Assessing the Interaction Between Eukaryotes and Prokaryotes in Simulated Microgravity Conditions

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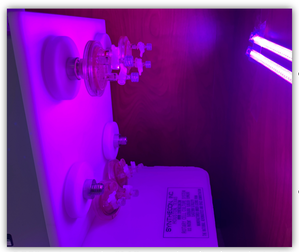
Introduction

- In nature, bacteria can develop relationships with eukaryotic organisms, either synergistically or antagonistically.
- Most bacterial studies are in monoculture which does not accurately represent normal communities.
- Introducing the space environment (microgravity and increased radiation) to these systems of organisms can adjust these dynamics.

Bobtail squid with *Vibrio fischeri* luminescing. Todd Bretl Underwater Photography

- Multiple models for these interactions have taken place.
  - One heavily studied example is the symbiotic relationship between the Bobtail Squid and *Vibrio fischeri* which is used as a model system for space.
  - Bacteria have also been incorporated into plant grow systems for space-based life support systems to recycle waste products and generate an organic rhizosphere.
- This research begins to analyze the interaction of a forced contamination event of *Chlorella vulgaris*, a microalgae, and *Escherichia coli* in simulated microgravity (SMG) with a NASA-developed Rotary Cell Culture System (RCCS).

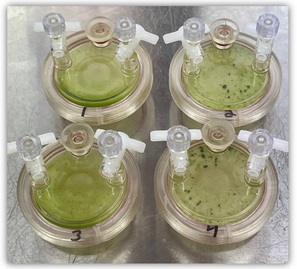


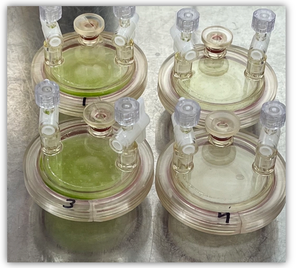
RCCS setup with SMG samples (top) and static control samples (bottom)

- *C. vulgaris* is a promising candidate in space-based bioreactors for resource recycling and vitamin supplementation for astronauts.
- Given the likelihood for contamination to occur in space, we aim to develop protocols to quantify and overcome contamination of a pure culture.

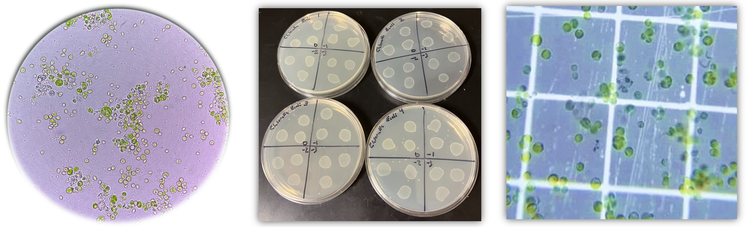
Forced Contamination

- *C. vulgaris* was grown with *E. coli* on an RCCS with, daily, 8hr illumination cycles.
- The starting mixture consisted of 25mL Proteose media, 22.5mL of dense *C. vulgaris* culture, and 2.5mL of an *E. coli* overnight grown in 3mL of nutrient broth media.
- The 10:1 ratio between the two organisms was chosen with the intention of a contamination event which initially starts at a low quantity.
- Each HARV contained 10mL of mixed culture with two for SMG and two for control conditions.
- Samples were monitored for one week before extracting samples for a range of assays:
  - RNA Extraction / Transcriptomics
  - Optical Density (OD) for total cell count estimation
  - Serial dilutions (SD) to quantify *E. coli* growth
  - *C. vulgaris* cell counts with a hemocytometer
  - Chlorophyll Extraction
- During the experiment, the SMG samples experienced clumping whereas the gravity samples only settled to the bottom.
- Upon removal of the samples, *C. vulgaris* cells had a significant portion adhered to the HARV membrane which could not be extracted.
- Each HARV volume was split with:
  - 3x 100μL aliquots for OD
  - 1x 100μL aliquot for SD
  - 1x 100μL aliquot for TB
  - 3x 2mL aliquots for RNA Extraction
  - 2x 1mL aliquots for Chlorophyll Extraction
- Nutrient Broth plates for serial dilutions were made to quantify the bacterial load after the one-week time period. Alternatively, a hemocytometer was used to begin counting the density of *C. vulgaris*.



