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

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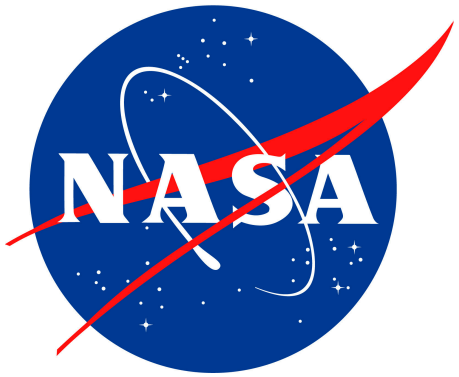
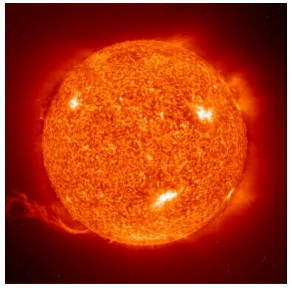
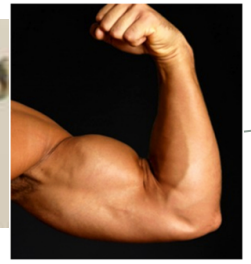


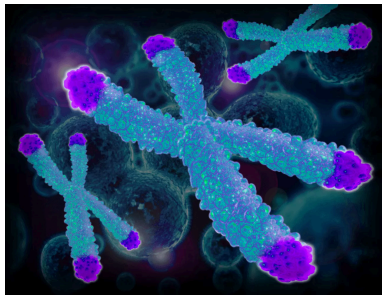
Spaceflight dramatically alters human physiology



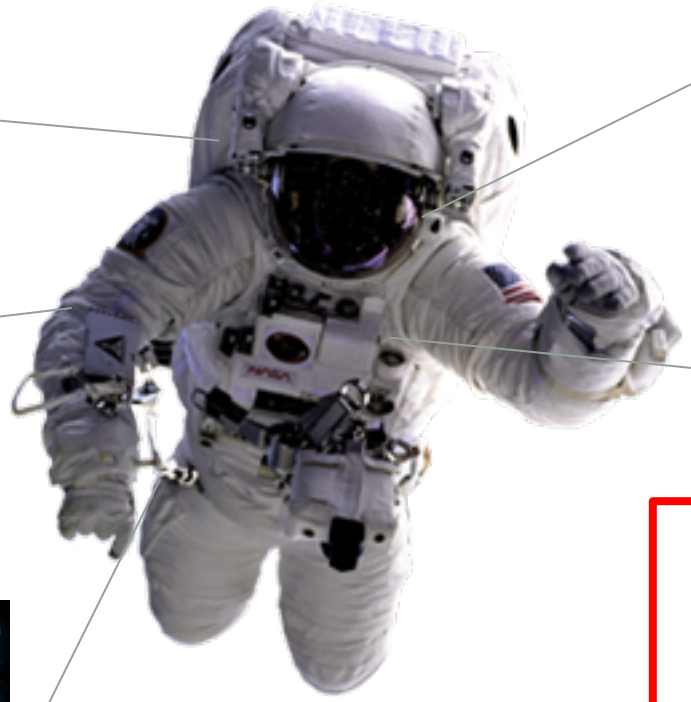
Increased radiation risk (oxidative stress)

