Increased bacterial virulence under zero gravity conditions

Tyler M. Shadid and Eric Paul
Southwestern Oklahoma State University, Weatherford, OK 73096

Abstract

Space flight exposes astronauts to tough environments before, during, and after the journey. These stressors increase susceptibility of astronauts to opportunistic pathogens. Bacteria are armed with a large arsenal of virulence factors that give the bacterium the ability to attach to host cells and maneuver around the host environment, facilitating colonization and immune avoidance. One such set of virulence factors is the presence of motility organelles, including flagella (swimming) and pili (twitching). This project examines normal bacteria under zero gravity conditions encountered in space and their ability to cause disease. *Pseudomonas aeruginosa* is a bacterium found in soil, water and on the skin that can cause urinary tract, lung, and kidney infections. *Escherichia coli* is a bacterium found in the gut that contributes to gastrointestinal and urinary tract infections. These microbes were grown in conditions mimicing zero gravity and normal gravity conditions. We then conducted twitching assays on test microbes to examine if space conditions enhance disease establishment. After extended growth periods, *P. aeruginosa* grown under zero gravity conditions showed a significant difference in the spreading/twitching growth on 0.3% agar motility plates, as well as an significantly different colony morphology, compared to cultures grown under normal gravity conditions. *E. coli* cultures had a much less pronounced difference in the two conditions studied. These results led us to believe that in addition to a weakened immune system, some of the pathogens show increased virulence under zero gravity conditions. We are working towards understanding the molecular aspects of the twitching assay to determine the genes responsible for this differential effect.

Introduction:

The human race has always been fascinated with exploration from simply searching for food and shelter in prehistoric times to travelling and mapping the globe in the 15th-17th centuries. The ultimate frontier to explore in the twenty-first century is space (1). With this intriguing opportunity comes novel problems and stressors that must be researched thoroughly before we can openly investigate the borders of our solar system and beyond. The combined stressors of space travel take an immunological toll on the human body possibly setting the stage for an opportunistic infection.

Pseudomonas aeruginosa:

is a ubiquitous Gram negative bacterium that is an important opportunistic human pathogen that is capable of causing disease in humans whose immune system has been compromised, who have sustained major trauma, or who are inflicted with cystic fibrosis (2).

Escherichia coli:

is also a ubiquitous Gram negative bacterium common to the intestinal tracts of mammals. There are several virotypes that can cause disease in a human host including the O157:H7 strain.

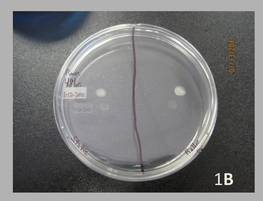
Materials and Methods

Simulated Zero Gravity:

Two separate environments were used to grow cultures of both *P. aeruginosa* and *E. coli*. For normal growth environment 100mL glass screw cap bottles were filled with 50mL of tryptic soy broth (TSB) media. They were inoculated with single colony forming units of respective bacteria and left on the bench top. For the simulated zero gravity environments the same bottles and media were used and then the cultures were placed on a Wheaton Bench Top Roller (Pic. 1A) at a select speed.

Twitching Motility Assay:

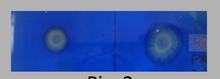
The cultures were allowed to grow for 72 total hours and samples were taken every 24 hours. The samples were stabbed into 0.3% agar plates (Pic. 1B) and incubated for 48 hours in 25°C. After incubation the plates were removed and growth was measured in millimeters. The plates were then stained with Commassie blue dye and sealed with parafilm for refrigerated storage.



Picture 1A: Wheaton bench top roller
Picture 1B: Image of twitching motility assay plate.

Results:

The zones of growth from the motility assays for each of the three trials were measured and the data was used to create the respective column graphs. The measurements for *P. aeruginosa* (Pic. 2 and Fig. 1) assays show a statistical difference while those for *E. coli* (Pic. 3 and Fig. 2) are not as pronounced or statistically relevant.



Pic. 2

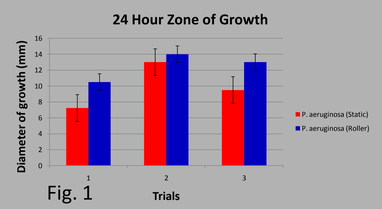
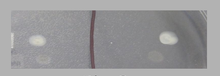


Fig. 1



Pic. 3

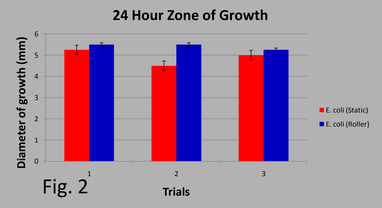


Fig. 2

Picture 2: Image of *P. aeruginosa* growth.
Figure 1: Column graph of *P. aeruginosa* zones of growth
Picture 3: Image of *E. coli* growth.
Figure 2: Column graph of *E. coli* zones of growth

Discussion

Type IV pili (Tfp) are used for bacterial twitching motility and responsible for intermittent jerks or gliding-like movements along the solid and liquid surfaces (3). Tfp is involved in bacterial adhesion and biofilm formation and is significant for *P. aeruginosa* virulence. There are at least 35 known genes required for *P. aeruginosa* Tfp and twitching function. Hence the difference in the twitching assays between the strains incubated under zero gravity and normal conditions could indicate a significant difference in bacterial virulence.

E. Coli on the other hand did not show significant difference in the twitching assays, indicating minimal change in virulence.

The difference in growth of *P. aeruginosa* from the zero gravity simulated environment compared to the static environment have interesting future implications.

Future:

Two new assays in the works include a slow killing assay utilizing the nematode *Caenorhabditis elegans* and its rate of death after consuming two different strains of *P. aeruginosa* that have been grown under the two environmental conditions (4). Also a plant assay using lettuce that has been injected with one of the two strains of *P. aeruginosa* as well (5).

Acknowledgements

We would like to thank Dr. Arden Aspedon for providing the bacterial cultures and Dr. Andrea Holgado for providing the nematodes as well as their invaluable suggestions during the project.

References Cited

(1) Setlow, Richard B.. "The Hazards of Space Travel." *European Molecular Biology Organization Reports* 4.11 (2003): 1013-1016. Print.
(2) He, Jianxin et al., "The broad host range pathogen Pseudomonas aeruginosa strain PA14 carries two pathogenicity islands harboring plant and animal virulence genes." *Proceedings of the National Academy of Science* 101.8 (2003): 2530-2535. Print.
(3)Wall, D., and D. Kaiser. 1999. Type IV pili and cell motility. Mol Microbiol. 32:1-10. Print
(4) Tan, Man-Wah et al., "Pseudomonas aeruginosa killing of Caenorhabditis elegans used to identify P. aeruginosa virulence factors." *Proceedings of the National Academy of Science* 96 (1999): 2408-2413. Print.
(5) Walker, Travis S. et al., "Pseudomonas aeruginosa - Plant Root Interactions. Pathogenicity, Biofilm Formation, and Root Exudation." *Plant Physiology* 134 (2004): 320-331. Print.