Investigating amino acid-modulated motility of the zebrafish bacterial isolate, *Aeromonas veronii*

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Abstract

Animals are colonized by communities of microorganisms that influence the health and development of their host. However, the mechanisms of host colonization are still underexplored. To investigate this, previous work in the lab used experimental evolution to adapt a bacterial symbiont, *Aeromonas,* to the zebrafish gut. These experiments led to the identification of a novel gene, *spdE*, which significantly impacts host colonization. We found that evolved isolates with mutations in *spdE* had faster rates of motility and increased host immigration. Sequence analysis revealed that the protein, SpdE, has a domain for sensing extracellular signals and a diguanylate cyclase domain which produces an intercellular signaling molecule that regulates motility. Further biochemical investigation identified that the signal SpdE senses is hydrophobic amino acids, specifically proline, valine, and isoleucine. To further investigate the relationship between SpdE-dependent *Aeromonas* motility and environmental amino acids, we developed a new technique ("exploration assay") which is designed to measure differences in motility between strains or conditions. Using the exploration assay, we compared motility of wild type and *spdE* knockout strains in different amino acid environments. From our results, we found that the wild type strain is more motile in the presence of these amino acids. However, even in the absence of amino acid signal, the *spdE* knockout is more motile than the wild type. From these data, we have created a model for how SpdE regulates motility in response to amino acids which offers novel insights into *Aeromonas* biology and the mechanisms of host colonization.

Background

Previous work in the lab used experimental evolution to adapt a bacterial symbiont, *Aeromonas,* to the zebrafish gut. These experiments led to the identification of a novel gene, *spdE*, which significantly impacts host colonization. We found that evolved isolates with mutations in *spdE* had faster rates of motility and increased host immigration. Sequence analysis revealed that the protein, SpdE, has two domains, a domain for sensing extracellular signals and a diguanylate cyclase domain which produces an intercellular signaling molecule that regulates motility. Further biochemical investigation identified that the signal SpdE senses is hydrophobic amino acids, specifically proline, valine, and isoleucine. Mass spectrometer quantification of the intracellular cyclic di-GMP revealed that amino acids decreased the concentrations of c-di-GMP.

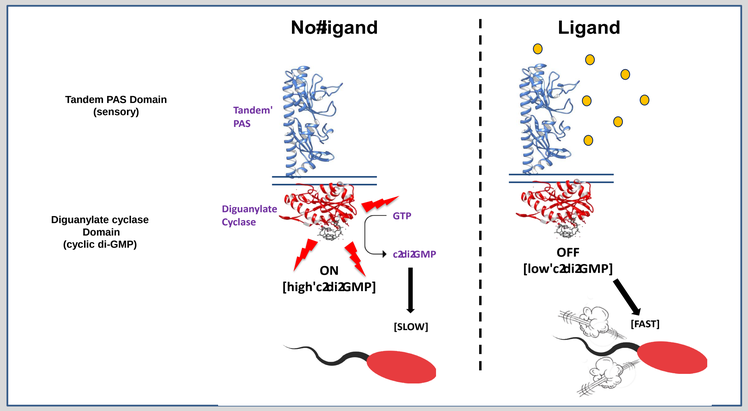


Figure 1. Model of SpdE Structure and Function

Goal

To measure differences in motility of the wild type strain of *Aeromonas* and its *spdE* knockout to understand how SpdE regulates motility in response to amino acids.

Methods

Cultures of the wild type strain of *Aeromonas* and its *spdE* knockout were grown overnight, washed, and incubated with different amino acid solutions for 3-4 hours. Using a Rainin Liquidator, supernatants of bacterial cultures were transferred into pipette tips and halfway submerged in bacterial culture in a 96-well plate. Over time, bacteria "explore" into the supernatant in the submerged tips, as a measure of motility. Resulting supernatant containing bacteria was added into a 96-well plate containing tryptic soy broth. A plate reader was used to measure growth curves for all wells in the plate.

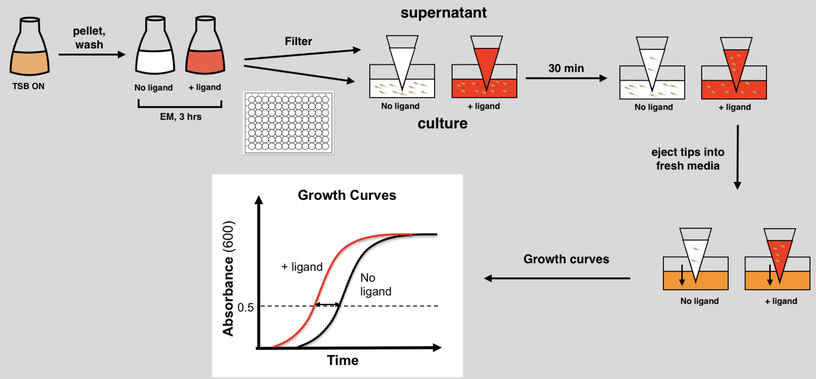
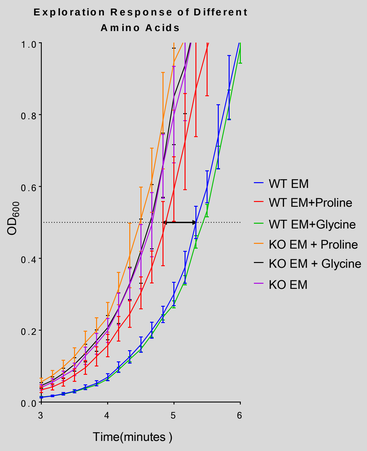