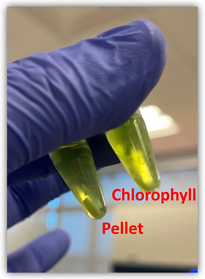


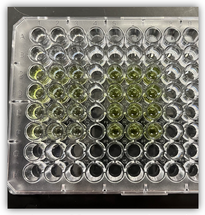
Gravity samples (left) and SMG samples (right) before (top) and after (bottom) removing the culture for testing. Clumping from SMG HARVs and adhered cells in the gravity condition are visible.

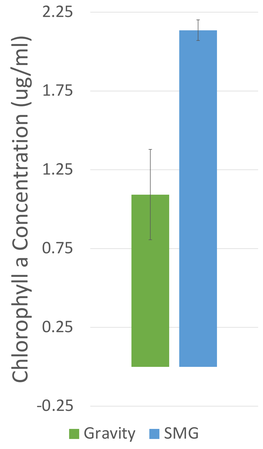


100X image of *C. vulgaris* experiencing clumping (left) and overgrown serial dilutions of *E. coli* (center). An example of *C. vulgaris* on a hemocytometer for plate counting (right), adapted from Colorado-Reyez et al., 2020.

- Chlorophyll was extracted following Zavřel, Sinetova & Červený, 2015. The chlorophyll a content was quantified between the two conditions using absorbances at 665nm and 720nm.
- The results showed SMG having a significantly higher chlorophyll concentration than gravity. However, this difference can be attributed directly to the adhered cells that were unable to be removed.







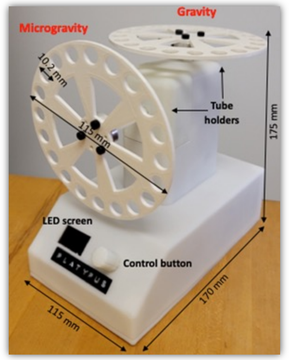
Extracted chlorophyll content (left) placed into a 96 well plate (center). The SMG samples are the right grouping of samples. The chlorophyll a content between all gravity and SMG samples shown (right)

Exploring Bacteria in Martian Regolith

- A recent field of study in development is the utilization of nitrogen fixing bacteria to enhance the nutrient content of Martian regolith for improved plant usage.
- Most research regarding regolith studies have studied direct use of estimated compositions for Mars and the Moon.
- These studies have determined the basic ability for plants to germinate without additives. However, this process occurs at a much slower rate than Earth
- By incorporating bacteria, a synergistic connection could be made to enhance the soil quality as time passes for future generations of plants.

Clover grown in Martian regolith simulant with nitrogen fixing bacteria (left) and without synergistic bacteria (right).

- Initial findings have indicated that bacteria can be successfully incorporated into regolith simulant (Harris et al., 2021).
- After making alterations to the EagleStat, a 2D clinostat previously developed, Martian soil simulants with bacterial colonies can be placed under simulated Martian gravity. These samples can then incorporate plants seeds, such as *Arabidopsis thaliana*, to begin transcriptome analyses that can be used to understand the stress experienced by the plants.



The current EagleStat designed for bacteria in liquid suspension.

Future Research

- Determine a method to fully retain all the culture during the removal process.
- Increase the illumination amount to increase *C. vulgaris*' growth rate and recomplete the experimental workflow to obtain statistical differences across trials.
- Determine, obtain, and practice culturing nitrogen fixing bacteria along with Martian soil simulant to begin preliminary studies growing the specialized bacteria and complete physiological assays for later analysis.

References

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