Organisms throughout nature have been identified to exhibit a variety of collective behaviors. Ants form colonies and can harvest foods and soils, bees form swarms and can create hives, decorator crabs can harvest materials and camouflage itself with its surroundings. However, collective behaviors have only been observed in organisms with hierarchical organizations. To understand how harvesting behaviors exist in organisms as a collective, we study the California blackworm as an organism with a unique behavior mechanism and investigate the properties behind how and why this occurs.

### Introduction

When observed as a single organism, the California blackworm is still observed to be able to gather algae around itself. The analysis conducted showed the existence of clusters using a relative cluster formula. The graph shows evidence to support harvesting behavior. When observed as an individual, the California blackworm also exhibits the harvesting behavior for other materials, such as microplastics. This show an implication for a larger scale application, such as water purification in a public health setting.

### Results

- When observed as a single organism, the California blackworm is still observed to be able to gather algae around itself.
- The analysis conducted showed the existence of clusters using a relative cluster formula.
- Relative cluster:
  - Number of components in the frame / Maximum number of components
- The graph shows evidence to support harvesting behavior.
- When observed as an individual, the California blackworm also exhibits the harvesting behavior for other materials, such as microplastics.
- This show an implication for a larger scale application, such as water purification in a public health setting.

### Conclusions

- Future application for cleaning water for clump extraction
- Understanding rules in biology and application to swarm robotics
- Future work with more materials, sorting potential
- Theory of how they grab with mechanisms behind grabbing
- California blackworms can provide evidence of harvesting behavior through trials conducted, has potential to be applied in public health

### Acknowledgments

The authors are grateful to Dr. Bhamla and Bhamla Lab for providing helpful mentoring and resources. The authors would like to thank NSF and ACE REU (Dr. Hammer) for support through OCE-1851723.

### References

**INTRODUCTION**

*Pseudomonas aeruginosa* is an opportunistic pathogen commonly sourced in the lungs of cystic fibrosis (CF) patients. Previous studies have demonstrated that mutation within *ssg*, a gene found in *P. aeruginosa* is involved in the synthesis of lipopolysaccharide O-antigen, both integral membrane components of Gram negative bacterial cells.

Previous studies have demonstrated that mutation within *ssg* determines the stacked (wild-type) or clumped (mutant) aggregate assembly type. The clumped aggregate is inferred to result from an increased surface hydrophobicity due to loss of *ssg* function. After previous literature confirms the integral role of *ssg* in the synthesis of Lipopolysaccharide.

**METHODS**

- **Design of pME6032:ssg construct** with *Benchling* software.
- **Transformation of S-17 (E. coli) and PAO1::*ssg* with pME6032::*ssg* PCR amplification.
- **Sequencing of S-17:** pME6032::*ssg* isolate
- **Gel electrophoresis**
- **Induction of PAO1::*ssg* with IPTG**
- **Imaging of aggregates using Zeiss LSM 880 Confocal Laser Scanning Microscope.**

**RESULTS**

- **Gibson Assembly Protocol**
  - a) pME6032, E. coli: 10 µg/mL tetracycline
  - b) pME6032, *P. aeruginosa*: 200 µg/mL tetracycline
  - c) pUCP18::gfp, *P. aeruginosa*: 200 µg/mL tetracycline, 300 µg/mL carbenicillin

  Schematic of Gibson Assembly, utilized to construct plasmid.

  **Assembly and transformation verification**

  (a) Initial amplification of Gibson Assembly products (lanes 4, 5) against PAO1::*ssg* (lane 3). Run against 100 bp reference ladder (lanes 1, 6).
  (b) Isolated and amplified plasmid from transformed S-17 cells. Lanes 11 as 1kb reference ladder, lane 2 PAO1 amplified *ssg*, lanes 4-10 amplified S-17::pME6032::*ssg*. Gels of 10% agarose, using 6x dye.

**CONCLUSION**

Following rounds of PCR, electrophoresis, transformations, and sequencing, our pME6032::*ssg* construct was successfully transformed into PAO1::*ssg* cells. Verification was also present by use of tetracycline supplemented plates, wherein only cells containing the pME6032 gene were viable. With microscopy, complemented cells displayed the following:

- Stacked aggregate assembly type in both PAO1 and PAO1::*ssg* pME6032::gfp, pucP18::gfp
- Differential threshold for expression of *ssg* by IPTG.

