Effects of spaceflight and simulated microgravity on a host-pathogen system

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Spaceflight dramatically alters human physiology

- Increased radiation risk (oxidative stress)
- Reduction in bone density and muscle mass
- Telomere lengthening
- Immune system decrement
- Visual impairments and changes to ocular pressure
- Cardiovascular risk, inability to regulate blood pressure upon return to Earth
Immunity and spaceflight

Studies in astronauts show:

- Elevated neutrophils (innate immunity)

- Increased and/or decreased T-cell and monocyte counts (depending on mission duration and sample collection methods)

- Reactivation of latent Epstein-Barr virus, varicella-zoster virus, and cytomegalovirus in astronauts during flight
  - Sign of autoimmune disorder
Spaceflight affects bacterial pathogens, too

• Increased antibiotic resistance in *E. coli* (Tixador et al. 1987, Lapchine et al. 1986)

• Increased virulence in *Salmonella typhimurium* (Wilson et al. 2008) and *Pseudomonas aeruginosa* (Crabbé et al. 2011) and others

• Morphological changes to *E. coli*, including higher cell count after growth, thicker cell envelope, and increased cluster formation (Zea et al. 2017)
**Serratia marcescens** is a relevant pathogen

- *Serratia marcescens* is a ubiquitous pathogen, found in all environments including your bathroom, hospitals, the ISS, and other spacecraft
  - Primarily found in water supply of spacecraft (Ott et al 2004)
- Nosocomial – typically not considered pathogenic, but can be infectious in immunocompromised patients (or astronauts)
  - Causes urinary tract infections, upper respiratory infections, can be lethal in very immunocompromised people
Spaceflight increases virulence and *in vivo* growth of *S. marcescens*

Spaceflight-exposed bacteria are more lethal than ground control bacteria ($X^2=38.92$, $P<0.0001$)

<table>
<thead>
<tr>
<th>Hour post-infection</th>
<th>P-value (compared to ground)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.0009 *</td>
</tr>
<tr>
<td>15</td>
<td>0.0002 *</td>
</tr>
</tbody>
</table>

Gilbert et al. in press
Simulated microgravity (SMG) increases bacteria virulence

SMG DB11 reduces survival by 3.4 fold (P=0.0002) compared to the RWV control from the same subculture

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<tr>
<td>9</td>
<td>0.008</td>
</tr>
<tr>
<td>12</td>
<td>0.019</td>
</tr>
<tr>
<td>15</td>
<td>0.009</td>
</tr>
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</table>
Spaceflight gene expression varies from SMG

- *Serratia marcescens* grown in LB on ISS for 5 days (SpaceX-14, April-May 2018)
- Fixed in RNAlater in-flight and frozen
- Extracted RNA on ground and compared qPCR results

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Change In SMG</th>
<th>Fold Change in Spaceflight</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>asnB</td>
<td>28.84</td>
<td>3.96</td>
<td>asparagine synthetase B - Catalyzes the conversion of aspartate to asparagine</td>
</tr>
<tr>
<td>FlgG</td>
<td>2.09</td>
<td>0.82</td>
<td>Helps form the filaments of bacterial flagella</td>
</tr>
<tr>
<td>secY</td>
<td>2.06</td>
<td>0.86</td>
<td>Essential for protein secretion across the cytoplasmic membrane</td>
</tr>
<tr>
<td>hslU</td>
<td>2.31</td>
<td>1.68</td>
<td>Heat shock protein (ATPase) that is expressed in response to cell stress</td>
</tr>
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</table>
Asparagine pathway

In simulated microgravity:
Asparagine synthetase B: overexpressed
Asparagine tRNA ligase: overexpressed
Asparaginyl beta-hydroxylase: overexpressed

Is increased asparagine synthesis responsible for the increased growth and accelerated mortality of SMG and spaceflight bacteria?
Possible role for asparagine in increased virulence?

Asparagine catabolism - Inhibits T-cell response and mediates virulence in *Salmonella typhimurium* (murine model) (McLaughlin et al. 2017)

Asparagine essential for proliferation of *Francisella tularensis* and *Mycobacterium tuberculosis* within macrophages (Gesbert et al. 2013, Gouzy et al. 2014)

From McLaughlin et al. 2017 – *Salmonella* competes with T cells for asparagine, T cells cannot proliferate/activate in response to infection due to local depletion of the key resource.
Bacteria in SMG utilize asparagine and glutamine differently

![Graph showing Extracellular Asparagine (g/L) and Extracellular glutamine (g/L) over Hours growth]
Does early asparaginase treatment reduce virulence?