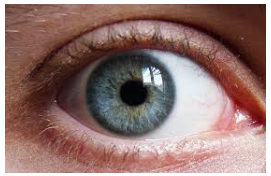


Reduction in bone density and muscle mass

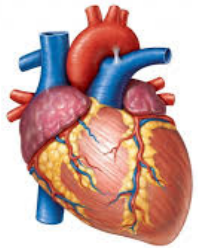


Telomere lengthening

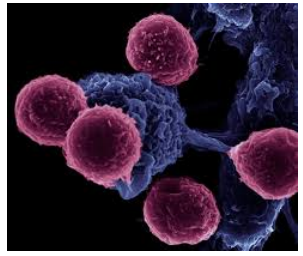




Visual impairments and changes to ocular pressure



Cardiovascular risk, inability to regulate blood pressure upon return to Earth

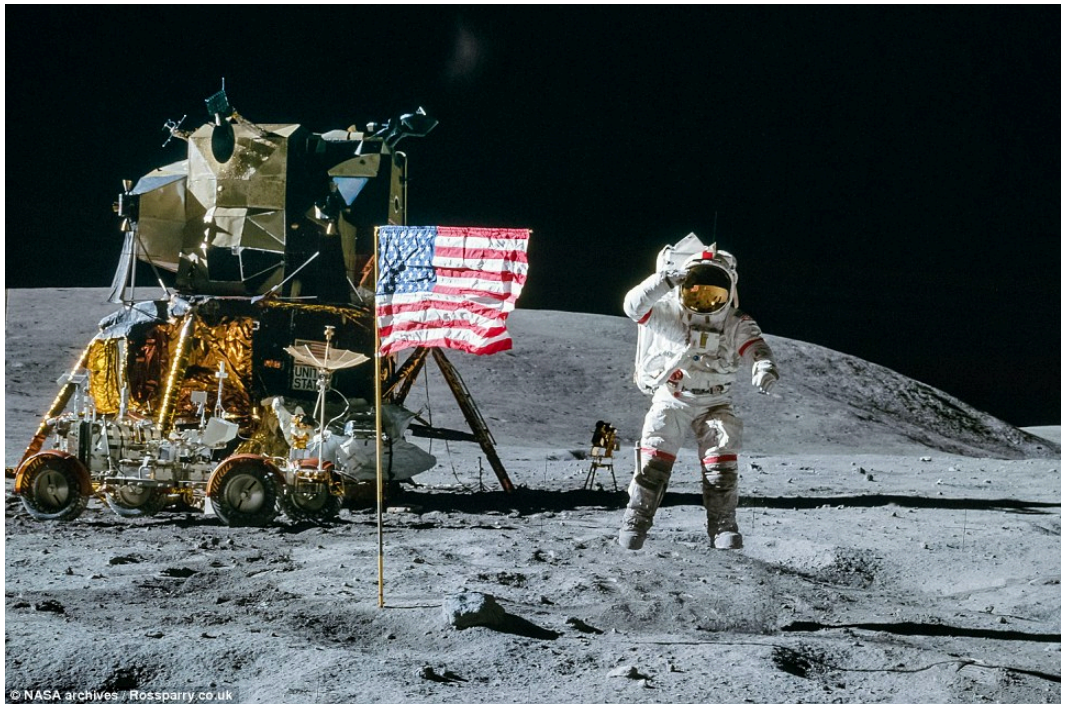


Immune system decrement

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

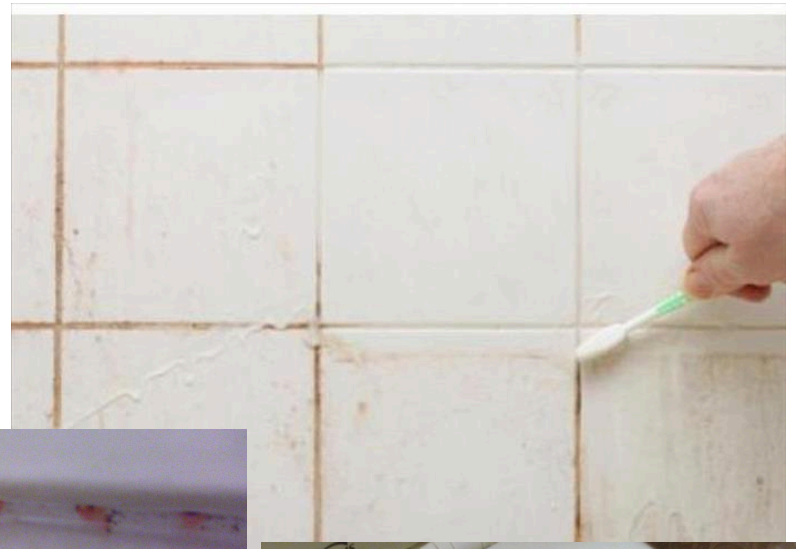


Spaceflight affects bacterial pathogens, too

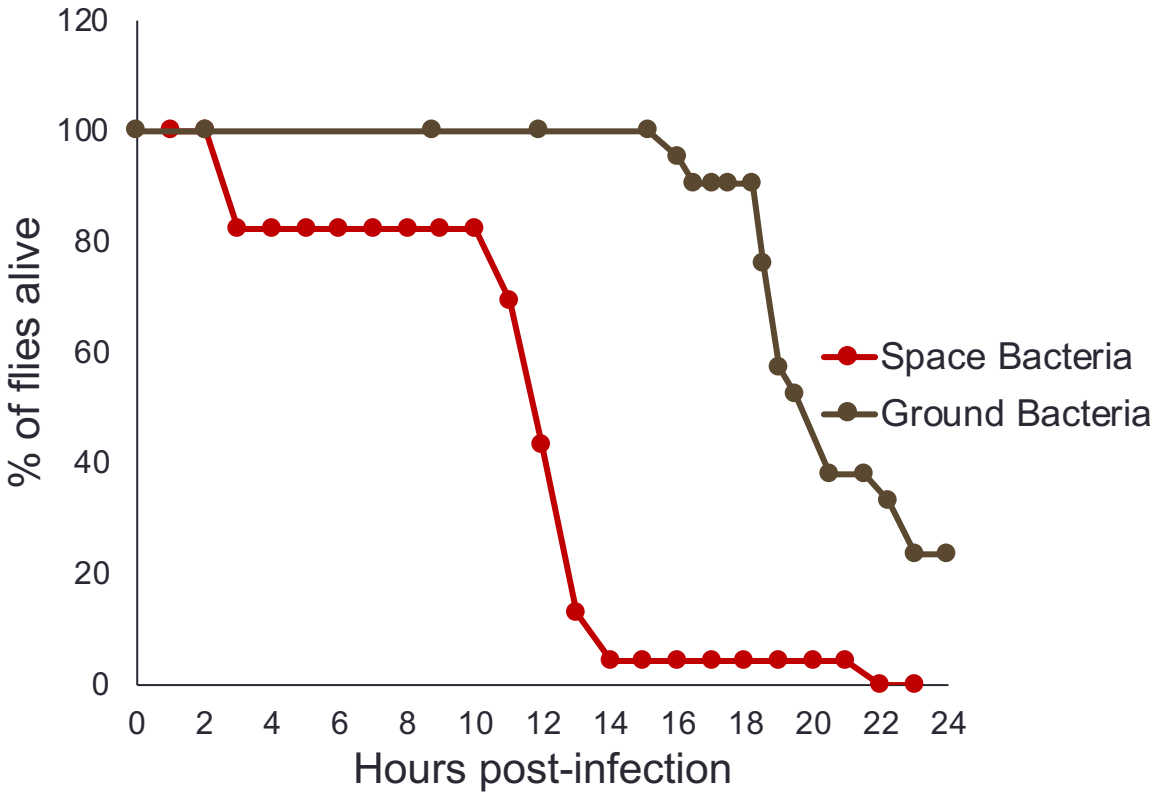
- Increased antibiotic resistance in *E. coli* (Tixador et al. 1987, Lapchine et al. 1986)
- Increased virulence in *Salmonella typhimurium* (Wilson et al. 2008) and *Pseudomonas aeruginosa* (Crabbé et al. 2011) and others
- Morphological changes to *E. coli*, including higher cell count after growth, thicker cell envelope, and increased cluster formation (Zea et al. 2017)

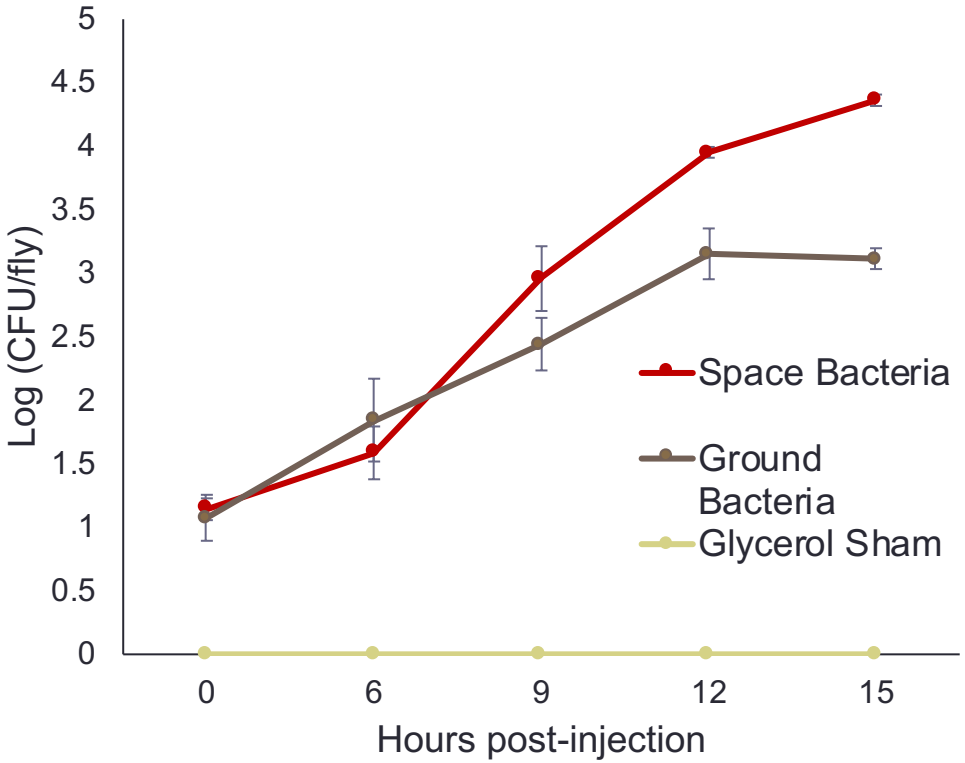
Serratia marcescens is a relevant pathogen