Our results reinforce both the integral role of IPTG and differing inputs for *ssg* transcription, as well as the role of *ssg* expression in spatial organization as a result of surrounding environment. Potential continuations for the project include the following:

- Molecular modeling for *ssg* gene product, including charge and polarity evaluations.
- Antimicrobial susceptibility testing to observe differences across two aggregate types.
- Homology mapping of PAO1 in comparison to other *Pseudomonas*, CF strains.
- IPTG threshold testing for *ssg* expression.

**ACKNOWLEDGEMENTS AND RESOURCES**


Funds for this research were provided by the Center on Materials and Devices for Information Technology Research (CMDITR), the NSF Science and Technology Center No. DMR 0120967, and NSF OCE-1851723. Many thanks to Dr. Sheyda Azimi for mentorship and guidance, Dr. Steve Diggle for PI support, and Georgia Institute of Technology’s Center for Microbial Dynamics and Infection (CMDI) for facilities and technology/software.

**REFERENCES**


**CONCLUSION**

Following rounds of PCR, electrophoresis, transformations, and sequencing, our pME6032:ssg construct was successfully transformed into PAO1:ssg cells. Verification was also present by use of tetracycline supplemented plates, wherein only cells containing the pME6032 gene were viable. With microscopy, complemented cells displayed the following:

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- Antimicrobial susceptibility testing to observe differences across two aggregate types.
- Homology mapping of PAO1 in comparison to other *Pseudomonas*, CF strains.
- IPTG threshold testing for *ssg* expression.
Zooplankton Elemental Composition in the Amazon River Plume Region

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I. Summary

The Amazon River generates an extensive surface plume, the Amazon River Plume (ARP), that reaches hundreds of kilometers into the Western Tropical North Atlantic. Within the ARP, distinct phytoplankton habitats are generated by the interplay of various physical and chemical factors. The impact of these factors on higher trophic levels has not been studied. We measured the elemental composition of five size fractions of zooplankton, a critical link between phytoplankton and upper trophic levels in the ocean. Specifically, we measured the nitrogen, carbon and phosphorus content, in addition to the isotopic composition ($\delta^{15}N$ and $\delta^{13}C$) of zooplankton collected during cruise EN614 in spring 2018. We found statistically significant differences in zooplankton phosphorus content, C:P and N:P ratios between planktonic habitats and day vs. night samples. Further, we integrated these results with stable isotope data to assess the degree and nature of nutrient limitation among each habitat.

II. Who Cares?

• The ARP creates distinct phytoplankton habitats based on biogeochemical factors like surface salinity, temperature and nutrient distribution. The link between habitats and higher trophic levels, like zooplankton, is unknown.
• Zooplankton are primary and secondary consumers and connect ocean trophic levels and influence nutrient cycling in the ocean.
• Isotopic ratios ($\delta^{15}N$ and $\delta^{13}C$) help track an organism’s trophic position and food source.

III. Study Site

Figure 1: Amazon river plume (a) EN614 cruise track and phytoplankton habitats and (b) satellite image. Red represents the ‘young plume core’, yellow ‘western plume margin’, purple ‘outer plume margin’ and blue ‘oceanic water’.

IV. Methods

Figure 2: (below) Three boxplots displaying the effect of collection time on (a) C:P ratio (p=0.005), (b) N:P (p=0.002) and (c) C:N (p=0.089).

Figure 3: (above) Four boxplots of (a) P content (p=0.005), (b) C:N (p=0.032), (c) $\delta^{15}C$ (p=0.005) and (d) $\delta^{15}N$ (p<0.005) separated by habitat type.

