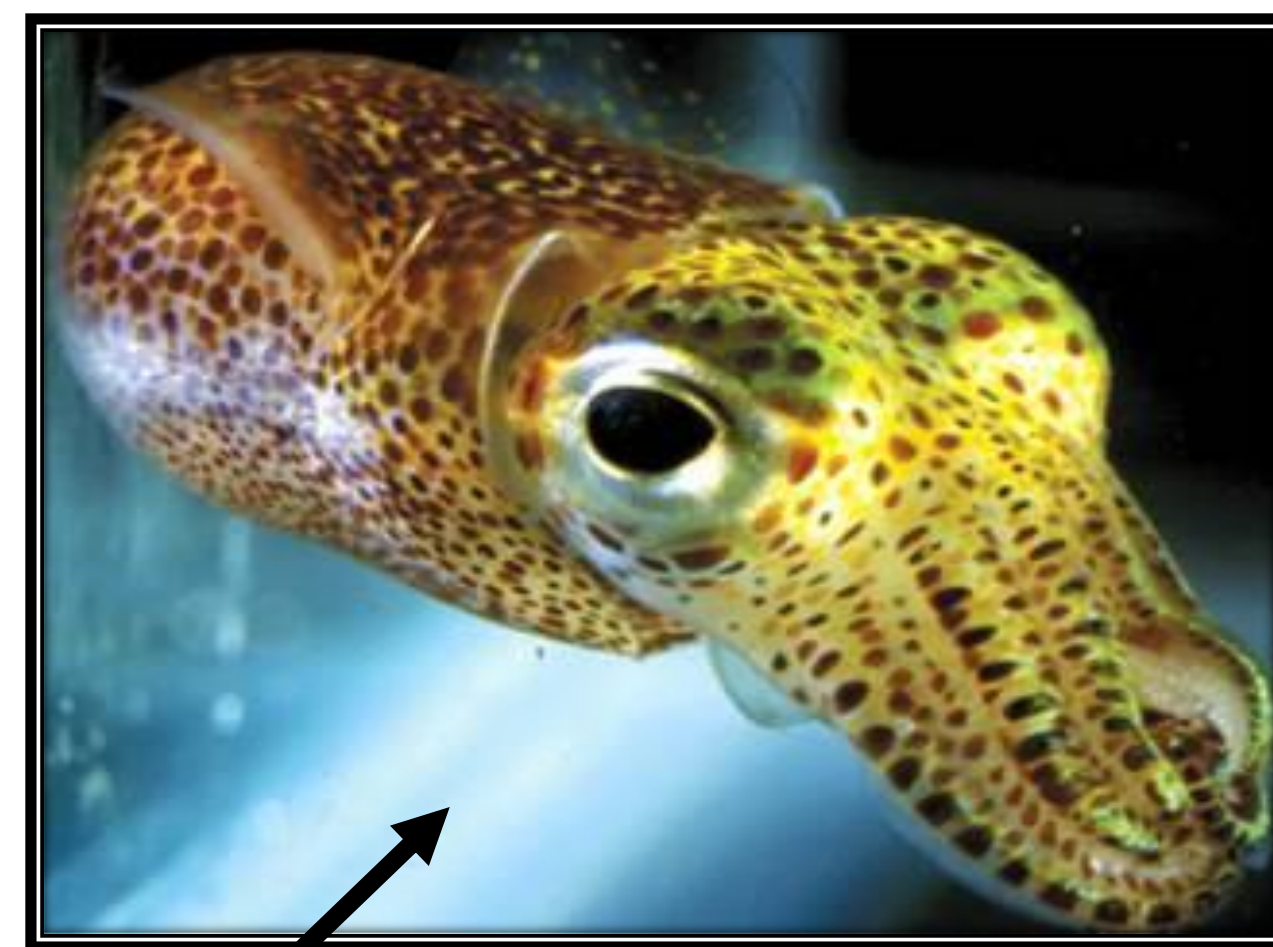
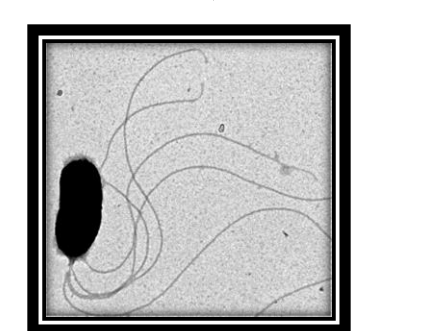


