Hypermotile Screen Reveals Potential *Haloferax volcanii* Two Component Regulatory System

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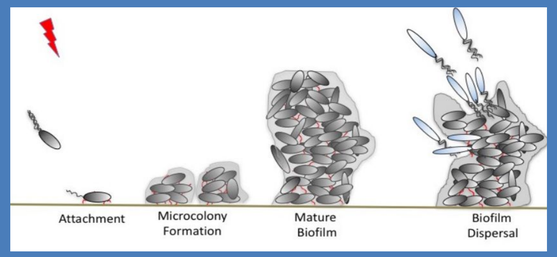
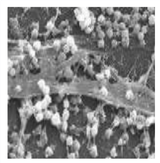
Abstract

A biofilm is a group of microorganisms sticking to surfaces and secreting extracellular polymeric substances that protect them from outer threats. This allows disease-causing bacteria like the cystic fibrosis pathogens or cells on our teeth, to hide from our immune system until the coast is clear. It also makes these cells up to 1000-fold more resistant to antibiotics. Finding ways to keep microbes from attaching, or to trick them into leaving biofilms, would be highly beneficial for the future of medicine. One trait that inhibits biofilm maturation, but is needed for biofilm dispersal, is their ability to swim. I screened a transposon library of the model archaeon *Haloferax volcanii* for hypermotility with a new technique, streaking the library down the middle of a low-agar motility plate, allowing me to identify 49 hypermotile mutants by picking mutants that reached the edges of the plates first. Genome analysis reveals that all but one hypermotile mutant contains a tn-insertion or other mutation affecting ,HVO_2248 and/or HVO_1357. While HVO_2248 is a hypothetical protein with no identified conserved domains, HVO_1357 appears to encode the receiver, and its flanking gene HVO_1356, the histidine kinase of a two-component regulatory system. This is exciting because it begins to explain a new archaeal signal transduction pathway that relates to motility. Also, these proteins have the potential to be effective drug targets to prevent biofilm formation or induce the dispersal of cells in an already existing biofilm making them susceptible to antibiotics.

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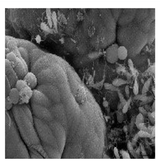
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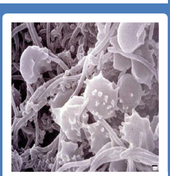
mouth

Figure 1. Biofilm formation and dispersal. Biofilm formation starts by cells attaching to surfaces generally initiated by stress.[1] They then aggregate into large structures encased by an extracellular polymeric substance that protects them. In this stage cells can be up to 500-1000 times more resistant to antibiotics than single cells. When the cells sense that the situation is less stressful, they form highly motile dispersal cells (light blue) that escape from the biofilm.[2] Electron micrographs of biofilms on a catheter, in a mouse gut or on a tooth.[1,5] (B) Biofilms associated with tooth decay contain both, bacteria and archaea but very little is known about archaeal biofilm formation and dispersal.[4]

Results

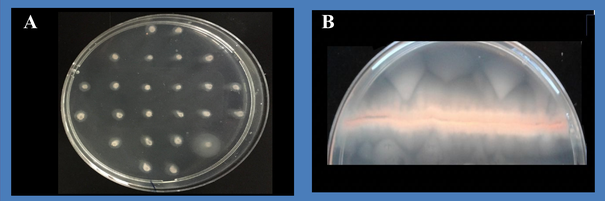

Figure 2. Motility screen. (A) *H. volcanii* colonies from transposon library were stabbed into motility (0.3% agar) plates and incubated for two days at 45ºC. (B) *H. volcanii* transposon library was streaked down the center of a motility agar plate and incubated for four to five days at 45ºC. Cells from halos furthest away from the center were streaked onto solid (1.5% agar) plates before re-stabbing individual colonies on motility plates to confirm hypermotile phenotype.