V. Results

• C:P and N:P lower in migratory (night) zooplankton
• C:N and P content decreases, as plume influence decreases (YPC to OSW)
• No difference in elemental ratios between zooplankton size fractions
• $\delta^{15}C$ and $\delta^{15}N$ peak in WPM

VI. Conclusions

• Non-migratory zooplankton are more limited by phosphorus than migratory zooplankton. This is contrary to the typical movement of phosphorus in the biological pump. Phosphorus is typically transported by migratory zooplankton and then excreted in the upper water column.
• Nutrients, specifically phosphorus, from the Amazon River dissipate as plume influence decreases. The zooplankton sampled from the YPC habitat displayed the highest phosphorus content by mass fraction.
• The YPC habitat is the most nitrogen stressed. This habitat lacks diazotrophs to supply zooplankton with fixed nitrogen.
• Zooplankton sampled from the WPM are possibly ingesting larger cells with higher $\delta^{15}C$ values.
• The WPM contains diazotrophs that produce low $\delta^{15}N$ values. Surprisingly, we found this habitat had the highest $\delta^{15}N$ values, possibly due to the lag between ingestion and incorporation of nitrogen in zooplankton tissues.

Citations

Plum et al. (in prep)

Acknowledgements

Thank you to Dr. Joseph Montoya and Erica Strope for mentorship in your lab and thank you to NSF and Georgia Tech for funding my REU experience.
The Effects of Ammonium Cycling on Benthic Alkalinity Flux in the northern Gulf of Mexico

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Introduction

The atmospheric and ocean exchange massive amounts of CO2, a weak acid, which produces a number of changes in sea water chemistry, especially the carbonate system, with important implications on marine micro- and macro-organisms (Kroeker et al., 2013).

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CO}_3^{2-} + \text{H}^+ \]

Sediments have been found to play a key role in ocean acidification especially in coastal environments (Cai et al. 2011). The carbon remineralization processes can release acidity through aerobic respiration, alkalinity through anerobic respiration, as well as reduced metabolites, and nutrients. Simultaneously, the reduced metabolites and the nutrient ammonium can be oxidized by dissolved oxygen to generate acidity (Dong et al., 2020).

Total alkalinity (TA) determines the capacity for water to resist changes in pH. Ammonium is an important contributor to benthic alkalinity flux. The denitrification process contributes to TA generation while producing a reduced metabolite (\(N_2\)), which will eventually be released into the atmosphere (Rice & Herman, 2012).

\[ \text{TA} = (\text{Proton Acceptors}) - (\text{Proton Donors}) \]

Methods

1. Ammonium diffusive and benthic flux display seasonal variations.
2. The geographical distribution of benthic alkalinity fluxes reflects the input of terrestrial organic matter and minerals from Mississippi River.
3. Seasonal variations in the ammonium flux are complex and may be attributed to variations in storms and seasonal discharge.
4. Higher discharge from the Mississippi leads to higher primary production and hypoxia that promote carbon remineralization in the sediment.
5. Differences between benthic and diffusive fluxes suggest ammonium does not contribute significantly to acidification and is mainly released into the overlying waters as nutrient.

Figure 2. Map of stations in north Gulf of Mexico.

References


Acknowledgements

I would like to thank Evan Magette, Martial Taillefert, Christophe Rabouille, Burno Bobled, Jordan Beckler, Hanna Bridgham, the NSF for support and guidance throughout this experiment.
Abstract

This research project tracks how neurons fire in the brain of Caenorhabditis elegans as they move and are exposed to different stimuli. Neurons are tracked manually as the worm moves in the channel. Manual tracking is a tool used to train an artificial intelligence program to track neurons as the worm moves. We hope to understand how the observed motions of the worms in reaction to different stimuli translate into the intricate functions of the worm's neural network. The goal of this project is to record if there is any spontaneous activity in neurons and whether high-quality tracking can eliminate false activity from neurons. After tracking was completed, the path of each neuron was mapped, and fluorescence of the red and green channels were plotted against time. This showed the neurons had no spontaneous activity and high-quality tracking can eliminate false activity from neurons. Future steps would be to train the artificial intelligence algorithm to track C. elegans neurons.

Methods

Worms are placed in microfluidic channels inside a PDMS device and exposed to different stimuli from each inlet. The flow rate of stimuli is controlled by a pressure box.

Background

Caenorhabditis elegans is a nematode which serves as a model organism because they are:

- ~1 mm long
- ~50 µm thick
- 302 neurons
- easy to genetically manipulate

Green fluorescence protein (gfp) are added to the C. elegans to make their neurons glow fluorescent under red and green light. Neural activity can be measured in the change in fluorescence from a base fluorescence ($\Delta F/F_0$) of a group of 20 pixels in a neuron.

