Bacterial Growth and Susceptibility to Antibiotics in Simulated Reduced Levels of Gravity

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ABSTRACT

Changes in bacterial growth and in their susceptibility to antibiotics during spaceflight have been observed since the beginning of the U.S. and Soviet space programs. Regarding bacterial growth, decreased lag phase and increased final population counts are common results of spaceflight experiments. In terms of susceptibility to antibiotics, spaceflight experiments have needed up to four times the antibiotic concentration, relative to Earth, to inhibit bacterial growth. These two phenomena are being investigated in the laboratory with the help of clinostats to simulate the reduced gravity environment around the bacterial cell. A novel method of inclining clinostats at different degrees permit to simulate 0, 1/6 and 1/3 g. Cultures of E. coli are being grown, separately, in BioServe’s Fluid Processing Apparatus (FPA) in these clinostats. Optical density measurements are taken throughout the cultures’ growths to assess lag phase duration and final cell concentration. These same bacteria are placed in different concentrations of Colistin Sulfate to assess their susceptibility to the antibiotic in these gravitational environments. For each of these environments, the Minimum Inhibitory Concentrations (MIC) is identified and compared to 1 g controls. Although testing continues to achieve statistical significance, the first tests have shown that E. coli grow to 9% larger final cell density in the 0 g simulation relative to the 1 g control. The antibiotic assessment test is currently under way.

BACKGROUND

Multiple spaceflight experiments have shown that in space, bacteria can grow to increased final cell populations, compared to 1 g controls, and can proliferate in antibiotic concentrations that on Earth would kill them. In a parallel fashion, a Drug-Resistant Bacteria problem here on Earth is taking the lives of over a hundred thousand people around the world, every year – costing the U.S. Government alone over US$20B a year in excess healthcare cost. One of this project’s aims is to use the spaceflight bacterial phenomena to gain and translate knowledge to the problem on Earth.

HYPOTHESES

1. E. coli culture’s final cell count is dependent on gravitational regime: as gravity decreases, the final cell count increases.
2. The Minimum Inhibitory Concentration (MIC) of antibiotic (Colistin Sulfate) required for E. coli increases as gravity decreases, i.e. the lower the gravity, the higher the antibiotic concentration needed to inhibit bacterial growth.

EXPERIMENT VARIABLES

- Bacterial species and strain: Escherichia coli ATCC® 43571 (non-motile)
- Growth Medium: Medium E supplemented with 5 g/L Glucose
- Incubation temperature: 30°C
- Definition of Minimum Inhibitory Concentration: Lowest antibiotic concentration that inhibits bacterial growth after being incubated for 32 hours at 30°C with a starting cell concentration of 5.0 x 10^8/cell/mL
- Antibiotic: Colistin Sulfate

TEST PROCEDURE

1. A mother culture of E. coli is grown in Medium E supplemented with 5 g/L Glucose at 30°C until it reaches its exponential growth phase.
2. At that time, it is diluted to reach a 1.25 x 10^9/cell/mL concentration.
3. Several test tubes (BioServe’s Fluid Processing Apparatus – FPA shown above) are filled and installed inside of BioServe’s Group Processing Apparatus (GAP – shown below).
4. The GAPs are placed in clinostats inside an incubator at 30°C. Three clinostats at different inclinations – to simulate different gravitational regimes – and horizontal ground controls are used.
5. Optical density measurements at 600 nm are taken with a spectrophotometer to assess bacterial growth. With that data, growth curves are produced for each gravitational environment.
6. For each gravitational regime, a sample is taken during exponential growth phase at around 5.0 x 10^8/cell/mL and distributed through five GAPs, each with a different concentration of Colistin Sulfate.

These cultures are allowed to incubate until the MIC is identified and compared to that of the 1 g control.

REDUCED GRAVITY SIMULATION

- Reduced gravity environments were simulated using clinostats, devices that continuously rotate a fluid solid body, re-orienting the gravity vector.
- Clinostats don’t remove the gravity vector but simulate the quiescent environment around a cell characteristic of reduced gravity.
- Clinostats have been used for gravitational microbiology experiments of different kinds.
- The clinostat rotational speed must be calculated based on multiple variables, including cell density and volume, medium density and viscosity, cell sedimentation velocity, supplement and byproducts diffusion coefficients, etc.
- The clinostat must rotate fast enough so the particle doesn’t sediment off its zone of depletion and slow enough to ensure it is not centrifuged out of the clinostat.

Using a coordinate system [x, y, z] rotating in the angular velocity as around a y axis, a summation of forces analysis including weight, buoyancy and centrifugal force yields two second order, non-homogeneous partial differential equations. The one for the x axis is:

\[ \frac{\partial^2 x}{\partial t^2} + \frac{f x}{\omega^2} - \frac{m^2}{m} \varphi = -\frac{m^2}{m} \varphi_{syst} \]

where f is the cell’s Stoke’s radius, m is its mass and m^2 is its buoyancy-corrected mass.

- The solution to these equations describes a particle circular motion with radius q:

\[ q = \frac{v_{red}}{\omega} - 2g \omega^2 \delta \rho \]

where q is the cell’s sedimentation velocity and \( \delta \rho \) is the density difference between the cell and fluid.

- This bacterium cell’s circular motion has to have a smaller radius than that of the sphere described by the particle’s Brownian motion.
- Partial gravity, i.e. 1/6 and 1/3 g are attained by inclining the clinostat at an angle \( \theta \), where the value of \( \theta \) is between 9° and 90°. One third g, or Martian gravity, is generated when the clinostat is inclined at a 19.5° angle; one sixth, or lunar gravity, when it is at a 9.6° angle. Details and validation of the system is reported in Ref. 33.

RESULTS

Example of E. coli Growth Curves at Different Simulated Levels of Gravity

Colistin Sulfate Minimum Inhibitory Concentration at 1 g

Colistin Sulfate Minimum Inhibitory Concentration at Different Simulated Levels of Gravity

FUTURE WORK

- More growth curves are currently being produced to achieve statistically significant values.
- Colistin Sulfate MICs at different simulated reduced levels of gravity will be identified.
- This investigation will be expanded to analyze Gentamicin Sulfate, an antibiotic with a different mechanism of action.
- This research will be expanded to include Bacillus Subtilis to have a gram positive model.

CONCLUSIONS

- Although more experiments are currently being conducted to achieve statistical significance, so far data indicates that there is a direct relationship between gravitational regime and E.coli proliferation: the lower the gravity, the higher final cell concentration.
- First tests show final cell concentration of E. coli to be 9% higher than the 1 g horizontal controls.
- This data suggests, although bacteria could proliferate better in Lunar and Martian settlements compared to Earth, the worst case scenario could be the microgravity environment.

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