## Introduction



*Euprymna scolopes*

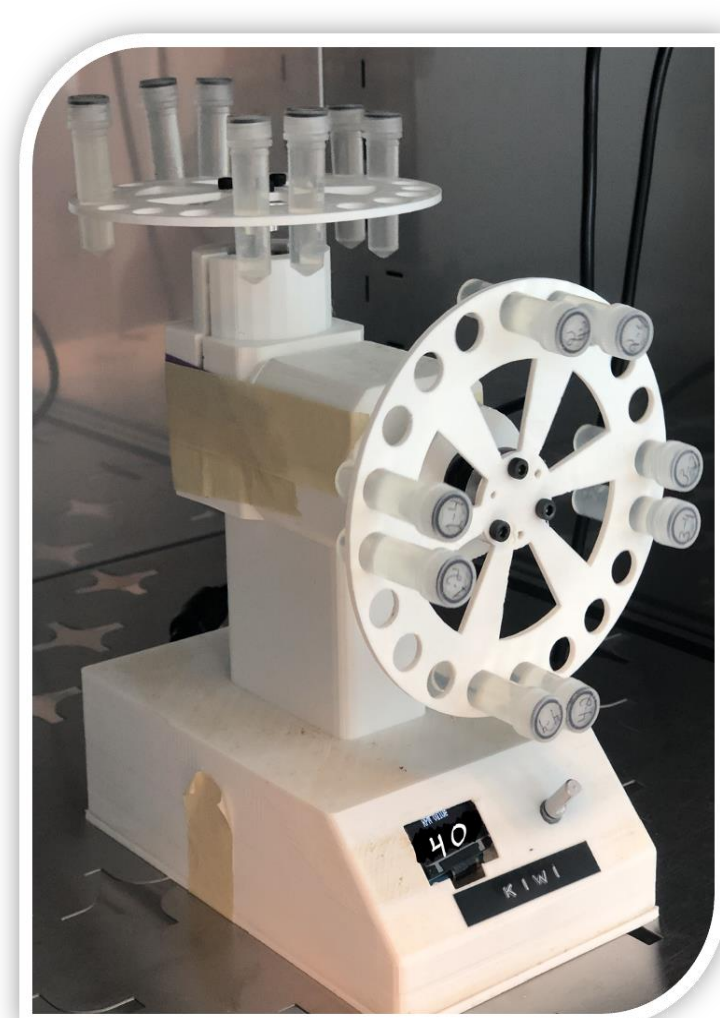


*Vibrio fischeri*

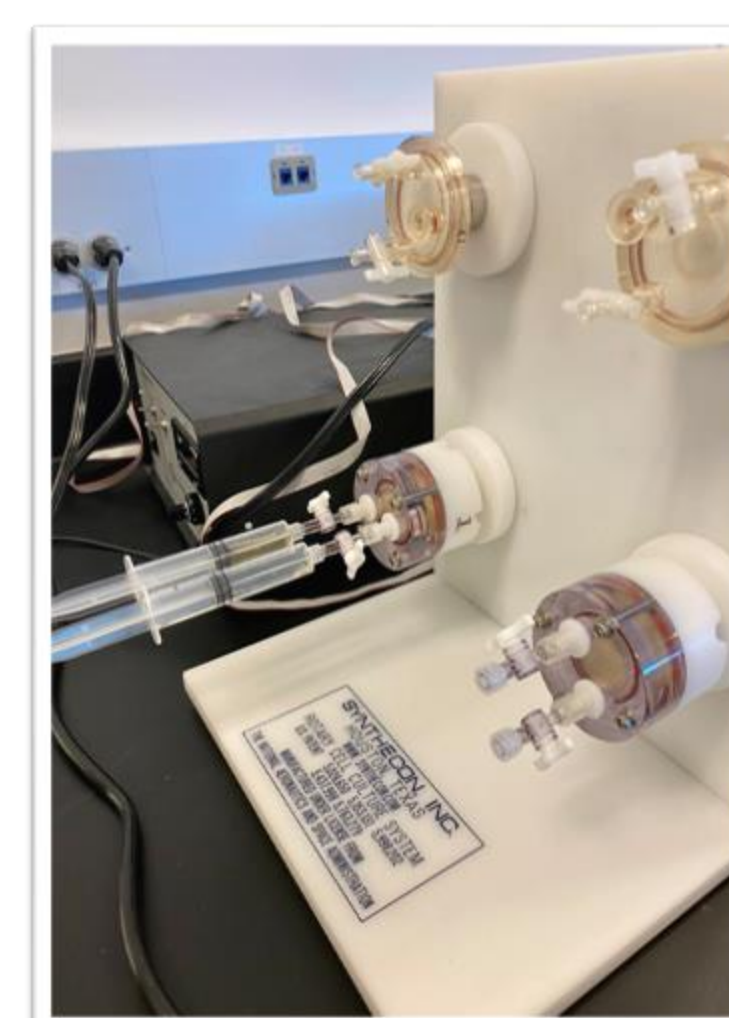
- Changes in environmental conditions represent a challenge for all terrestrial organisms, including the organisms involved in mutualistic associations (when both organisms obtain a benefit from each other).
- Changes in the environment might include fluctuations in gravity and microgravity which represents a new frontier for space biology research.
- This study utilized *Vibrio fischeri*, a beneficial symbiotic bacterium of squids and monacanthid fishes.
- Previous microbiology studies observed altered virulence and antibiotic resistance in response to space stressors
- Further microgravity study is needed to include virulence-related phenotypes such as biofilm formation to determine bacterial plasticity and adaptation
- The survival strategy of *Vibrio fischeri* biofilm formation will serve as a mutualistic model system for this microgravity study

### Microgravity Simulations

- An EagleStat and a Rotary Cell Culture System (RCCS) were utilized to simulate microgravity conditions.