Data Analysis

The path of a neuron is tracked across the screen as the worm moves.

Summary

So far, when there has been no stimuli applied to the C. elegans in the microfluidic channel there is no spontaneous activity in the neurons. Additionally, the tracking seems to be high quality since there is not much false activity recorded.

Acknowledgements

This work was conducted in collaboration with Dr. Yun Zhang’s Lab at Harvard University.

The Aquatic Chemical Ecology REU program is funded by the NSF OCE 1851723 grant

References

(1) A Transparent window into biology: A primer on Caenorhabditis elegans
(2) Hermaphrodite - Nervous System - General Overview
Introduction

Phytoplankton are primary consumer microorganisms; they are a fundamental part of marine ecosystems because they are a major food source for many other small species. Since they are such small organisms, they are often overlooked when people think of important marine creatures, but without phytoplankton there wouldn’t be any other marine life.

The goal of this project is to determine the movement of phytoplankton when exposed to a Burgers vortex (Figure 9). Three different species, Orgasol, Stephanopysis, and Coscinodiscus wailesii, were put into different levels of this vortex and recorded to see how their movement differed. This is important because if people can find a way to manipulate the movement of phytoplankton to their needs it can be used for things like other research or aquaculture. It can create more sustainable areas with fisheries because they will be able to supply naturally occurring food for other creatures, without having to add any foreign substances to the water.

Methods and Materials

The apparatus used was a clear box with two paddles opposite each other where the paddles would spin and pull water into them to create a Burgers vortex. This is shown in Figure 4 with green dye that was dropped in the middle of the paddles. The dye tapering down as it’s being pulled towards the sides is illustrating the flow of the Burgers vortex. There are 4 different speeds of the paddles that were used, the higher the levels the stronger the vortex became. Two cameras were used to film, one on top and one on the side, about 6 different segments of videos with 400 frames per segment were taken for each level. The data was collected using MATLAB. DLTv5’s tracking for the raw tracking and two scripts, one to combine the camera’s tracks into one image and another to output all the kinematics.

Three different particles were used during this experiment, Coscinodiscus wailesii, Stephanopysis, and Orgasol. C. wailesii and Stephanopysis are the phytoplankton species used, Stephanopysis is a chain shape while C. wailesii is a circular hockey puck shape. Orgasol was the control for this process, it is a spherical neutrally buoyant plastic particle that was expected to demonstrate the exact flow of the vortex (Figures 1, 2, and 3).

Results

The mean relative velocity is seen to increase as the level increases. This was expected since if the particle is following the flow it will move at a similar speed to the turbulence (Figure 6). For all three species they were statistically similar to each other, but different by level.

For the net gross duration rate (NGDR) there is a statistical difference between most levels but not between the particles. This was also an expected result since while doing the tracking it was noticeable that most particles were being tracked up until about similar frames but when increasing the levels, the particles disappeared faster (Figure 7).

When looking at the trajectory flow alignment (TFA) C. wailesii is statistically the same throughout all levels while for Orgasol and Stephanopysis level 3 is statistically different than all other levels. It is expected for level 1 to follow the path poorly because the pull of the turbulence is small so it’s easier for the particles to fall off the path. Also, it makes sense that C. wailesii follows the path better as the level increases for the same reason that the force of the turbulence will pull the particles more when its stronger. Stephanopysis has a similar pattern to Orgasol, where the first level is the highest and the lowest being level 3.

Something that is surprising is that the actual phytoplankton are following the path better than the control. This is surprising because Orgasol is a neutrally buoyant plastic particle meant to show the control of a lifeless particle floating in the vortex to see how its movement can be controlled (Figure 8).

Conclusions

All three particles followed similar patterns for each of the kinematics. Since phytoplankton do not have any anatomy to assist them in moving out of currents this was expected, and the slight differences can be justified in the different shapes and sizes of each species. Looking to future work, discovering why Orgasol followed the flow the worst is a useful finding that will assist in finalizing the data found here. Also, it could be useful to repeat this process with different species of different shapes to expand on these findings.