<table>
<thead>
<tr>
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<th>Injected w/ 32nL H₂O</th>
<th>Injected w/ 32nL L-asparaginase</th>
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<tr>
<td>RWV simulated microgravity bacteria</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>1g RWV control bacteria</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Glycerol sham</td>
<td>30</td>
<td>30</td>
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- Inject flies with bacteria grown in the RWV
- One hour following infection, flies were then injected with 32nL of L-asparaginase from *E. coli* (300 units/mg, Sigma)
- Survival measured for 24 hours
Injection of asparaginase delays mortality with SMG bacteria

- Survival is significantly different during the period that we typically see the SMG flies die faster than the control flies (focusing on when the LD50 of the two treatments becomes significantly different)

- Flies injected with asparaginase have delayed mortality, and survival is more statistically similar to the 1g-infected flies
How does presence/absence of amino acids affect growth and virulence?

Davis minimal defined media +aspartate +glutamine +aspartate +glutamine

Measure growth every 24 hours for 72 hours (reduced growth rate in minimal media)

Take sample at 24 hours for qPCR and injections
Aspartate alone increases growth in SMG

Summary

- Aspartate allows for greater growth in SMG at hour 24 (p=0.0098)
- No difference in other conditions, likely that cell proliferation cannot happen with these amino acids alone
AsnB still overexpressed in limited nutrient media

- Asparagine synthetase is overexpressed in SMG only when both glutamine and aspartate are present (at 24 hours of growth)
  - Recapitulates the expression levels seen in nutrient rich growth media
- L-asparaginase overexpressed in SMG
Amino acid presence alone affects virulence

- Flies injected with SMG Aspartate + Glutamate (24 hour) die slightly faster than the 1g Aspartate+Glutamate
- No difference between SMG and 1g when only using minimal media
Proposed mechanism of virulence in *S. marcescens*

In low shear modeled microgravity:

- Increased expression of *asnB* (asparagine synthetase)
- Increased consumption of both glutamine and asparagine
- Increased expression of several L-asparaginase genes
- Increased metabolism of key amino acids in the asparagine pathway
- This pathway is linked to bacterial virulence, but this is the first time it’s been implicated in altered gravity
Conclusions and future directions

• Spaceflight and simulated microgravity increase virulence and *in vivo* growth of *Serratia marcescens*

• Asparagine catabolism-related genes are implicated both in spaceflight and in simulated microgravity, and are likely related to the increased virulence of *S. marcescens* in altered gravity

• Asparaginase treatment mitigates the rate of mortality after infection with *S. marcescens*, possible use as a countermeasure?
  • Asparaginase currently used as a cancer treatment in acute leukemias, Hodgkin disease, melanosarcoma – potential future role for bacteria infections

• Upcoming studies will focus on further studying the involvement of asparagine pathway in increased virulence via *asnB* knockouts

• Start translating asparagine/asparaginase work to mammalian cell models
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<td>astL</td>
<td>2.35</td>
<td>asparagine tRNA ligase (regulates asparagine synthetase)</td>
</tr>
<tr>
<td>asbh</td>
<td>4.67</td>
<td>aspartyl/asparaginyl beta-hydroxylase (shown to be involved in late stage LPS biosynthesis)</td>
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<td>FliE</td>
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<td>Involved in biogenesis of flagella</td>
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<td>nudE</td>
<td>2.00</td>
<td>Enzyme superfamily that helps remove potentially toxic metabolites and stress-induced signaling molecules from the bacterial host</td>
</tr>
<tr>
<td>lpxD</td>
<td>2.04</td>
<td>Lipopolysaccharide biosynthesis gene, expression essential for biofilm formation, and decreased expression reduced bacterial attachment to cultured airway epithelial cells</td>
</tr>
<tr>
<td>tatB</td>
<td>2.08</td>
<td>Reduced expression results in slowed growth, impaired cytochrome oxidase c activity, and increased susceptibility to intracellular infection.</td>
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<td>Heat shock protein (ATPase) that is expressed in response to cell stress</td>
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<td>dnaK</td>
<td>2.00</td>
<td>Involved in the heat shock response, deletion decreases bacteria cell survival</td>
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Genes selected primarily from previous spaceflight literature (Nickerson et al., Wilson et al. 2007)
Asparaginase in leukemia treatments

- Anti-tumor treatment: asparaginase causes a depletion of asparagine, which is essential to leukemia cells

- Lack of asparagine leads to inhibition of protein synthesis, causing cytotoxicity of cancerous cells (but not healthy cells)

- Used to treat acute lymphoblastic leukemia, Hodgkin disease, acute lymphocytic leukemia (in children), melanomasarcoma, and others

- Also breaks down glutamine into glutamate, which targets cancer cells that express asparagine synthetase

Credit: Fung and Chan 2017 DOI:10.1186/s13045-017-0509-9