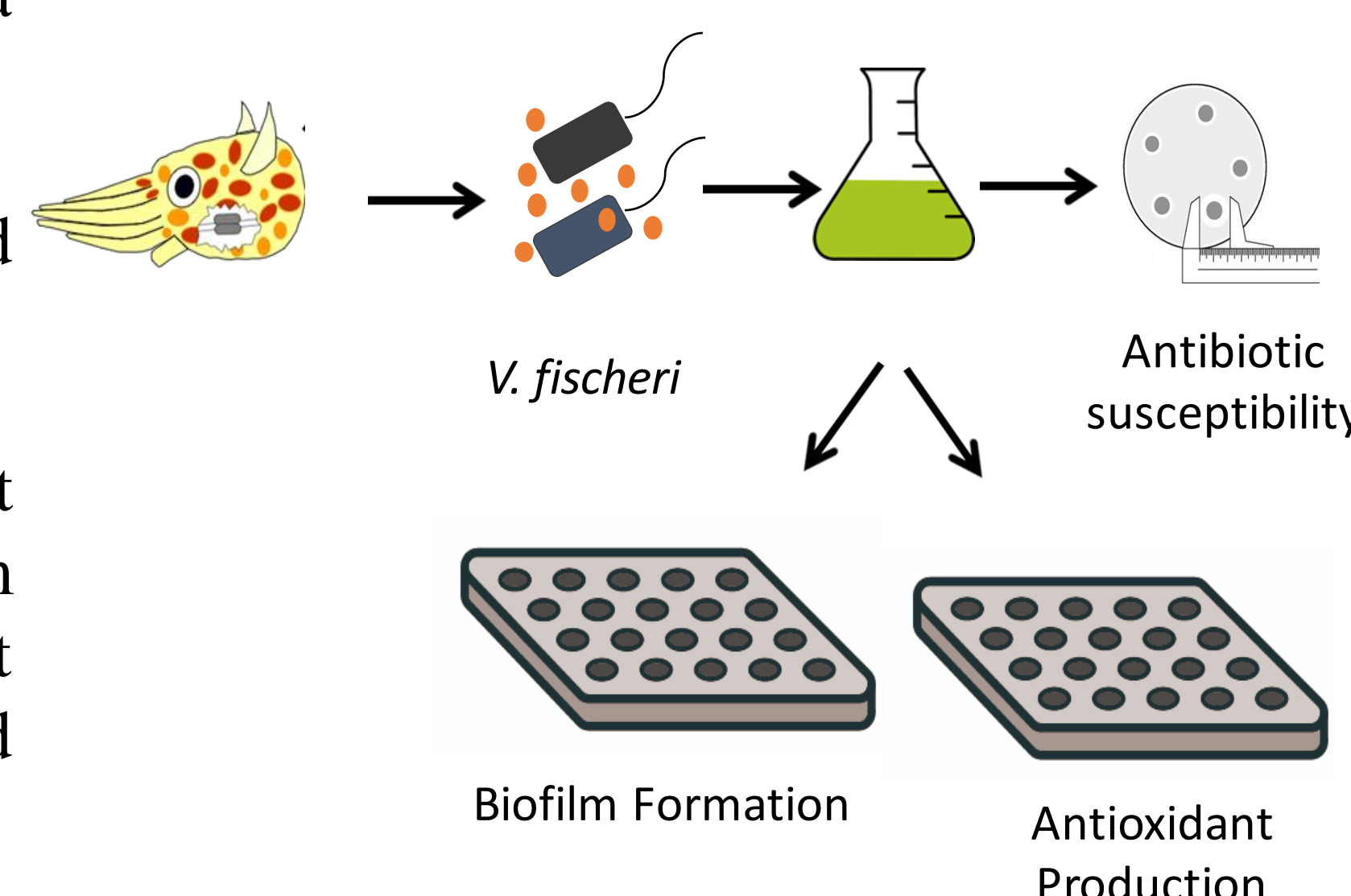
EagleStat



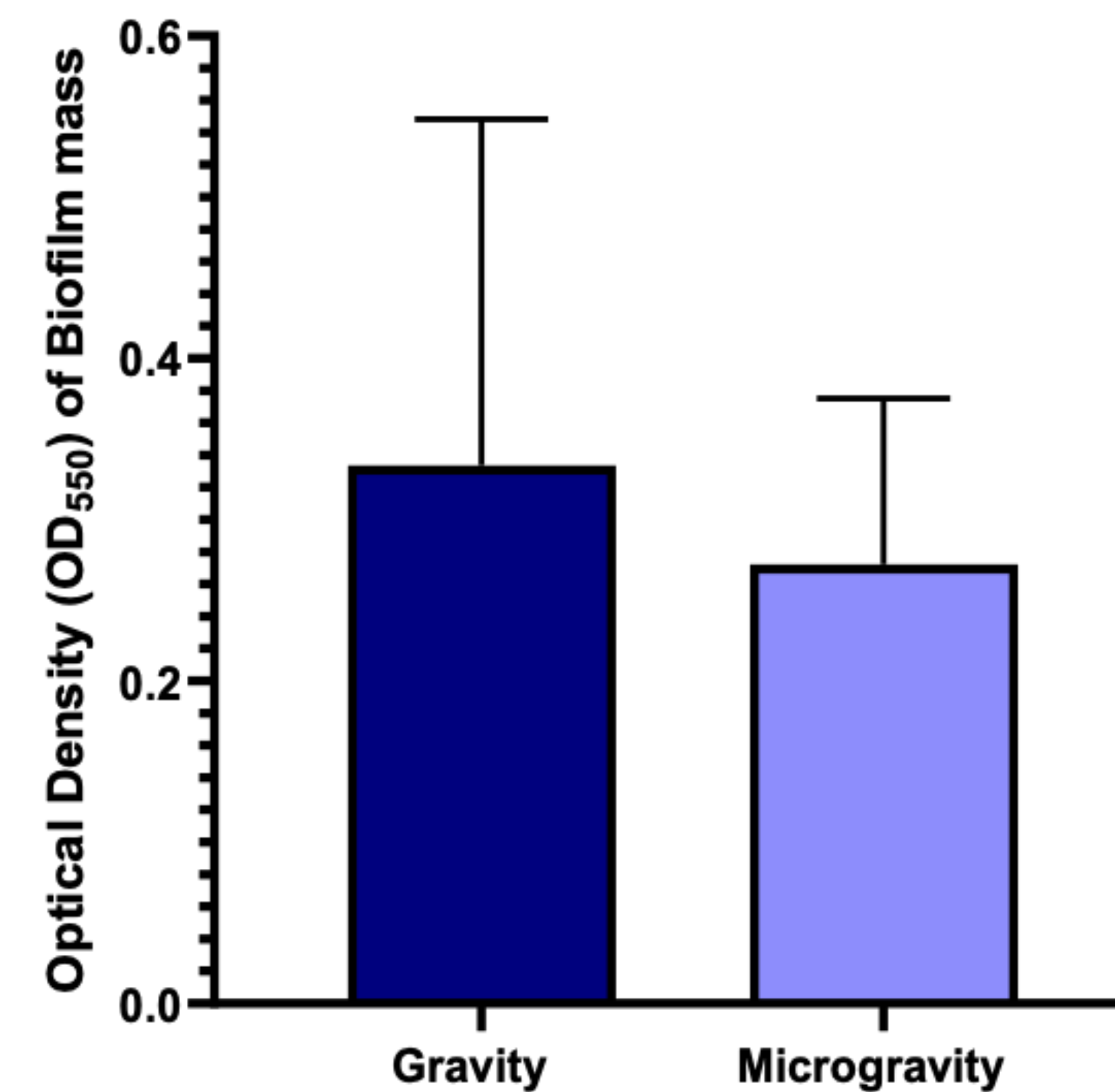
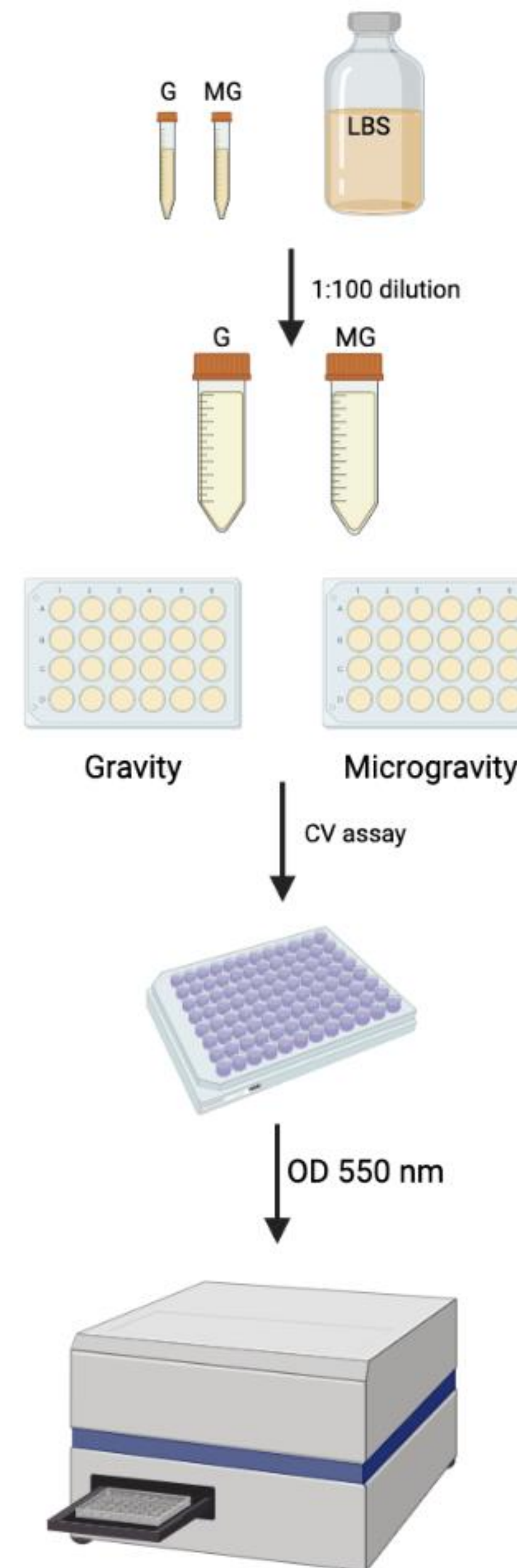
RCCS

## Material and Methods

- V. fischeri* was harvested from a squid
- The bacteria was then cultured for experimentation
- The cultures were used to test antibiotic resistance, biofilm formation, and antioxidant production in microgravity and gravity conditions