Contact

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References

- Figure 1: Proto Credit: Anna Cerf Slagelse et al.
- Figure 2: Proto Credit: Dr. Lee Kueblew et al.
- Figure 3: Proto Credit: ARRAY commercial et al.
- Figure 4: Proto Credit: Dr. David Huang et al.
- Figure 6: Proto Credit: D. Webster, L. Wang, D. Webster, L. Wang, et al.
- Figure 8: Proto Credit: D. Webster et al. et al.
- Figure 9: Proto Credit: D. Webster et al. et al.
- Figure 10: Proto Credit: D. Webster et al. et al.

Figure 11: Final 3D Tracks. Left to right: C. wailesii, Stephanopysis, Orgasol
Top: X-Y axes, bottom: Z-Y axes
Light Catalyzed Oxidation of Dissolved Manganese on Iron Oxides

Emily Piwowarski1, Qian Wang, PhD2
1North Carolina State University, 2Georgia Institute of Technology

Abstract
Manganese oxide interactions, particularly those which cause marine sediment deposition, store geochemical information about past and present systems. Alternative to the established biotic route1 of manganese oxide production by microbial processing, an abiotic, photocatalytically-driven pathway using iron oxides was probed for this study. Because manganese oxide and photoactive iron oxide deposits are commonly coincident, researchers investigated the manganese oxide yields in the presence of natural light conditions and iron oxides.

Photooxidative catalysis of manganese in solution with iron oxides produces the same tunnel-structured manganese oxides that are observed to be naturally concomitant with iron oxide deposits,3,4 with potential to compete with bioprocessing yields.3,4 Succeeding a previous study using this method, lab-synthesized goethite was doped with variable fractions of aluminum to approximate naturally occurring minerals.3 This iron oxide is abundant in nature, and has shown to be photoactive at near-red wavelengths.3

Introduction
SEM-EDX analysis showed even distribution of manganese oxides on the goethite surface. This reinforces that dissolved manganese oxides at the water-mineral interface, adsorbing onto the surface of the mineral and producing the stratified deposition in ferromanganese nodules observed in nature.5 UV/Visible spectroscopy (Figure 5 and 6) show that oxygen in solution is a strong driver of manganese oxidation extent. Oxidic conditions resulted in a near-tenfold increase in manganese oxide yield versus that of anoxic conditions. For both oxygen treatments, the zero aluminum experimental control was the same proportion for driving manganese oxidation extent. Elevating aluminum doping fractions reduced manganese oxidation, excepting a statistically similar outcome for the anoxic 7% and 13% goethite conditions. Importantly, iron oxides in nature contain up to approximately 33% aluminum impurity by molar ratio.2 And notably, while the oxic goethite experiment with 18% aluminum by molar ratio added produced low concentrations of manganese oxide, the anoxic experiment of the same treatment did not produce any measurable quantities of the target solid.

Methods and Materials
Aluminum was added in 0, 0.03, 0.07, 0.13, 0.18 molar fractions to iron oxide solution.2 Figures 5 and 6 give resultant aluminum doping fractions following dialysis and washing with deionized (DI) water. SEM-EDX spectroscopy confirmed goethite reagent purity. To elucidate the role of oxygen in solution, oxic experiments were conducted in the air while anoxic conditions used N2 purging gas. For each condition, solutions of .01 g/L goethite, 175 mL DI water, and 3.6 mL dissolved manganese solution were prepared and stirred continuously during the eight-hour experiment. A 450 W Xe-arc lamp simulated natural light. Following hourly sampling, pH was adjusted to near 7.5 with 0.1 M NaOH to approximate seawater conditions. UV/Visible spectroscopy on these samples revealed change in concentrations of dissolved manganese and manganese oxides at each hour. Following the reaction, solutions were vacuum filtered and dried. TEM and SEM-EDX spectroscopy characterized the distribution of target product on the reacted solids.

Results

Conclusions
Manganese oxides are strong oxidizers, and their presence in seawater strongly effects redox chemistry and metal and organics cycling.2 As such, combined biotic and abiotic yields of manganese oxides in anoxic, euphotic zones result in more strongly oxidizing chemical environments. Low-light and suboxic zones would not experience this same forcing on solution chemistry. Because this pathway has shown that manganese oxide production occurs abiotically to a significant extent under simulated natural conditions, future research could dope iron oxide with other common impurities to study even more closely approximated natural conditions.