- *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*



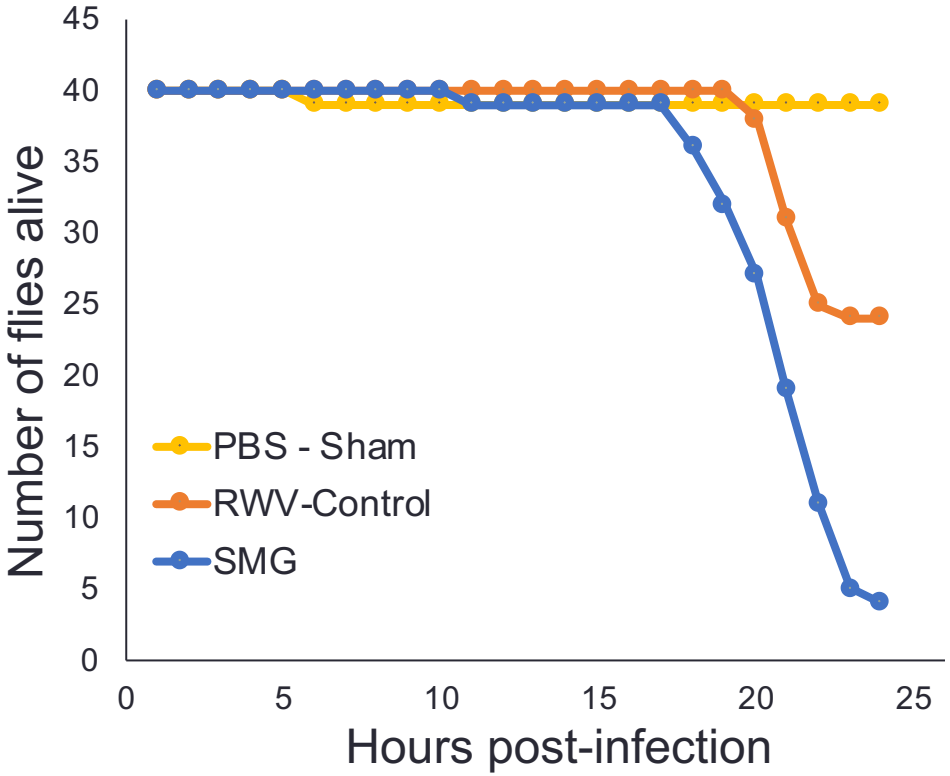


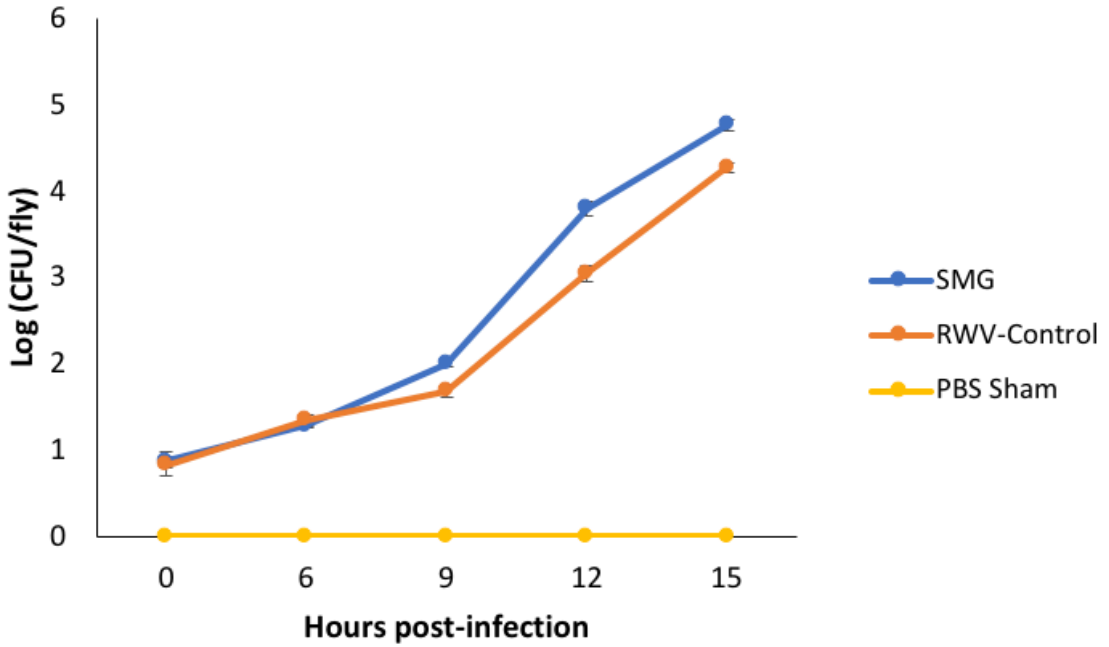
Spaceflight-exposed bacteria are more lethal than ground control bacteria (X^2=38.92, P<0.0001)

Hour post-infection	P-value (compared to ground)
12	0.0009 *
15	0.0002 *

Gilbert et al. in press

Simulated microgravity (SMG) increases bacteria virulence





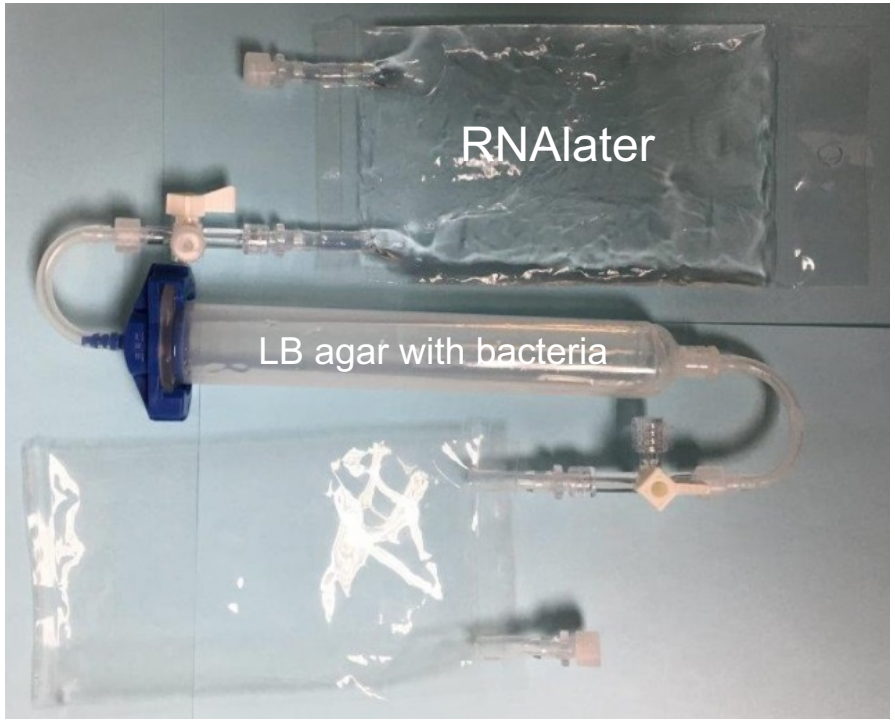
Hour post-infection	P-value (compared to ground)
9	0.008
12	0.019
15	0.009

