# Space Microbial Ecology


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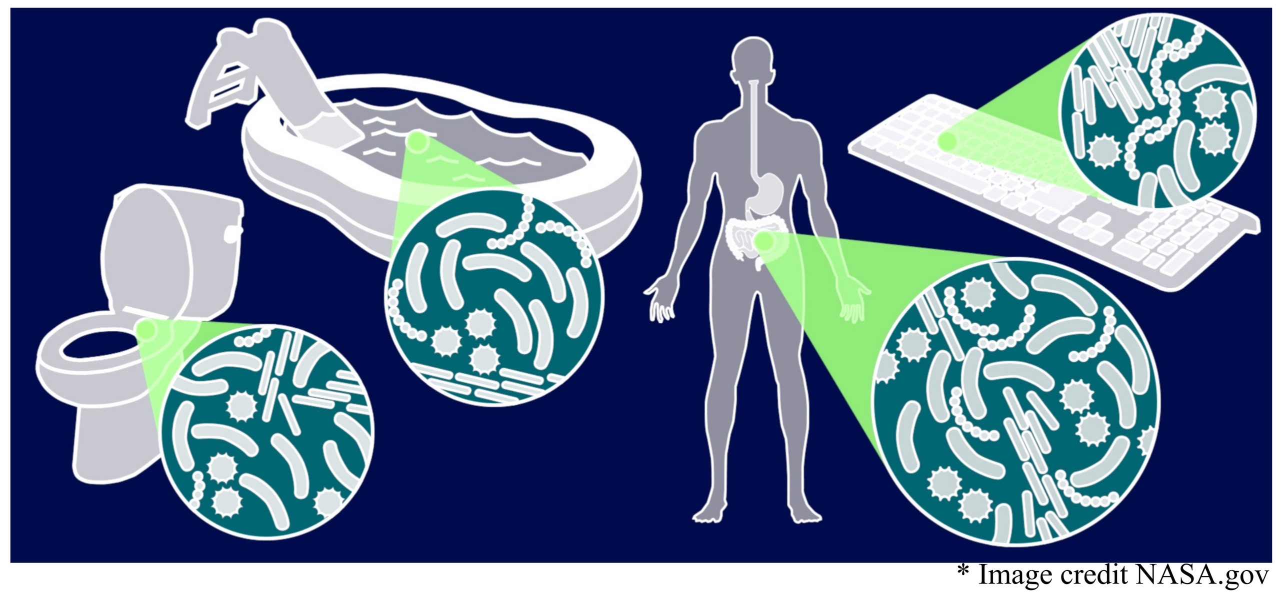
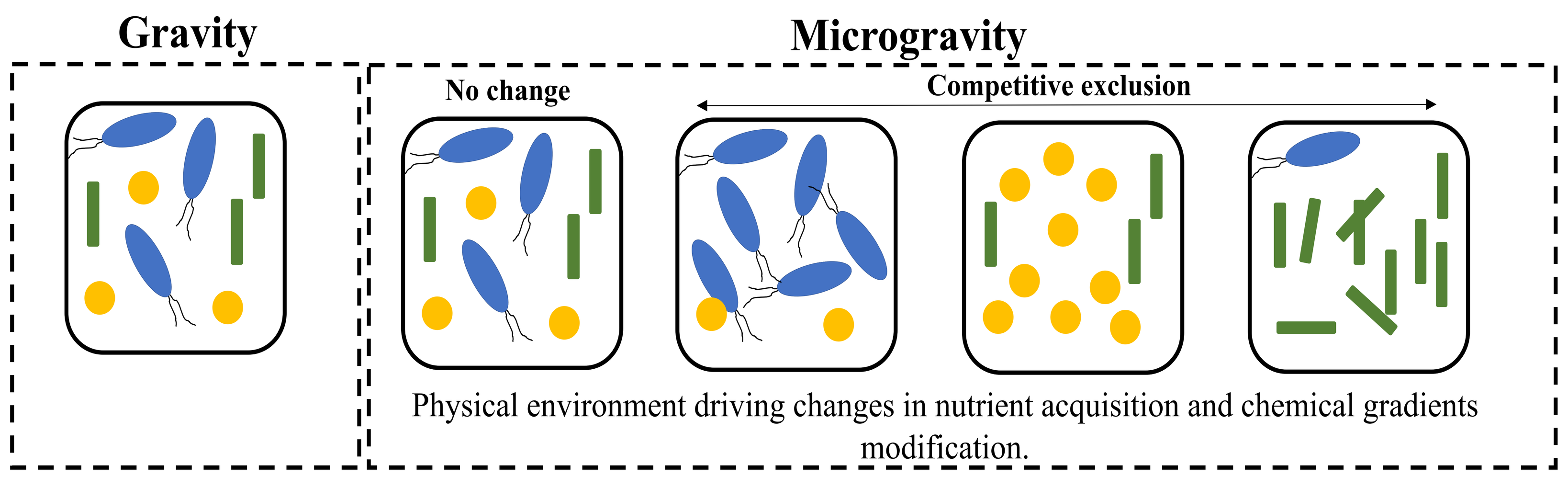
## Introduction