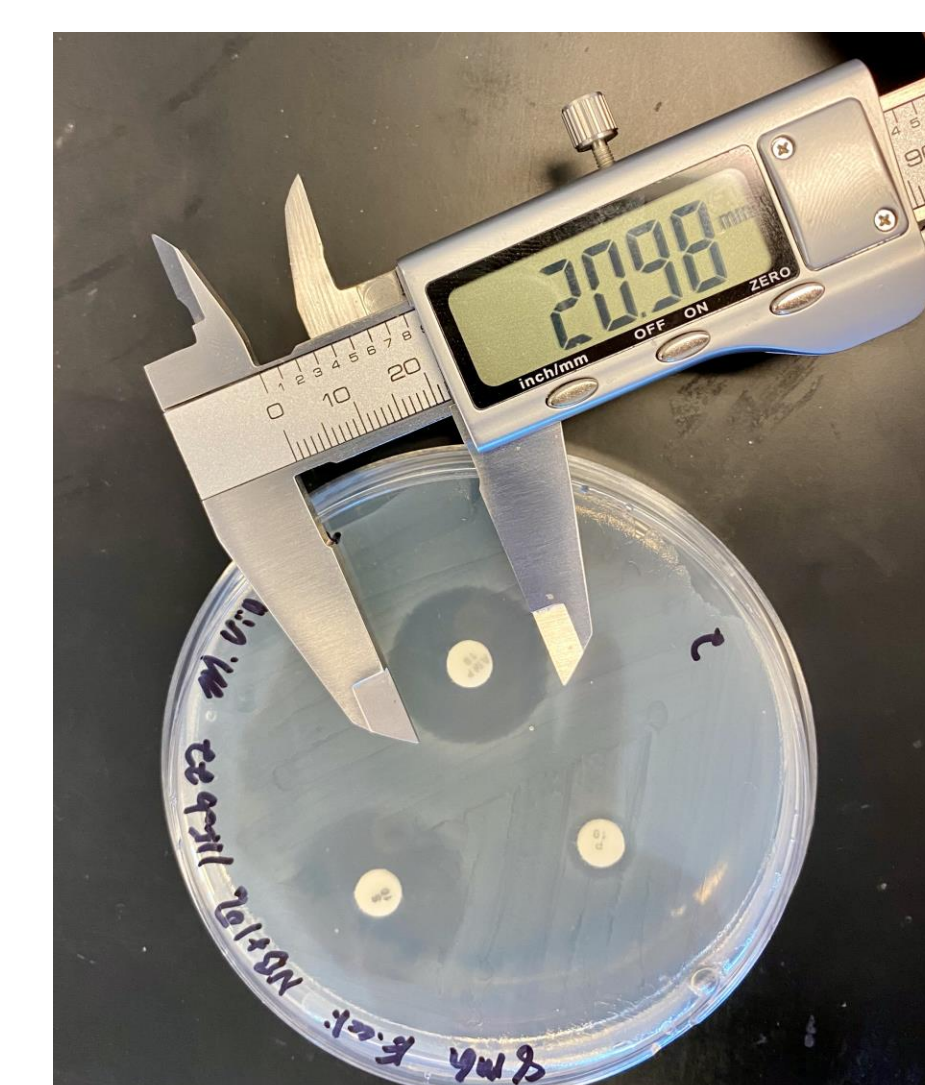
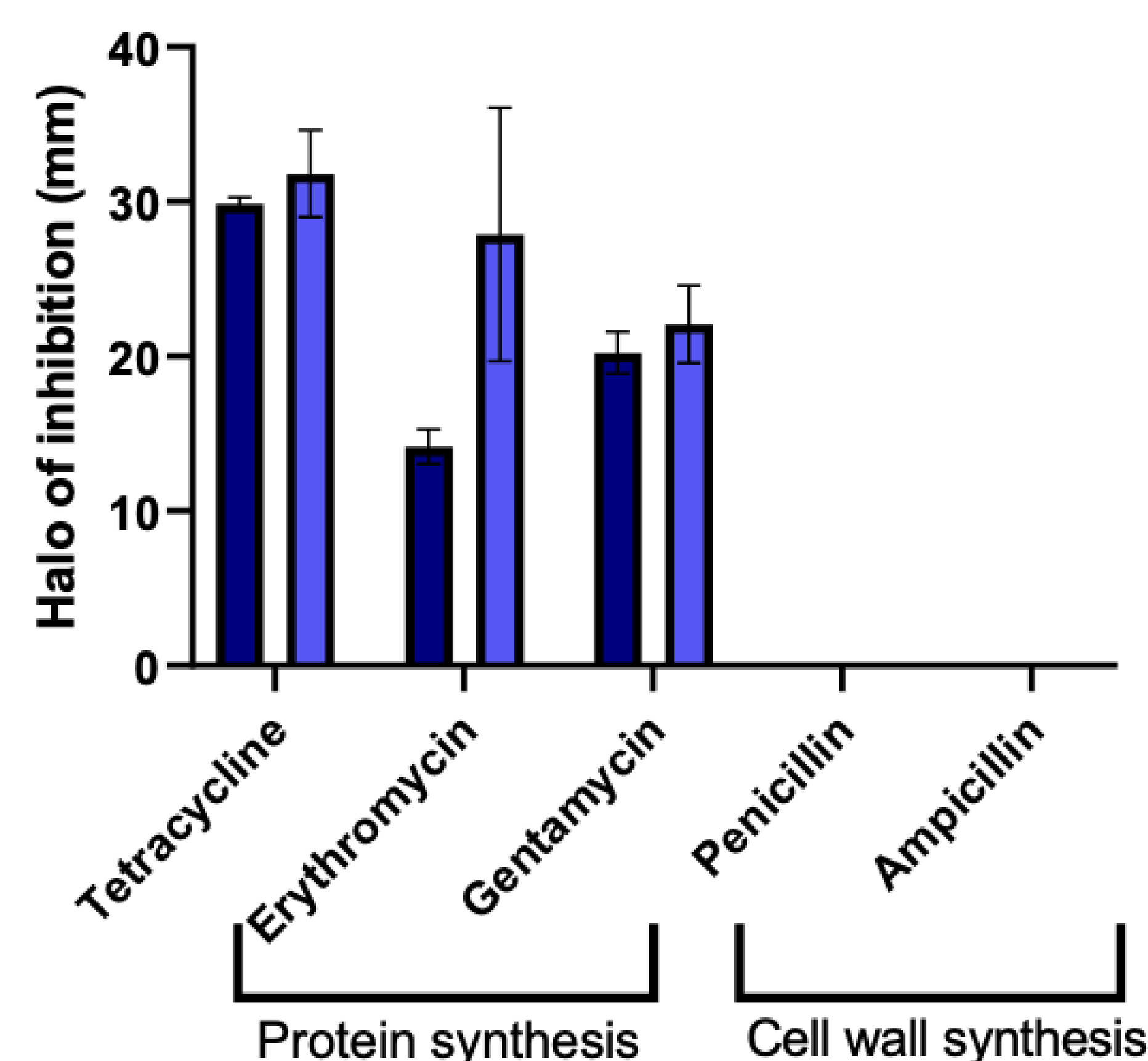