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

Gilbert et al. in press

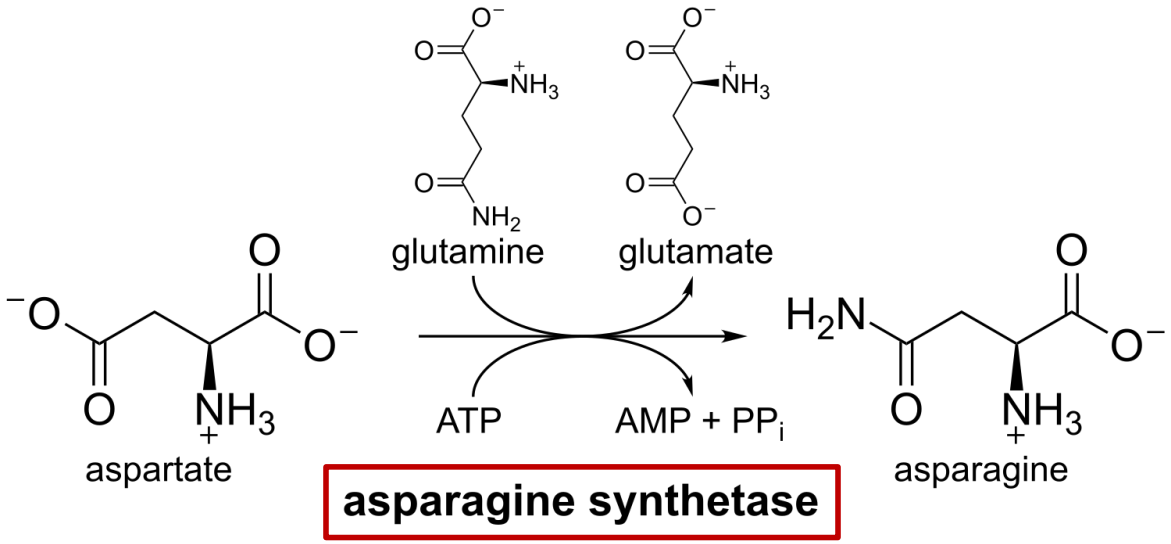
Spaceflight gene expression varies from SMG

- *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



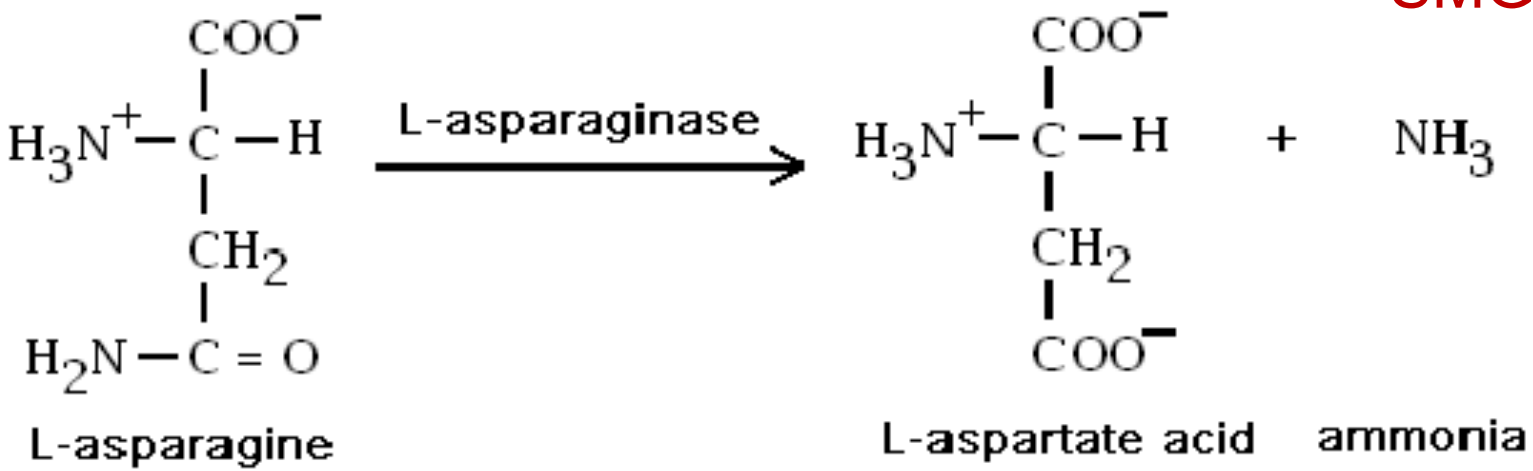
Gene Name	Fold Change In SMG	Fold Change in Spaceflight	Description
asnB	28.84	**3.96**	asparagine synthetase B - Catalyzes the conversion of aspartate to asparagine
FlgG	2.09	**0.82**	Helps form the filaments of bacterial flagella
secY	2.06	**0.86**	Essential for protein secretion across the cytoplasmic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

Asparagine pathway

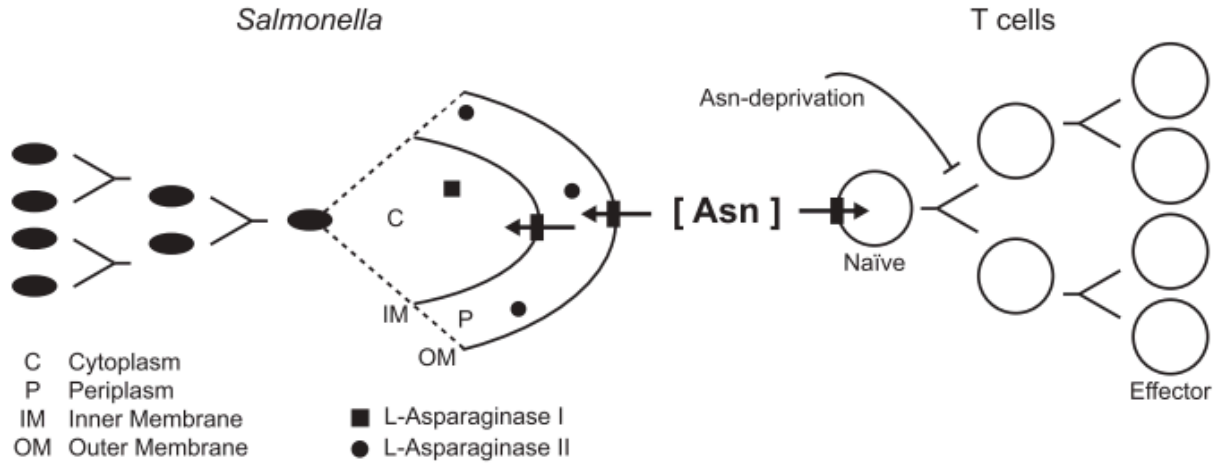


In simulated microgravity:
Asparagine synthetase B: overexpressed
Asparagine tRNA ligase: overexpressed
Asparaginyl beta-hydroxylase: overexpressed

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



Possible role for asparagine in increased virulence?