* Image credit NASA.gov

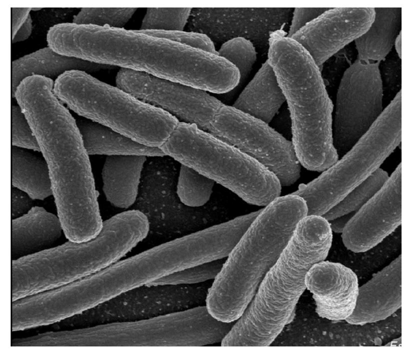
- The extreme environment of space induces stressors such as radiation, microgravity, and extreme isolation (Afshinnekoo, E., et al 2020)
- Long-duration human exploration space missions will expose astronaut and spacecraft microbiomes to space stressors (Mahnert, A., et al 2021)
- Simulated microgravity (sim $\mu$g) exposure has been shown to replicate space microgravity stressors (Topolski, C., et al 2022)



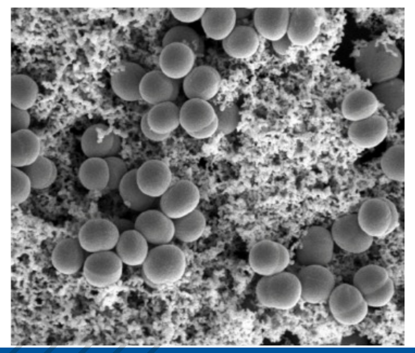

## Hypothesis

- <u>Hypothesis</u>: Microgravity exposure affects microbial ecology relationships to change colony growth, phenotypical characteristics, and virulence
- <u>Null Hypothesis</u>: Colony growth and phenotypical characteristics will not change under sim $\mu$G conditions.

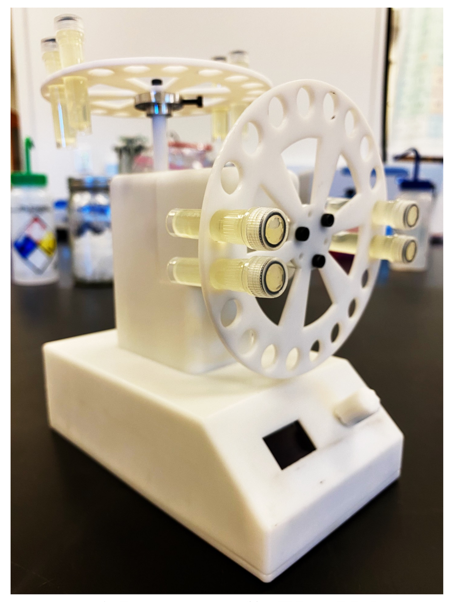
## Experimental Design



- *Escherichia coli*
  - Bacilli shaped
  - Gram Negative
  - Image credit National Institute of Allergy and Infectious Diseases