Figure 2. Exploration Assay Schematic

Results

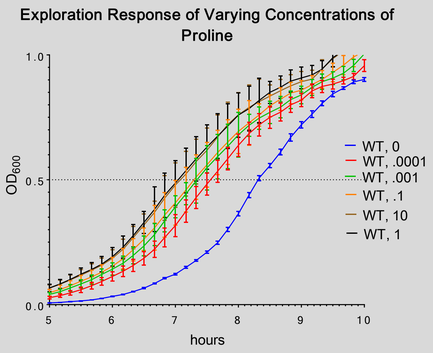
Figure 3. Bacterial Growth Curves. Dotted line represents OD 0.5



Increasing concentrations of ligand (proline) increase motility of wild type *Aeromonas.* These investigations revealed that SpdE's response to ligand is dose-dependent and sensitive to very low concentrations.

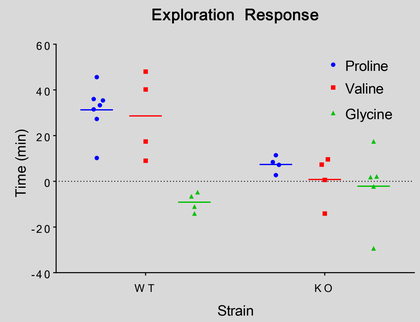
Black arrow in Figure 3 represents time difference that was plotted between growth curve with ligand (WT EM + Proline) and growth curve with no ligand (WT EM) at OD 0.5 that are shown by the red and blue lines. Blue line (WT EM) comes up later in comparison to red line for WT EM + Proline, so *Aeromonas* was faster in that amino acid compared to no amino acid control, because growth curves cross OD 0.5 sooner.

Figure 4. Growth curves with increasing concentrations of ligand.



Results

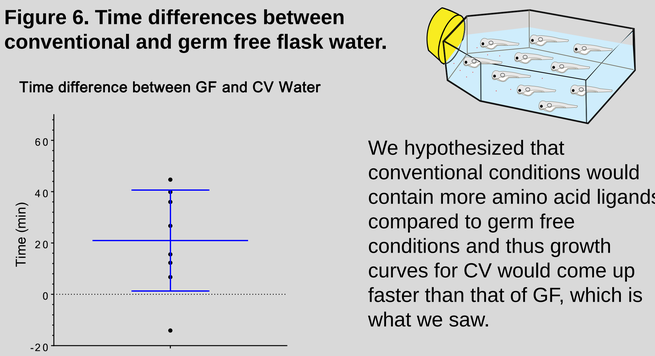
Figure 5. Time differences between amino acid conditions and its EM control



Data collected from multiple Exploration Assay experiments such as those from Figure 3. Points correspond to time differences between ligand and control environments. Positive values mean that *Aeromonas* was faster in that amino acid compared to no amino acid control. Overall, WT strain is more motile in response to ligands, while *spdE* KO is not.

In order to explore the relevance of SpdE in a more natural setting, we compared motility of *Aeromonas* in water collected from flasks of larval zebrafish that were raised germ free (GF; without microbes) or conventionally (CV; with a complex microbiota). We collected the flask water from fish that were 4-7 dpf (days post fertilization), filter-sterilized it, and used it for exploration assays.

Figure 6. Time differences between conventional and germ free flask water.



We hypothesized that conventional conditions would contain more amino acid ligands compared to germ free conditions and thus growth curves for CV would come up faster than that of GF, which is what we saw.

Future Directions

- In order to verify that water from conventionally-raised fish has higher amino acid concentrations than germ free fish, we are collecting filtered conventional and germ free water and measuring amino acid concentrations via mass spectrometry.
- We are further testing to see if amino acid concentrations in conventional and germ free fish water differ over developmental time (e.g between 4 and 7 dpf).

References

Robinson, C. D. *et al.* Experimental bacterial adaptation to the zebrafish gut reveals a primary role for immigration. *PLOS Biology* **16,** (2018).

All figures except for graphs made by Cathy Robinson