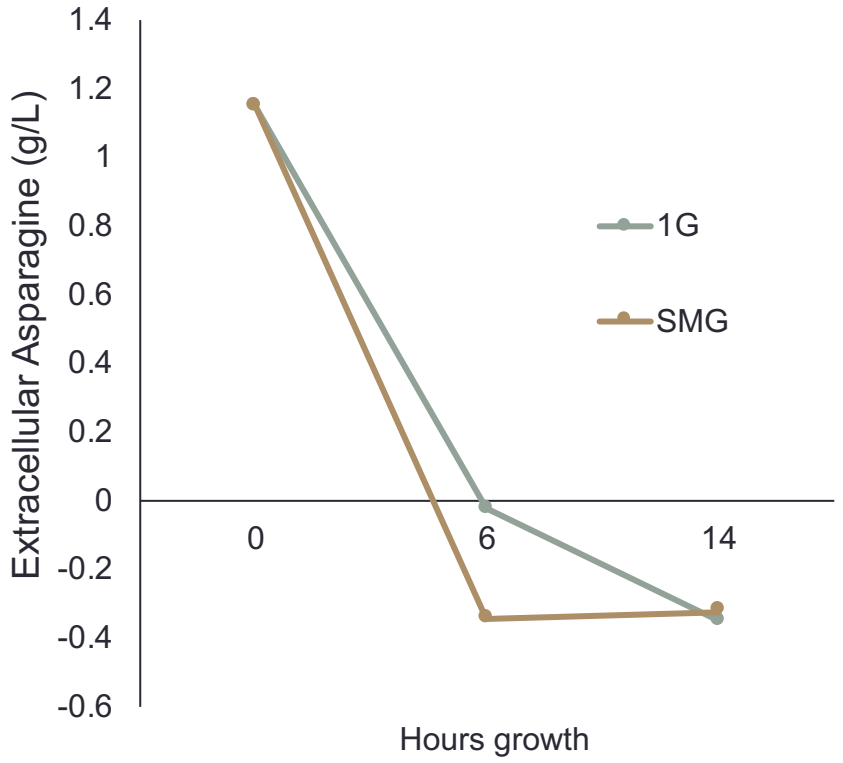


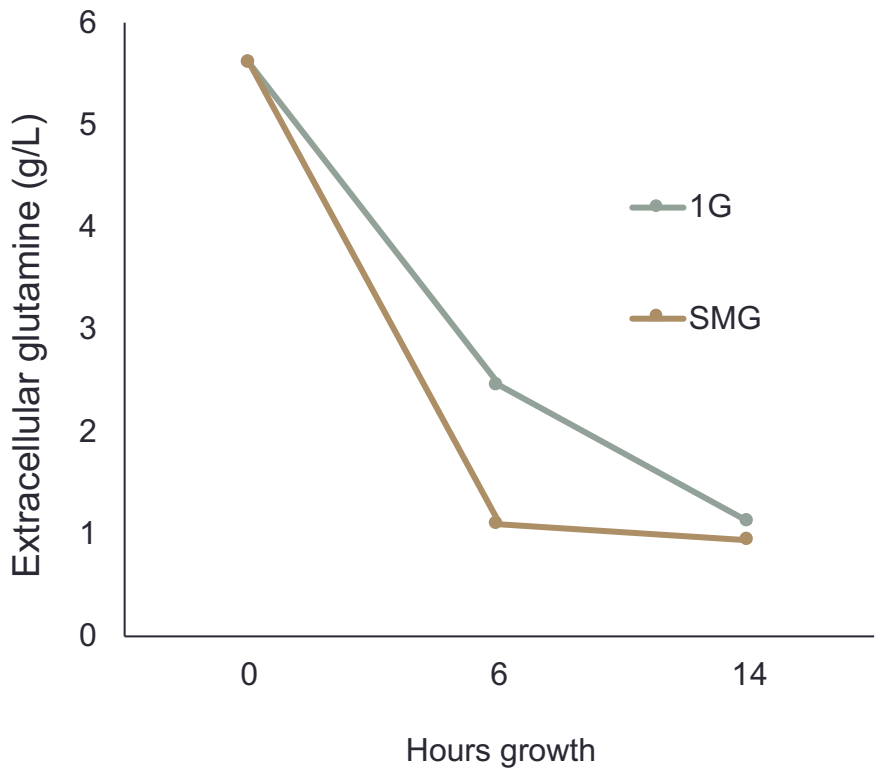
From McLaughlin et al. 2017 – Salmonella competes with T cells for asparagine, T cells cannot proliferate/activate in response to infection due to local depletion of the key resource

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)

Bacteria in SMG utilize asparagine and glutamine differently



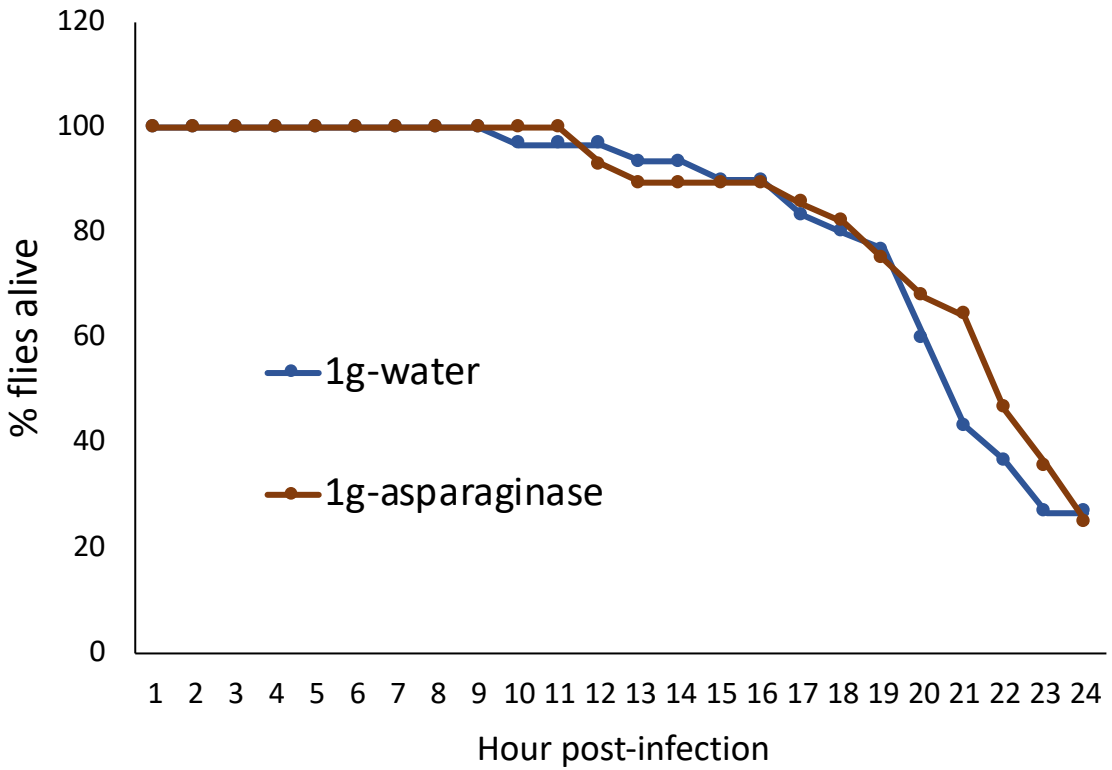


Does early asparaginase treatment reduce virulence?