## Biofilm Formation

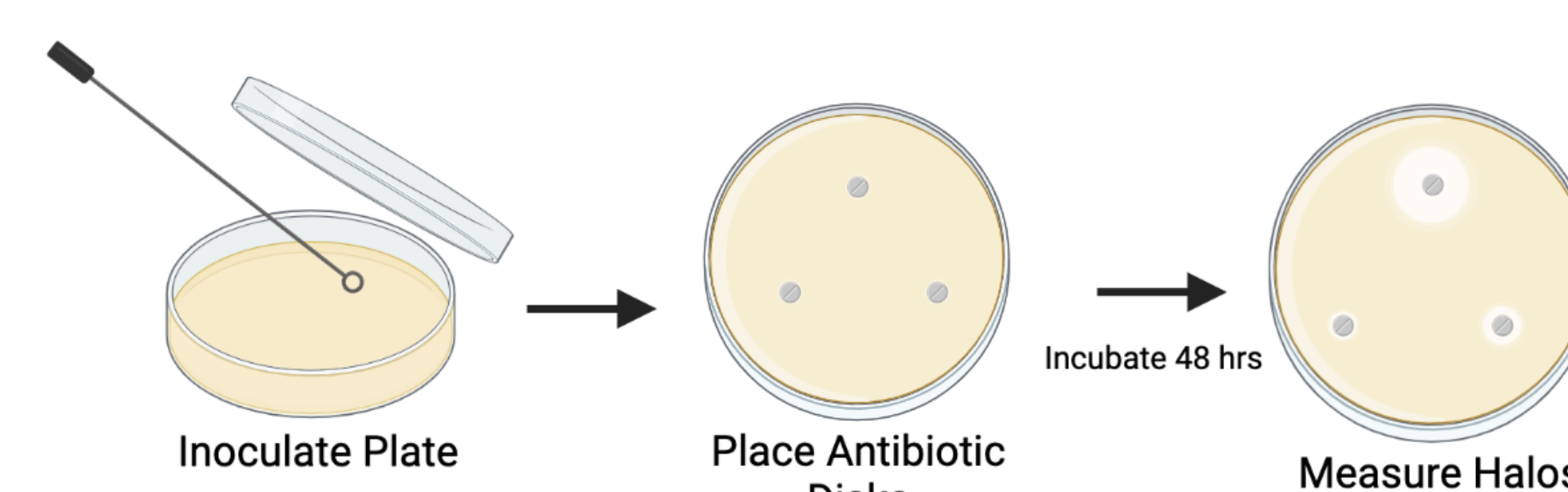


- V. fischeri* biofilm formation measures the structural development of the strain's membrane within 48 hours for both treatment and control
- Increased mass is due to successful colony formation with surviving factors like membrane integrity and nutrient utilization within a liquid media
- Data was collected via optical density readings conducted by spectrophotometer

