Biology research experience at the ERAU's Space Microbiology Laboratory

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Introduction

- The Aerospace Physiology program has created opportunities for students to not only get an education on the biological effects of flight and space but also to be trained in advanced research techniques using state-of-the-art equipment.
- The Space Microbiology Laboratory currently experiments with several bacterial species such as *Escherichia coli*, Arthrospira platensis, *Lactococcus lactis*, *Streptococcus salivarious*, *Candida albicans*, and *Candida parapsilosis* (isolated from a space station module and provided to the lab by NASA) using techniques to simulate microgravity conditions and measuring different endpoints such as growth dynamics, stress response measurement and differential gene expression analysis.

The lab aims to study how space affects the physiology and gene expression in bacteria.

Microgravity analog: 2D clinostat

- Clinostats work through the removal of the effects of gravity on cells to simulate microgravity environments.
- A specific rotation is required based on the time, distance from the internal rotational axis in the samples, and a constant.

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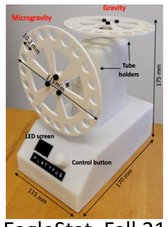
$$w \approx 138.951 * \sqrt[3]{L_0 * t}$$

$w = rotations\ per\ minute\ (rpm)$

$L_0 = initial\ displacement\ from\ the\ axis\ of\ rotation\ (cm)$

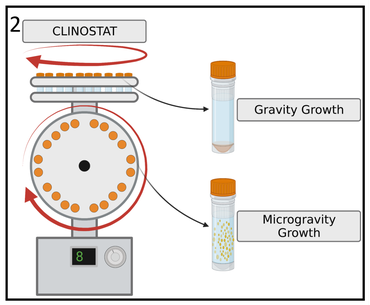
$t = time$ (minutes)

Equation for Rotations Per Minute Required for Clinostat to Simulate Microgravity



EagleStat, Fall 21

- The rotation of the samples around the vertical clinostat plate has no shear forces on the cells inside, as there is no air to create the forces, reducing the forces of gravity on the rotating cells.



EagleStat Microgravity Diagram

UV Spectrophotometer:

- The UV spectrophotometer is used to measure the optical density of the cells at specified wavelengths of light to estimate the concentration of cells and to create growth curves:

Escherichia coli:

- *E. coli* was used as a model bacteria to learn the use of the lab equipment, as well as in the Microbiology and Cell and Molecular Biology Lab courses.

Arthrospira platensis:

- *A. platensis* is a cyanobacterium that fixes (removes) CO_2 from the atmosphere, so its use as part of life support systems could be beneficial.

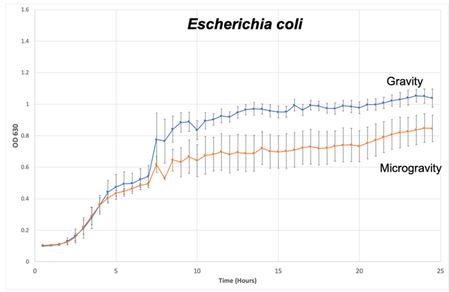
NASA Yeast Isolates:

- *Candida parapsilosis* is a pathogenic yeast. This strain was collected from a Russian space probe, and a comparison of its growth with *Candida albicans*, another closely related pathogenic yeast, could provide insights into long term effects of microgravity on pathogenicity.

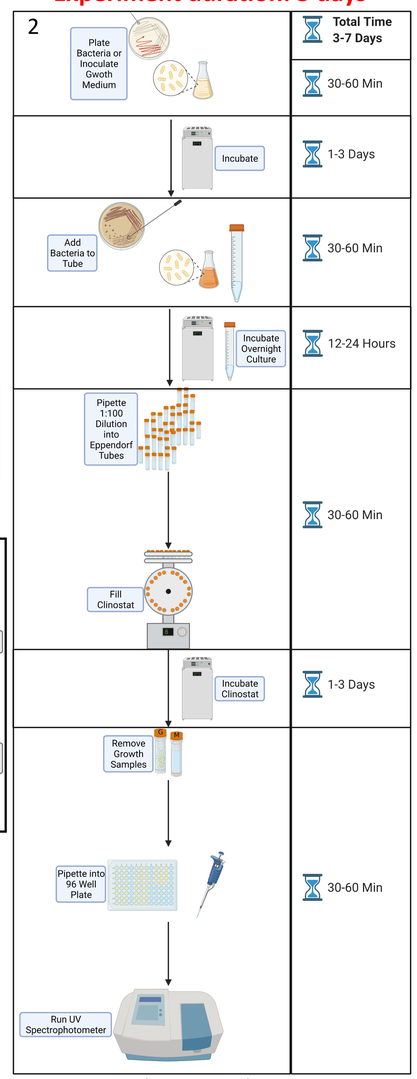
Escherichia coli

- *E. coli* is a great model organism for initial testing and starting in the lab
- First use as a student in lab extracting and purifying RNA from microgravity treated and gravity control *E. coli*
- Began working in the lab as independent students out of class, learning how to use the instruments using *E. coli* as a quick organism for testing