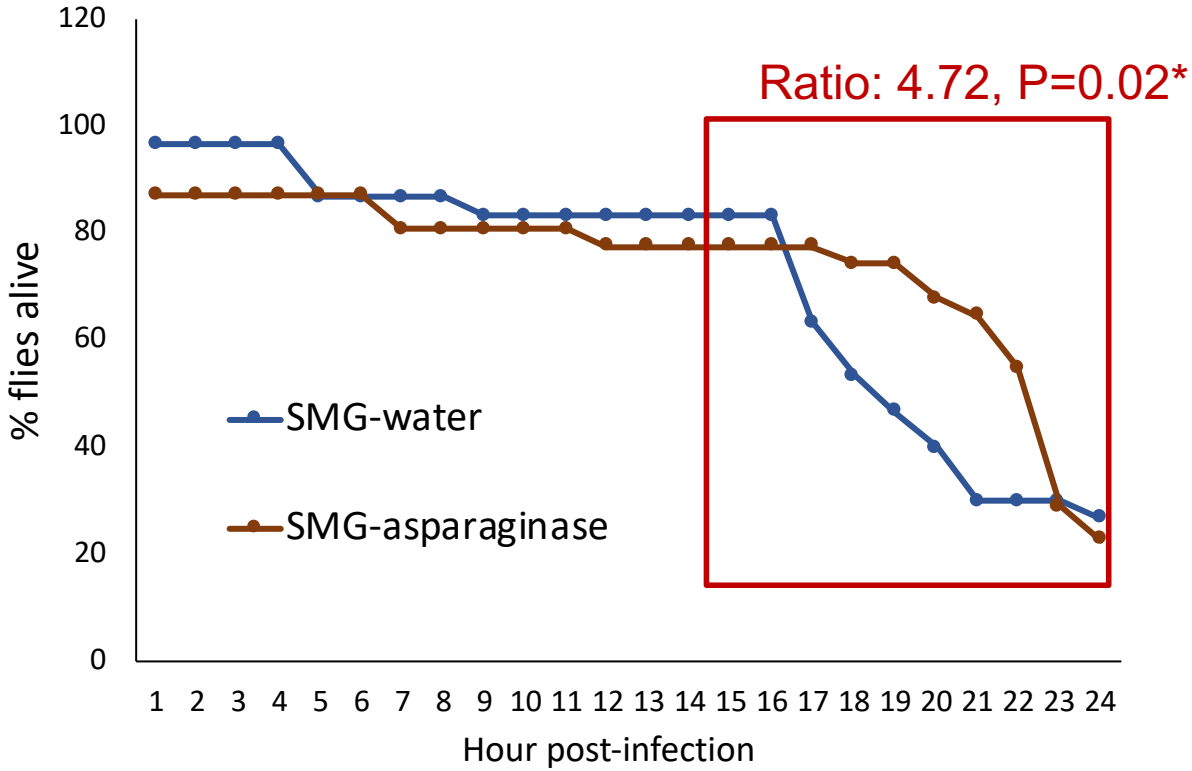
	Injected w/ 32nL H_20	Injected w/ 32nL L-asparaginase
RWV simulated microgravity bacteria	30	30
1g RWV control bacteria	30	30
Glycerol sham	30	30

- 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



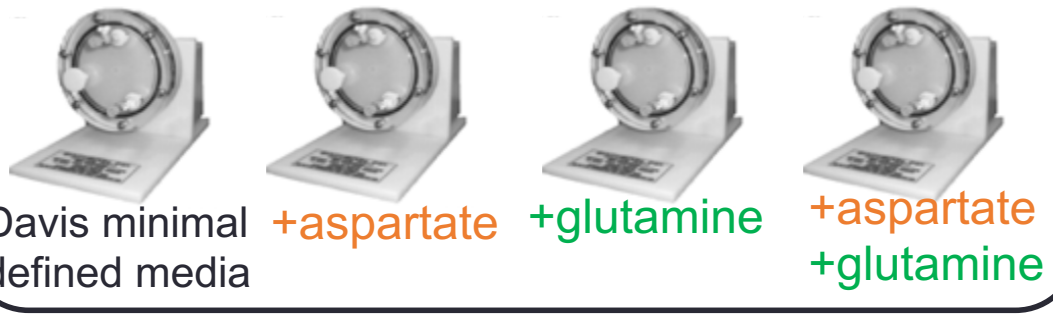
Ratio: 1.15, P=0.63



Ratio: 1.0, P=0.98

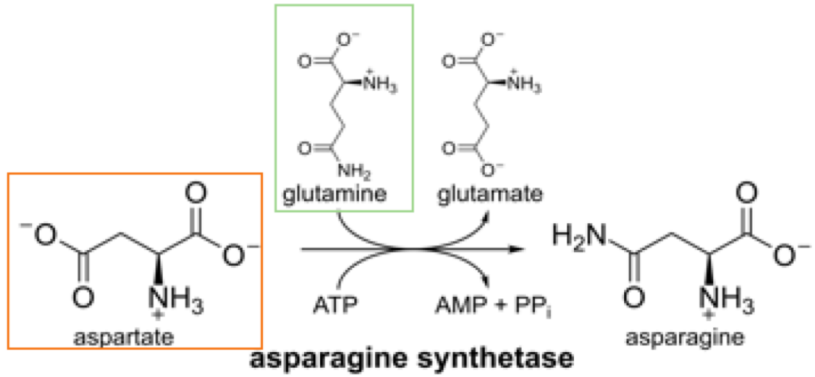
- Survival is significantly different during the period that we typically see the SMG flies die faster than the control flies (focusing on when the LD50 of the two treatments becomes significantly different)
- Flies injected with asparaginase have delayed mortality, and survival is more statistically similar to the 1g-infected flies

How does presence/absence of amino acids affect growth and virulence?