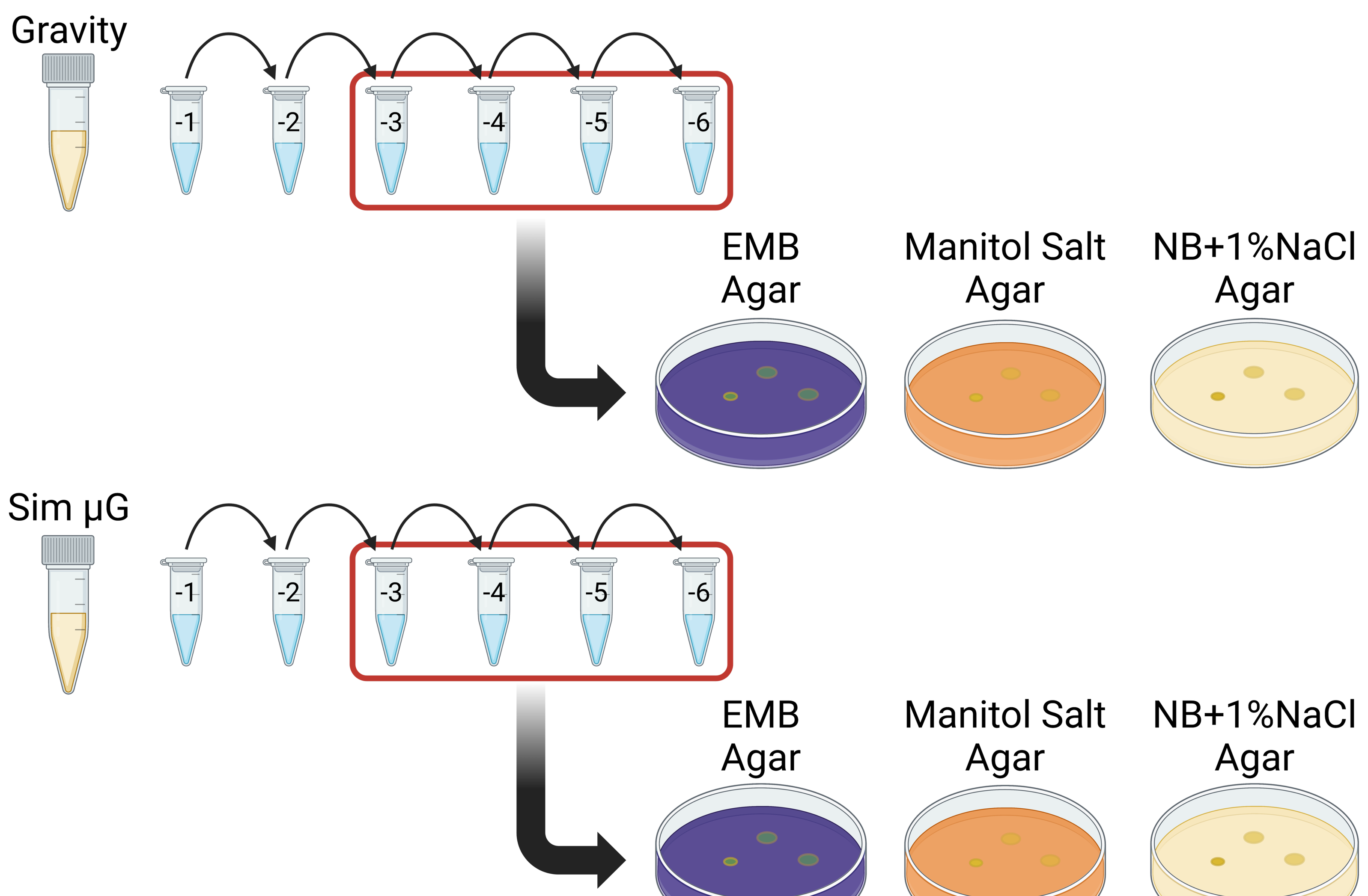


- *Staphylococcus epidermidis*
  - Cocci shaped
  - Gram Positive
  - Image credit Bernatova, S., Et al (2013)



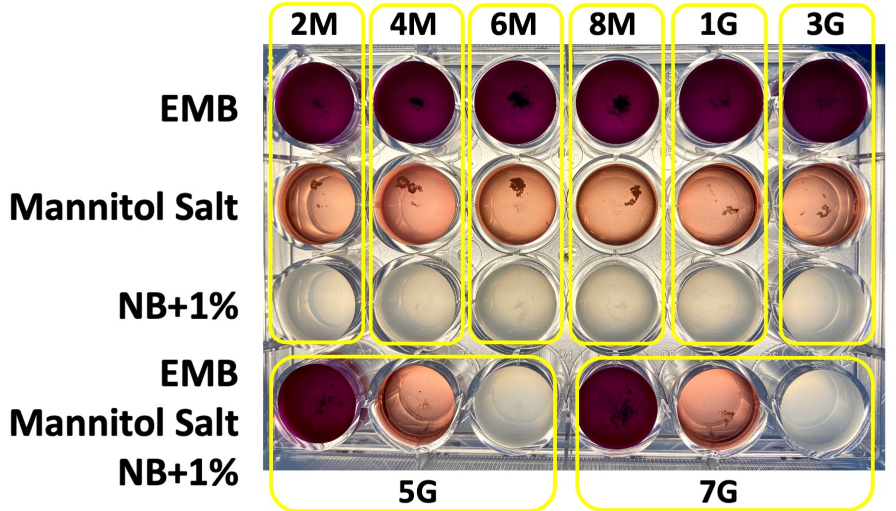
* Image credit ERAU Space Microbiology Lab