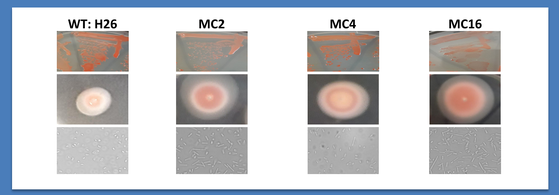

Figure 3. Colony and cell phenotypes. A subset of the hypermotile mutants have a distinct colony and/or cell phenotypes. Wild-type (wt) and mutant colonies were streaked onto 1.5% agar plates (top) or stabbed into 0.3% agar plates (middle) and incubated at 45ºC for four to five days. Liquid wt and mutant cultures were grown to an OD_{600} of ~0.4 and cells were observed using an inverted DIC microscope (bottom).

Table 1: Summary of Raw Genomic Data

Gene	Position	# of TN Mutants	non-TN HVO_2248 mutations	Non-TN HVO_1357 mutations	*Additional Non-TN mutations	Plate #
HVO_2248	2734434	6	-	1	1	P1,P3,P6,P10
	2734510	2	-	1	-	P1,P10
	2734437	1	-	-	1	P7
	2736353	1	-	-	-	P3
<HVO_2248	2734755	10	-	3	6	P1,P5,P7
HVO_1357	1867189	13	-	-	6	P1,P2,P4,P6,P7,P9,P10
	1867146	4	1	-	3	P2,P4, P5, P7
	1865880	2	-	-	-	P9,P10
HVO_0576	1147136	1	1	-	1	P7
HVO_1791	2285156	1	1	-	1	P10
HVO_2229	2723422	1	1	-	-	P10
HVO_2377	2871511	1	1	-	1	P10
HVO_2649	3126642	1	-	-	1	P10
HVO_A0546	621497	1	1	-	1	P10
HVO_1926	2404062	1	1	-	1	P7
Grand Total	15	46	7	5	23	N/A

A compiled database of the number of mutations within each gene. *Various point mutations , deletion of CRISPR3, point mutation or deletion of pHV3 and pHV4, deletion of provirus3, mutations in HVO_2380 or HVO_2377, and a deletion between HVO_A0128-A0016, HVO_A0127-A0017, HVO_A0124-A0111. The last column represents the original plate the mutant was take from.

Genomic Location of HVO_2248 and HVO_1357

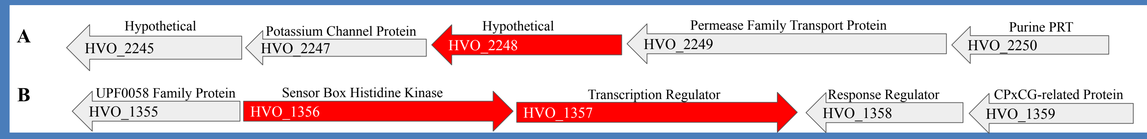

Figure 4. Protein Domains. HVO_1356 is a sensor box histidine kinase which also has a GAF and as PAS domain. HVO_1357 itself is a multidomain protein. It has receiver domain at the N-terminus, a Bat-type HTH domain at its C-terminus.

Figure 5. Two Component Regulatory System. (A) A membrane protein transmits an extracytoplasmic stimulus to a cytoplasmic histidine kinase, which after autophosphorylation transfers a phosphate to the regulator. This activates the regulator resulting in a transcriptional response.[3]

Conclusions and Future Considerations

I hypothesize that HVO_1357 and its genomic neighbours (HVO_1356 and HVO_1358) are part of a signal-dependent gene regulation cascade and may be the directly involved in transcription regulation of target genes. For my next steps, I will construct Hvo_2248 and Hvo_1357 knockout strains. Assuming that these knockout strains are also hypermotile, I will next perform transcriptomics of these strains. The identification of what this two component regulatory system is sensing may allow us to reveal proteins important for motility, biofilm formation as well provide additional information about the regulation of motility and/or cell morphology.

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