Experiment duration: 3 days



E. coli Growth Curve



Eaglestat Pipeline

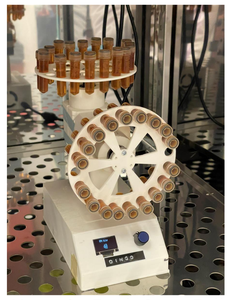
Arthrospira platensis

- Life support systems for space travel will need, among many things, the constant removal of CO_2 and supply of O_2.
- Cyanobacteria, like *A. platensis*, grow on mineral media using CO_2 as the source of carbon and light for energy.
- *A. platensis* growth can take up to 20 days and microgravity and modify it photosynthetic activity, therefore the need to simulate its growth under microgravity and characterize these changes.

Experiment duration: 30 days

NASA yeast isolate

- Frozen stocks of *C. albicans* and *C. parapsilosis* were thawed and streaked onto a plate of YPD media.
- After incubation at 37º C for 72 hours, a colony was inoculated into 2 mL of YPD broth.
- One mL of the overnight culture was diluted in a 1:100 solution of YPD to start the experiment.



Yeast in EagleStat, Fall 2022

- This solution was split into 40 2 mL tubes, 20 of which were placed in the vertical wheel of the clinostat, and 20 were placed in the control wheel, running at 40 rpm. Samples were collected periodically over 72 to measure absorbance at 630 nm.

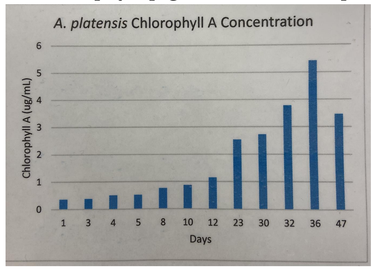
Experiment duration: 6 days

Results

Escherichia coli: The trials run with *E. coli* provided us with valuable data regarding the clinostat technique. We discovered that the standard Eppendorf tubes were not capable of minimizing the shear forces, as it was difficult to remove the air bubbles, and the caps would open during the trial. We switched to screw cap Eppendorf tubes, which greatly increased our ability to reduce shear forces and simulate microgravity.

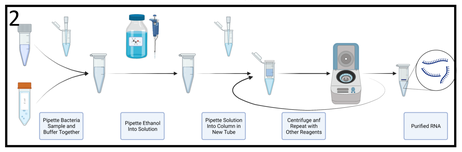
Arthrospira platensis: The chlorophyll extraction technique used produced data that accurately estimated the concentration of the Chlorophyll pigments in the samples.

- Fluorescence occurs when the electrons of a molecule absorb invisible light, giving the electrons more energy. When electrons are excited, they release the energy, returning to the original ground state. The energy that is released is slightly less than was absorbed, so it is no longer invisible, and a color is observed. Chlorophyll fluoresces a deep red color under UV light.



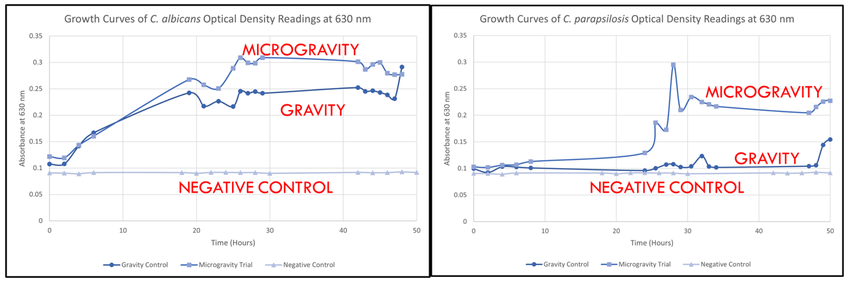
A. platensis Chlorophyll A Concentration Over Time

- RNA extraction produced a large amount of RNA; however, the gel showed that the RNA was degraded.



RNA Extraction Pipeline

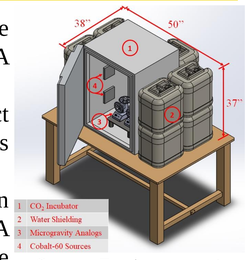
NASA yeast isolate



Growth Curves of ***C. albicans*** and ***C. parapsilosis*** Optical Density Readings at 630 nm

Future research

- We plan to move on to using the Rotary Cell Culture System (RCCS) to simulate microgravity with the NASA yeast isolates, which will facilitate better gas exchange.
- We also plan on moving to include radiation in our project so that we can simulate a space environment, as radiation is a key factor while living in space.
- Recently Embry-Riddle Aeronautical University has been developing an OMICS lab, which contains an RNA Sequencer, which we plan to use to perform in-house genetic analysis of bacteria grown in the Space Microbiology Lab.



Space Environment Simulator

References

1. Dedolph RR, Dipert MH. The Physical Basis of Gravity Stimulus Nullification by Clinostat Rotation. Plant Physiol. 1971;47(6):756-764. doi:10.1104/PP.47.6.756
2. Created with BioRender.com