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA, Smith HO. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* **6**:343–5.

Gonzalez JM. (2003). Silicibacter pomeroyi sp. nov. and Roseovarius nubinhibens sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. *Int J Syst Evol Microbiol* **53**:1261–1269.

Mather MW, McReynolds LM, Yu C-A. (1995). An enhanced broad-host-range vector for Gram-negative bacteria: Avoiding tetracycline phototoxicity during the growth of photosynthetic bacteria. *Gene* **156**:85–88.

Reisch CR, Stoudemayer MJ, Varaljay V a, Amster IJ, Moran MA, Whitman WB. (2011). Novel pathway for assimilation of dimethylsulphoniopropionate widespread in marine bacteria. *Nature* **473**:208–11.