- Overnight isolated cultures were mixed at a 1:1 ratio
- The mixed culture was run for 24 hours on an EagleStat (2D clinostat) at 37°C
- Both Gravity and sim $\mu$G samples were diluted to -6
- Drop plates were prepared on Eosin methylene blue (EMB), Mannitol salt, and NB+1% NaCl to select and count CFU's
- EMB selects for gram negative bacteria, Mannitol selects for gram positive bacteria, and NB+1% was a sham plate
- Plates were incubated for 24 hours, imaged, and counted to determine viable colony formation units (CFU's)



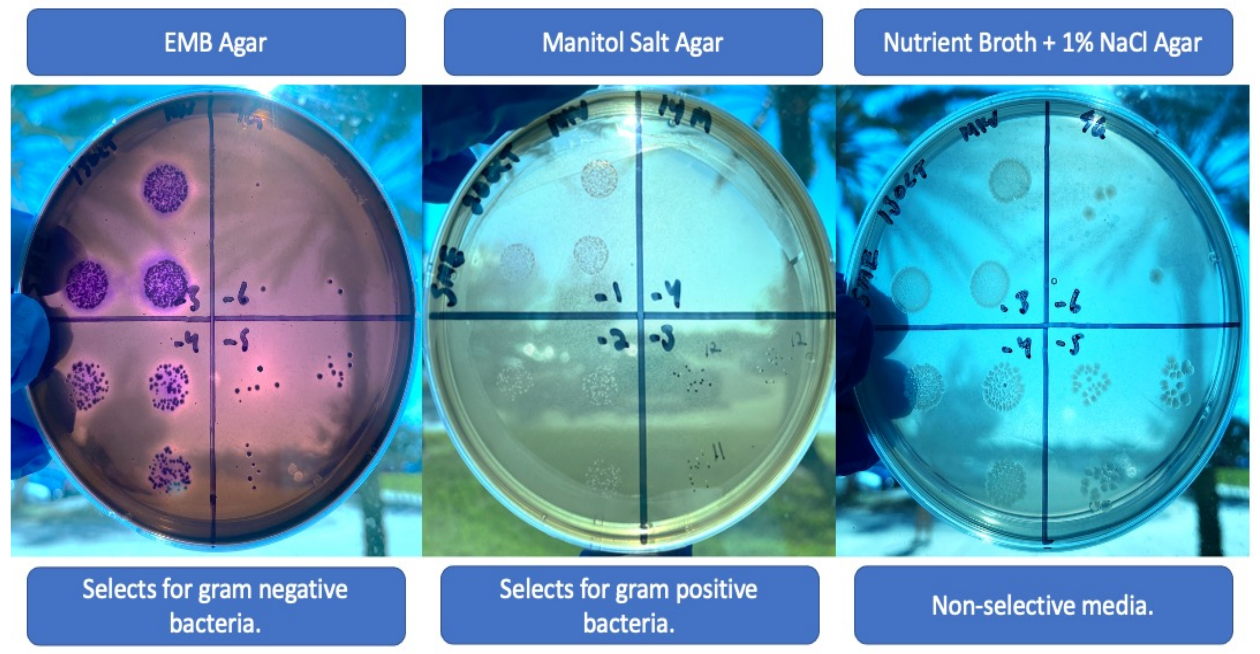

* Image created on BioRender.com

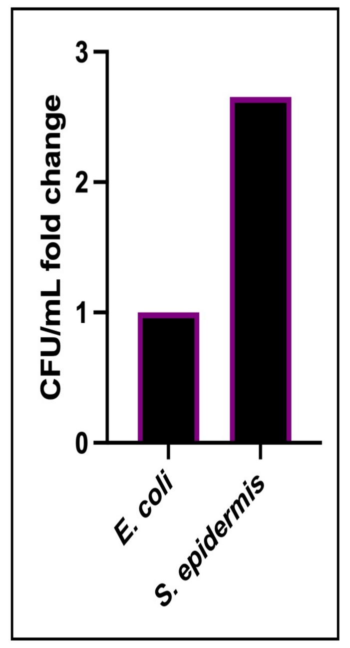
- Clinostat sample aliquots were plated on 24 well plates to test growth curve selective media viability
- Plate was run for 24 hours in a BioTek Synergy LX at 37°C
- Plate was set to continuous orbital mixing and read at 630nm every hour to measure biomass growth