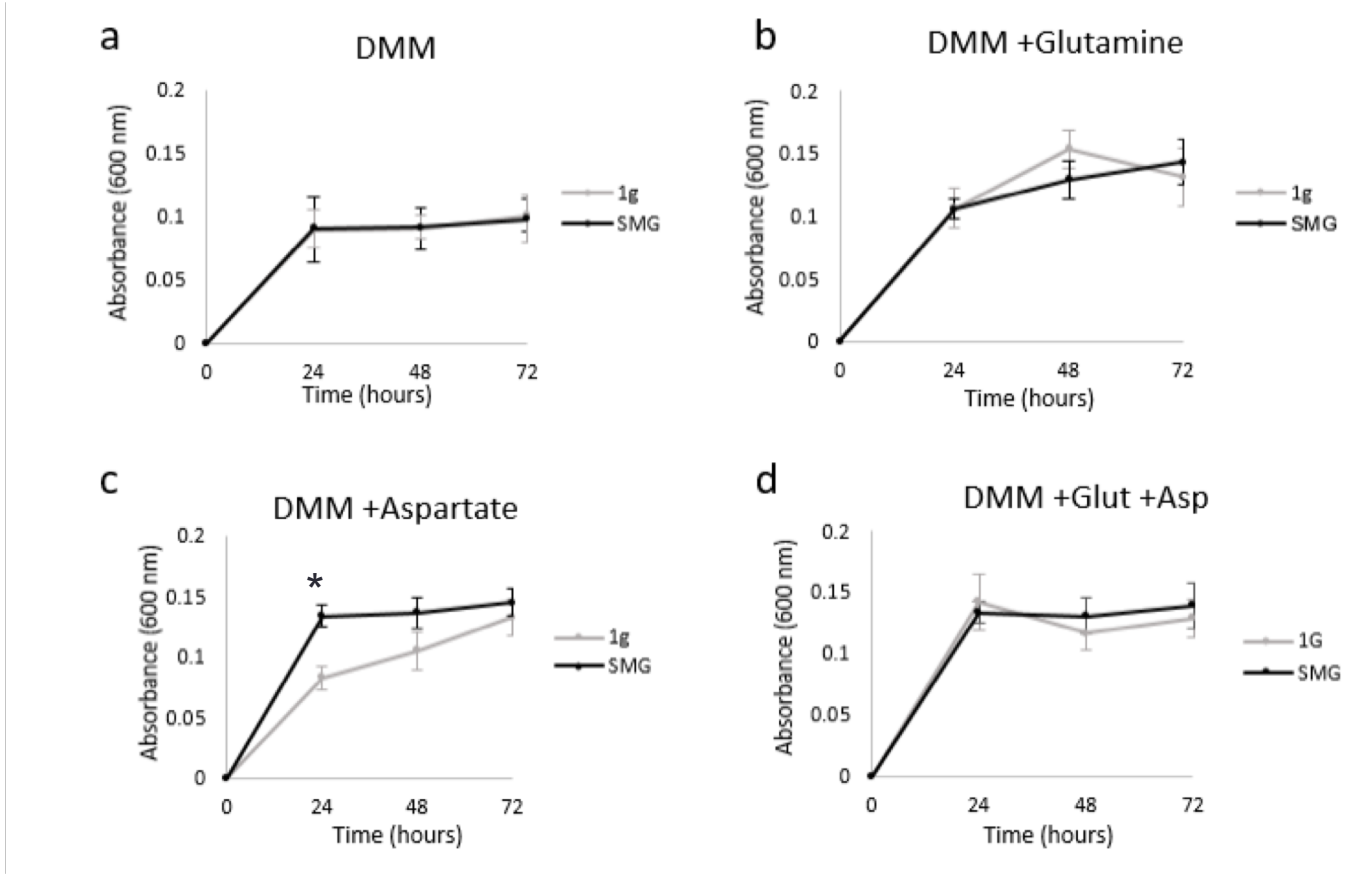


Measure growth every 24 hours for 72 hours (reduced growth rate in minimal media)

Take sample at 24 hours for qPCR and injections



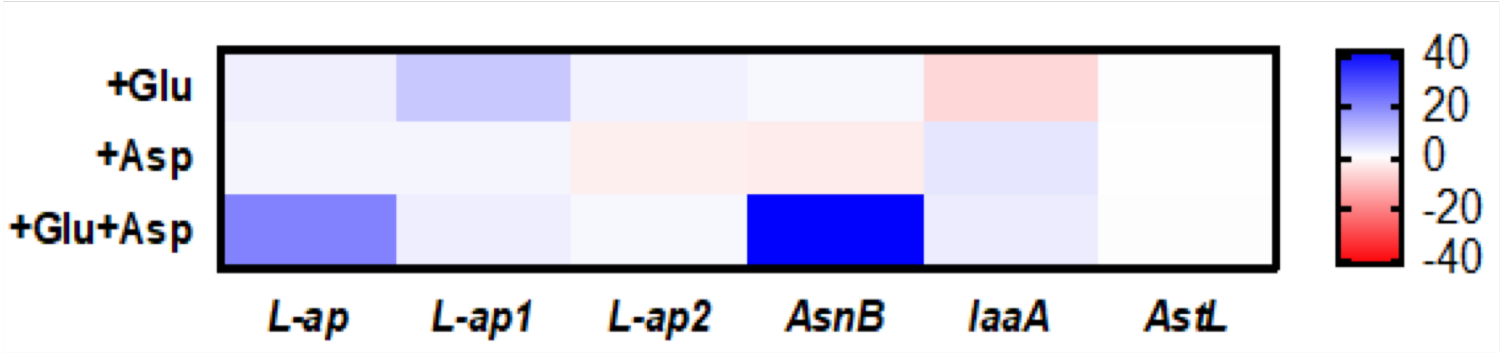
Aspartate alone increases growth in SMG



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

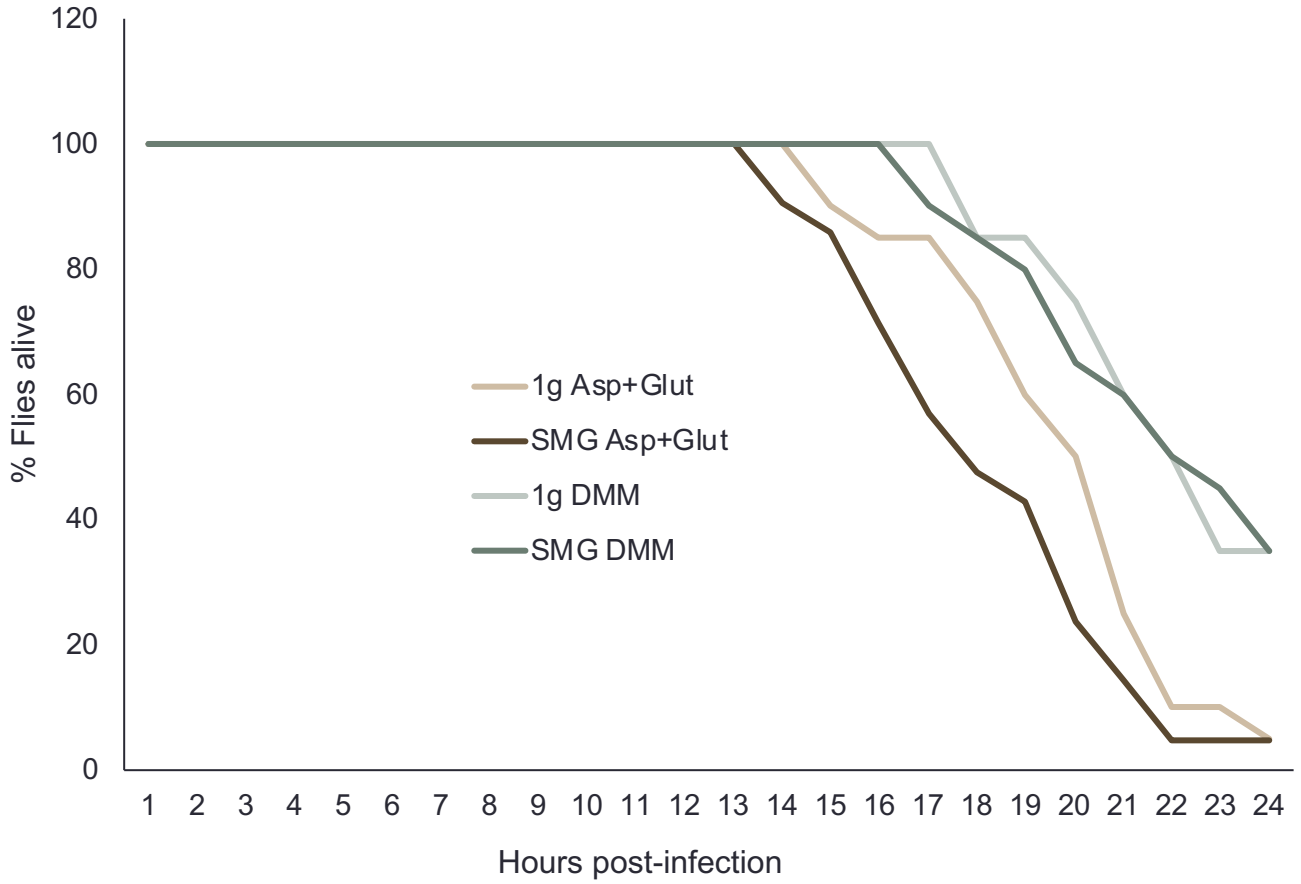
asnB still overexpressed in limited nutrient media