## Antibiotic Resistance

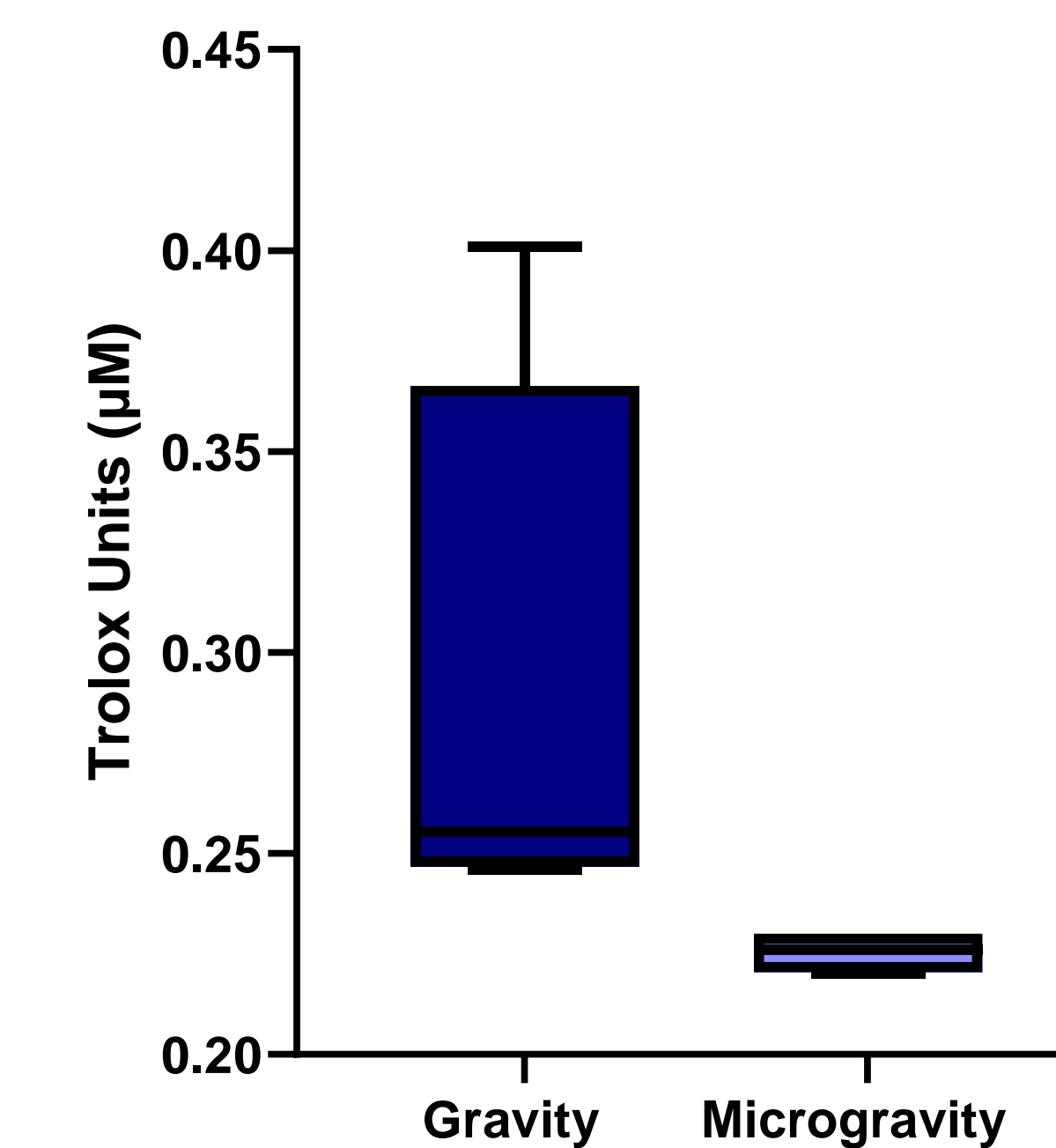
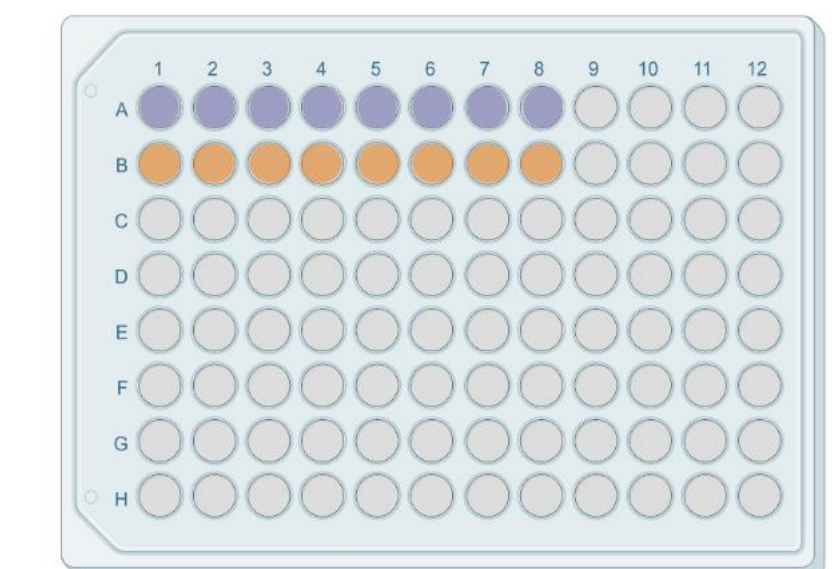


- V. fischeri* antibiotic resistance was measured using the Kirby Bauer method.



