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# Identifying V. Cholerae's Autoinducer to Manipulate Its Quorum Sensing

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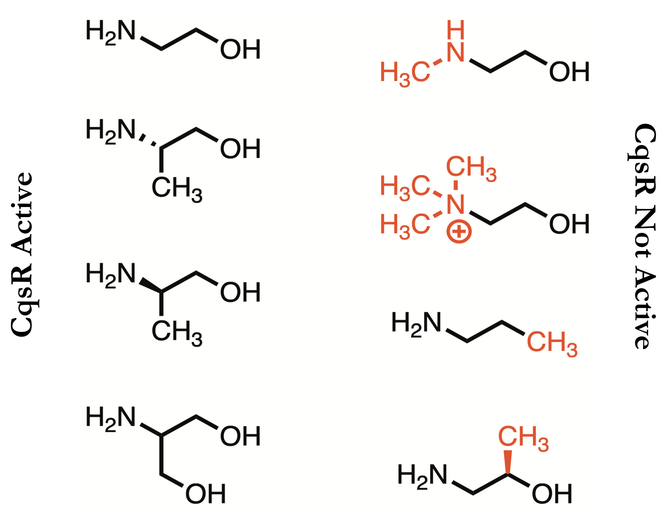
## Abstract

• Vibrio cholerae is a gram-negative anaerobic bacteria that inhabits brackish or saltwater areas.
• Causative agent of cholera, which results in acute diarrhea and dehydration.
• Uses quorum sensing, a cell density dependent method of communicating with other bacteria and regulating its entire lifecycle of infection. (gene expression of functions such as biofilm formation, virulence, and more)
• Vibrio cholerae determines bacterial population based on the secretion of several signaling molecules called an autoinducer from others of their kind.
• Upon adequate accumulation, they can deactivate their virulence and begin to leave their host's body to continue infecting other organisms.
•The goal of this experiment is to identify the structure of and isolate one of the autoinducer molecules. Current studies suggest there are four chemical inputs in V. cholerae, two are known (Ea-CAI1/CAI-1 circuit and the AI-2 circuit).

## Hypothesis

We believe that the autoinducer molecule shares many structural similarities to the chemical ethanolamine. In previous bioassays, ethanolamine has tested positive not only as a common contaminant in *Vibrio cholerae* supernatant but also as a significant source of bioactivity.



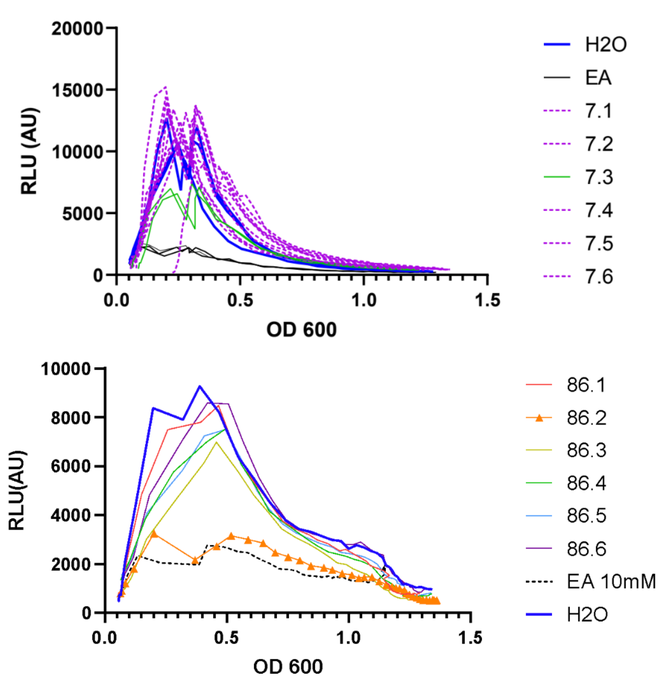

## General Workflow

Partial Purification of Bacterial Culture Supernatants

Bioassay of Semi-purified Fractions

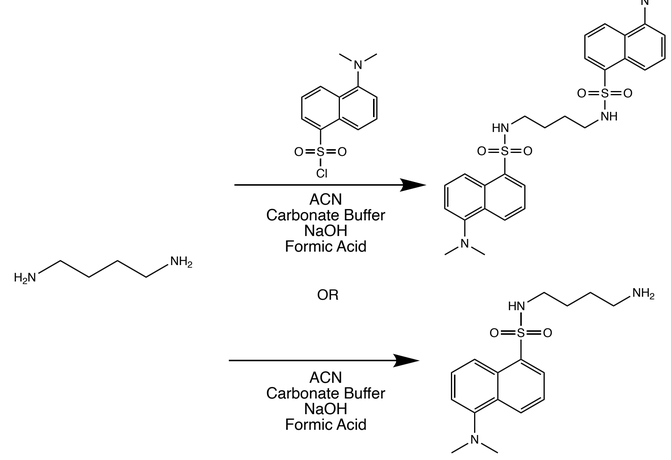
Characterization of Polyamine(s) in Active Fractions