References

Table 1. Final manganese oxide concentrations and standard error

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<th>Goethite Treatment</th>
<th>Final [MnOx] (μM)</th>
<th>Standard Error</th>
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<tr>
<td>Oxic 0% Al</td>
<td>59.7322</td>
<td>0.7892</td>
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<tr>
<td>Anoxic 18% Al</td>
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</tbody>
</table>

Figure 1. Ferromanganese crust deposition over geologic time.1
Figure 2. An anoxic goethite experiment.
Figure 3. SEM image of G18 following oxic reaction with manganese.
Figure 4. SEM image of G0 following anoxic reaction with manganese.
Figure 5. Manganese oxide production during oxic experiments. Aluminum doping fractions are given in the legend.
Figure 6. Manganese oxide production during oxic experiments. Aluminum doping fractions are given in the legend.
Figure 7. TEM image of G0 after oxic reaction with manganese.
Figure 8. TEM image of G25 after oxic reaction with manganese.
Climate Change Drivers Impact the Alpha and Beta Diversity of Prokaryotic Communities in Salt Marsh Porewater

Isabel Thornberry,1 Katherine Duchesneau,2 Genevieve Noyce,2 Patrick Megonigal2, and Joel E. Kostka2
1 Haverford College, 2 Georgia Institute of Technology School of Biological Sciences, 3 Smithsonian Environmental Research Center

Background
Salt marshes are anaerobic systems that sequester carbon due to high rates of primary productivity and low rates of decomposition.1 Microbes mediate critical biogeochemical cycles:
- Methanogens produce methane; methanothermophilic bacteria consume methane
- Methane is a potent greenhouse gas and one form of carbon released from salt marshes
- Methanogens compete with prokaryotes that have more favorable forms of respiration (ex. sulfate reducers) for organic carbon
- Methane emissions from salt marshes increase with warming, potentially indicating shifts in favored metabolic pathways2

Elevated atmospheric CO2 increases root growth in C3 plant communities2
- Plant physiology responses alter root exudates into soil, which provide labile carbon needed for microbial activity

Alpha diversity = Within sample (ex. richness, Simpson, Shannon Index)
Beta diversity = Between samples (ex. changes in community composition)

Site Description
- Salt Marsh Acclimation in Response to Temperature EXperiment (SMARTX): Simulate climate change drivers with whole-ecosystem warming and elevated atmospheric CO2
- Plant community in a brackish high marsh: Dominated by Schoenoplectus americanus (Bulrush, C3)
- Non-destructive porewater sampling allows for repeated sampling

Changes in Richness

Changes in Genera of Interest

Conclusions
- Depth is the most important factor explaining changes in alpha and beta diversity
  - Richness decreases with increasing sampling depth
  - Elevated atmospheric CO2 treatments are associated with increased richness near the surface
  - Plant-microbial interactions: Changes in plant physiology with additional CO2 likely contributes to changing richness
  - Warming treatment explains changes in community composition
  - Some genera of methanogens increase across the warming gradient, while less abundant sulfur reducers and oxidizers are negatively correlated with warming

Acknowledgements
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References
Evolved mutants survive 27-fold better than ancestor.

**Experimental evolution**

V. cholerae → E. coli → 3 hours at 37°C → Overnight at 37°C → Repeat 30X

Evolved mutants survive 27-fold better than ancestor.

**Results**

Mutants demonstrate increased survival

Confirmed viability of vasK1, vasK2 primers on T6+ Enterobacter sps.

**Conclusions**

- Manual confirmation of T6- Enterobacter sps. inactive T6 system
- vasK1, vasK2 primers viable on T6+ Enterobacter sps.
- Preliminary evidence of mutants surviving T6 mediated killing at higher rate than ancestor

**Future Work**

- Perform replicates to support preliminary evidence
- Investigate mechanism of T6-resistance genes
- Determine unique properties of Enterobacter sps. T6 system

**References**


**Acknowledgements**

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