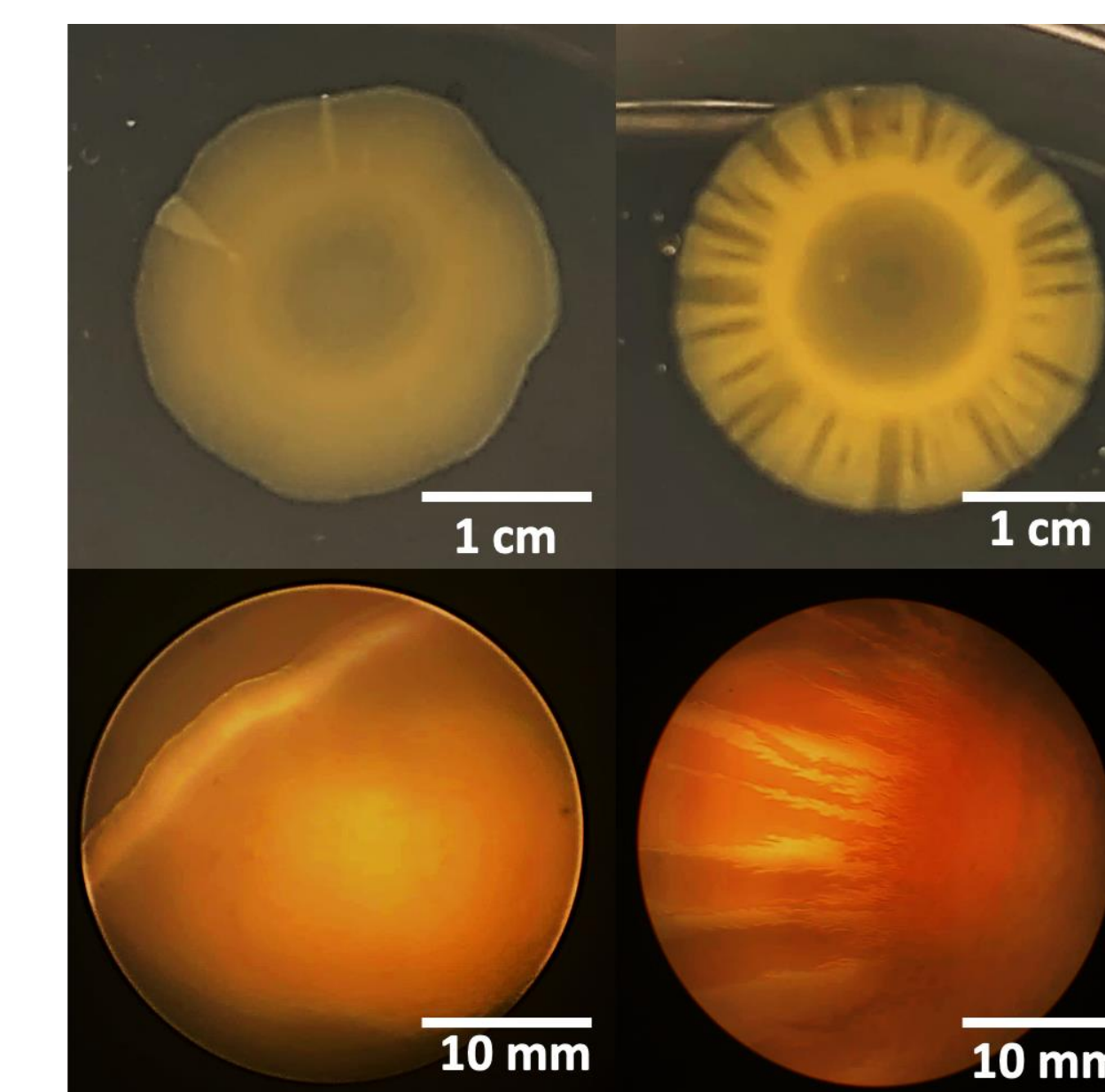
- Zones (halos) of clear media where no growth occurs were measured as indicative of the antibiotic disc limiting bacterial growth.

## Antioxidant Production



- V. fischeri* antioxidant production was measured using the Trolox assay
- The introduced radical is colored and reacts with the antioxidants and gets reduced with decrease in the color
- The ability of the antioxidant or test sample to decrease the color is measured by a spectrophotometer

## Future Research



- V. fischeri* develops colony morphological variation under simulated microgravity.
- Phenotypical features pictured here can be described as a filamentous margin
- The texture of the colonies grown in simulated microgravity are wrinkled compared to the smooth colonies grown in gravity
- Further research is needed to define the mechanisms behind these phenotypical characteristics, such as gene sequencing

## Expected Results

### Biofilm Growth

- Increased biofilm growth for simulated µg analogs
- Indicated by increased OD during spectrophotometry assay versus gravity analog

### Antibiotic Resistance

- Slight increases in inhibition zones around antibiotics for simulated µg analogs
- Indicated by relative size measurements between gravity and simulated µg analogs

### Oxidative Stress

- Downregulation of Catalase Enzymes with simulated µg analogs
- Indicated by spectrophotometry assay and comparing with gravity analogs

## References

<sup>1</sup>Ruby EG. 1996. Lessons from a cooperative, bacterial-animal association: The *Vibrio fischeri*-*Euprymna scolopes* light organ symbiosis. *Annu Rev Microbiol* 50: 591-624.  
<sup>2</sup>Nyholm SV, McFall-Ngai MJ. 2004. The winnowing: establishing the squid-*Vibrio* symbiosis. *Nature* 2: 632-642.  
<sup>3</sup>Soto W, Nishiguchi MK. 2014. Microbial experimental evolution as a novel research approach in the Vibrionaceae and squid-*Vibrio* symbiosis. *Front Microbiol* 5:593.  
<sup>4</sup>Soto W, Rivera FM, Nishiguchi MK. 2014. Ecological Diversification of *Vibrio fischeri* serially passed for 500 generations in novel squid host *Euprymna tasmanica*. *Microb Ecol* 67 (3): 700-21.  
<sup>5</sup>Ferguson GC, Bertels F, Rainey PB. 2013. Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. Insight into the niche specialist fuzzy spreader compels revision of the model *Pseudomonas* radiation. *Genetics* 195 (4):1319-35.