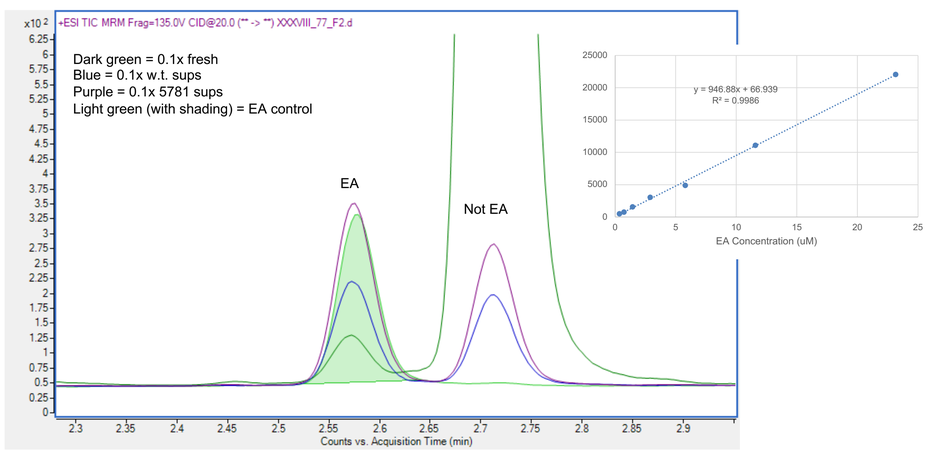
## CqsR Bioassay




Across numerous purifications via the Biotage, fraction 3 of each series has consistently shown itself to be active, as seen with the drop is RLU that is representative of quorum sensing being used to shut down *Vibrio cholerae's* bioluminescence. However, since switching to M9 minimal salt sups, activity has shifted to fraction 2.

## Dansyl Chloride Derivatization and QQQ




## Polyamine Quantification (μM) in Fractions

| Fraction | Active? | [Ethanolamine] | [Spermidine] | [Norspermidine] | [Putrescine] | [Diethanolamine] | [3-(nethylthio)-propylamine] | [Cadaverine] |
|---|---|---|---|---|---|---|---|---|
| 79.2 | No | - | 568.75 | - | - | - | - | - |
| 79.3 | No | - | - | - | - | - | - | - |
| 79.4 | No | - | - | - | - | - | - | - |
| 80.2 | Yes | - | - | - | 3624.08 | - | - | - |
| 80.3 | No | | | | 553.59 | | | |
| 80.4 | No | - | - | - | - | - | - | - |
| 81.2 | No | - | - | - | 44.69 | - | - | - |
| 81.3 | No | - | - | - | - | - | - | - |
| 81.4 | No | - | - | - | - | - | - | - |
| 82.2 | Yes | - | - | - | 1193.56 | - | - | - |
| 82.3 | No | - | - | - | - | - | - | - |
| 82.4 | No | - | - | - | - | - | - | - |
| 83.2 | Yes | - | - | - | 2537.98 | - | - | - |
| 83.3 | No | - | - | - | 524.27 | - | - | - |
| 83.4 | No | - | - | - | - | - | - | - |
| 84.2 | Yes | - | - | - | 1683.96 | - | - | - |
| 84.3 | No | - | - | - | - | - | 65. 54 | - |
| 84.4 | No | - | - | - | - | - | - | - |
| 85.2 | Yes | - | - | - | 3617.72 | - | - | - |
| 85.3 | No | - | - | - | 401.86 | - | - | - |
| 85.4 | No | - | - | - | - | - | - | - |
| 86.2 | Yes | - | - | - | 2558.77 | - | - | - |
| 86.3 | No | - | - | - | 279.20 | - | - | - |
| 86.4 | No | - | - | - | - | - | - | - |

## Future Studies - Polyamine Mutant Strains of *Vibrio cholerae*

| EA | Putrescine | Cadaverine | Diaminopropane | Spermidne / Norspermidine | |
|---|---|---|---|---|---|
| VC1554/ VCA013 | VCA0814 (speB)/VCA1063 (speC) | VC0281 (cadA) | VC1625 (dabD) | VC1623-VC1624 | Mutant Genotype |
| + | + | + | + | + | luxQ- cqsS- vpsS- |
| ? | + | + | + | + | luxQ- cqsS- vpsS- ΔVC1554 ΔVCA0136 |
| ? | + | - | + | + | luxQ- cqsS- vpsS- ΔVC1554 ΔVCA0136 ΔcadA |
| ? | - | + | + | + | luxQ- cqsS- vpsS- ΔVC1554 ΔVCA0136 ΔspeB ΔspeC |
| ? | - | + | - | - | luxQ- cqsS- vpsS- ΔVC1554 ΔVCA0136 ΔspeB ΔspeC ΔVC1623-1625 |

Tests on biosynthesis mutants of cholerae will be conducted to quantify polyamines in specific gene deficient specimens. The experiment will assist in correlating quantification of a polyamine/polyamine pathway to the activity of a fraction.

## Conclusion and Future Work

- Quantify polyamines in biosynthesis mutants of cholerae that lack specific polyamines
- Investigate putrescine as potential autoinducer or a molecule that is similar to it
- Refine MRM methods and calibration curves for polyamine quantification

## Acknowledgements

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