* Image credit ERAU Space Microbiology Lab

## Colony Formation Results

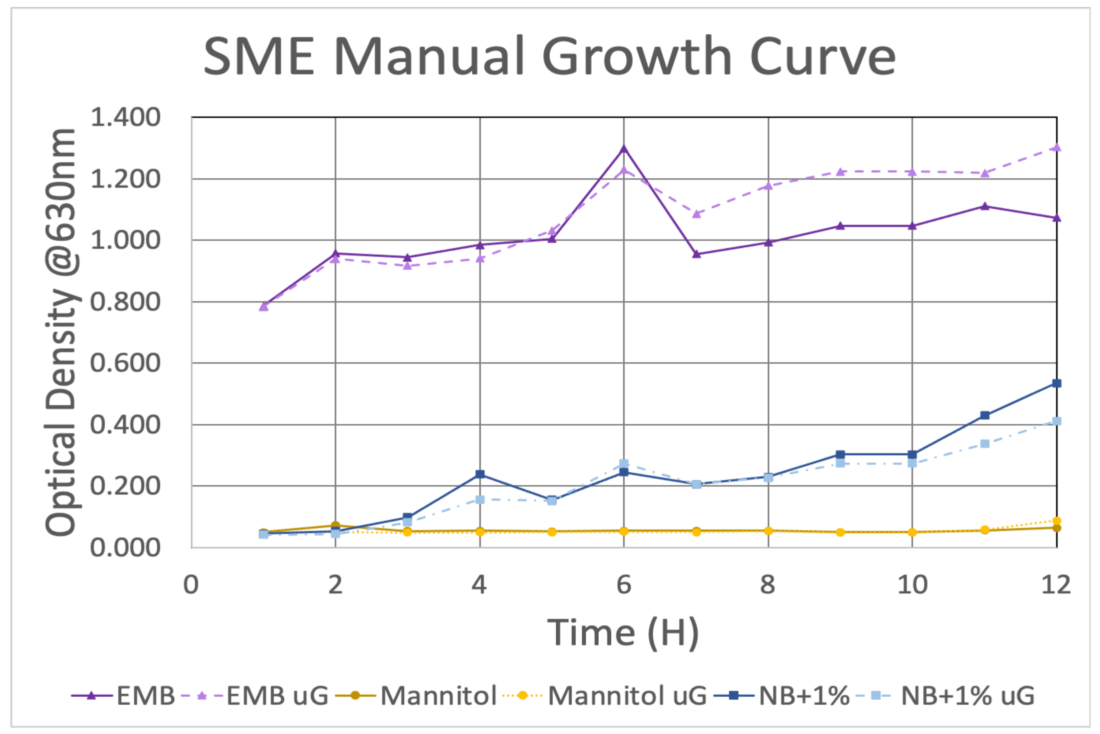



* Image credit ERAU Space Microbiology Lab

- 10 Gravity and 10 sim $\mu$G biological replicates tested
- Preliminary results indicate that *E.coli* growth was similar under gravity and sim $\mu$G
- Preliminary results indicate that *S. epidermidis* had a greater than 2-fold increase in colony growth after sim $\mu$G

## Growth Curve Results




- 4 Gravity and 4 sim $\mu$G biological replicates tested on BioTek Synergy LX
- 4 Gravity and 4 sim $\mu$G biological replicates tested by manual measurement hourly
- Results require further testing to normalize data

## Future Research

- *S. epidermidis* growth curve
- Selective media growth curve troubleshooting
- Antibiotic Resistance Testing
- Metabolite Testing


References:

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