Bacteria in Space Research: Testing the Effect of an Extended Non-Growing State on the Growth Dynamics of *Escherichia coli*

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**Bacteria in Space?**
- Bacteria can be used as biological models to study the effects of space-conditions, like radiation and microgravity, on cells.
- Several species of bacteria behave differently when grown on the ISS as compared to Earth, either by growing faster or by becoming more resistant to antibiotics (Kim et al. 2013).
- Information on how cells respond and after exposure to space conditions are necessary to improve the design of protective and living systems for future space exploration.

**The KhepriSat Project**, led by an interdisciplinary group of ERAU students, aims to study the growth dynamics of bacteria in a low Earth orbit system over a period of twelve weeks. The data from this experiment will contribute to our understanding of how increasing amounts of DNA damage affect cell growth.

**KephriSat Biological Payload**
- The KhepriSat will house 18 “cuvettes” that contain all the elements required by the selected bacterial model to grow.
- Cells suspended throughout nutrient solution
- Temperature control
- For more details, visit: KhepriSat: Payload for CubeSat Mission to Explore Effects of Radiation Exposure on Cell Growth

**Hypothesis**
- Space exploration requires crews to stay remain in space for extended periods of time. How will organisms react to a chronic exposure to high doses of radiation and microgravity?

**Fermentation Assessment**
- An array of bacterial and fungal species obtained from glycerol stocks were evaluated for gas production over a period of several weeks.
- If a microbe produced gas during this time period, their candidacy for our experiment was invalidated.
- Contrary to the patterns exhibited by both of the eukaryotic species, *E. coli* did not produce a significant amount of gas throughout the duration of the experiment.
- This confirms *E. coli* to be a potential candidate for the KhepriSat.
- Determining which media is optimal for inhibiting gas production will be evaluated in future experiments.

**Escherichia coli Growth Dynamics**
- For twelve weeks, the effects of an extended non-growing state on the growth dynamics of *E. coli* were investigated.
- Each week, three samples of *E. coli* were removed from an incubator and kept at 30°C.
- The growth of these samples was evaluated by measuring their optical density at 630 nm.
- The graph above represents the growth curves and plate counts collected for the past nine weeks.

This graph represents the lag phases of growth for each of the nine weeks. After Week 3, the curves stabilized from their initial decline.

**Where Are We Now?**
- Although this experiment has not completed a full 12 weeks of testing, preliminary results show that, after three weeks of long-term incubation, *E. coli* exhibits an extended lag phase.
- The lack of nutrients, coupled with a suboptimal growth temperature, may have stressed *E. coli*.
- The extended lag phase presented by these cells may be occurring to repair the damage that was accumulated during their long-term storage.
- Although plate counts show that the total number of cells in solution has decreased, it has not reached a critical level of importance.
- As whole colonies originate from single cells, only one cell must remain viable to ensure the success of the KhepriSat Project.

**Future research**
1. Characterization of optimal extended non-growing state conditions for *E. coli* and other bacterial models. What is the best way to preserve cell viability for long-term storage under simulated CubeSat conditions?
2. Study of the effect of space-relevant doses of ionizing radiation (gamma) on the growth of microbial models. How does the chronic exposure to radiation change bacterial growth?
3. Analysis of the bacterial gene expression regulation in response to simulated space conditions. Which genes are responsible for cell survival in space?

**Radiation Biology Laboratory**
- Counting colonies
- Using the plate reader to assess *E. coli* growth
- Using the biosafety cabinet