- 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

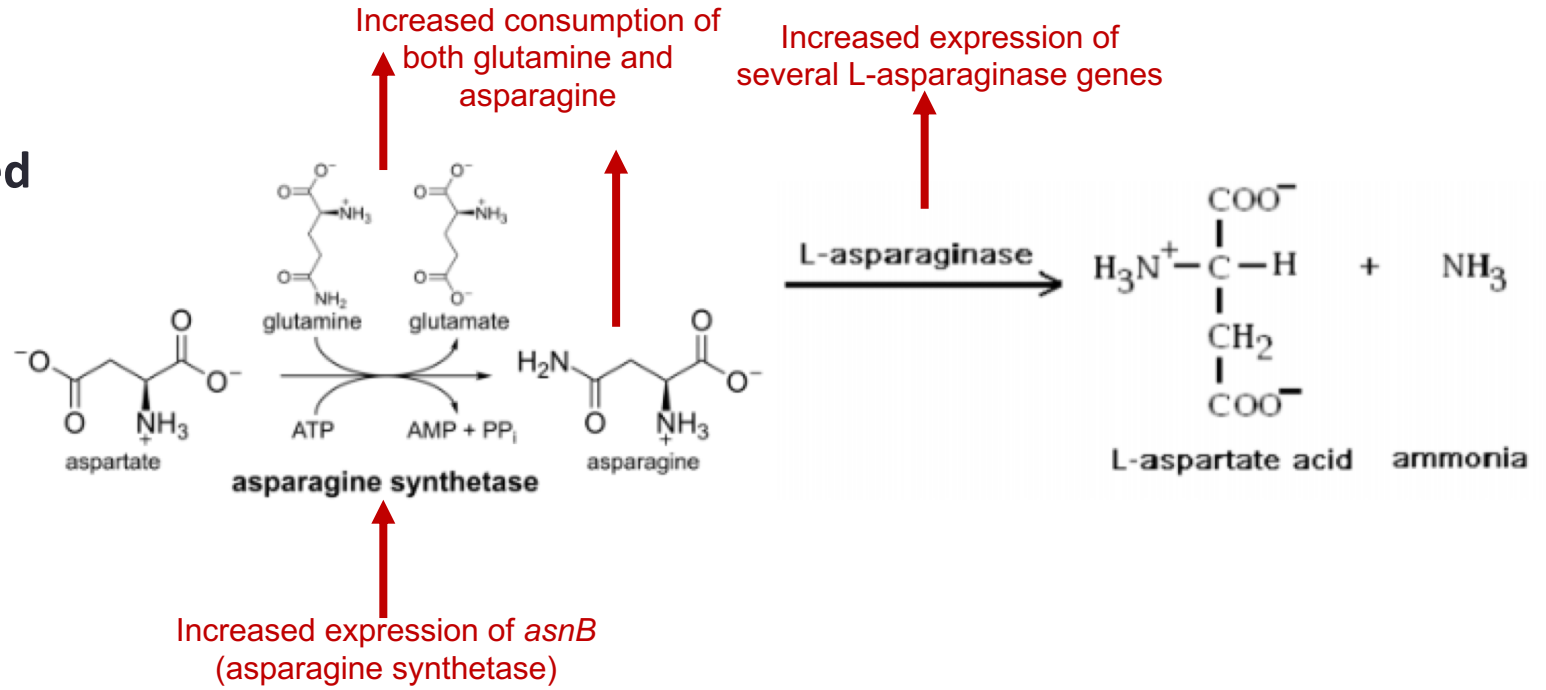
Amino acid presence alone affects virulence

- 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 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

Acknowledgements

Sharmila Bhattacharya
Medaya Torres
Christina Cheung
Amber Paul
Siddhita Mhatre
Janani Iyer

<u>Interns:</u>
Nicole Tanenbaum
Rachel Lo
Nhung (Mindy) Tran

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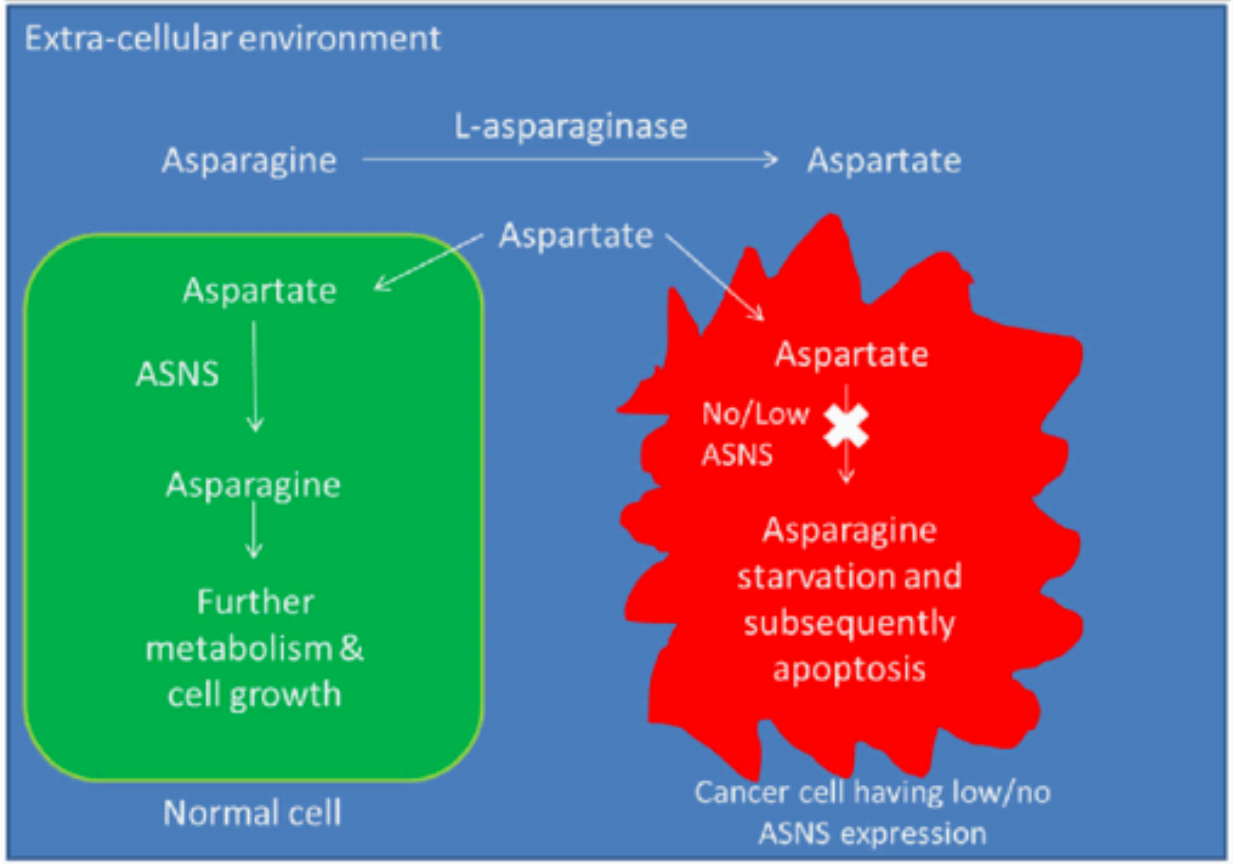
Gene Name	Fold Change In SMG	Description
asnB	28.84	asparagine synthetase B - Catalyzes the conversion of aspartate to asparagine
astL	2.35	asparagine tRNA ligase (regulates asparagine synthetase)
asbh	4.67	aspartyl/asparaginyl beta-hydroxylase (shown to be involved in late stage LPS biosynthesis)
FlgG	2.09	Helps form the filaments of bacterial flagella
secY	2.06	Essential for protein secretion across the cytoplasmic membrane
FliE	2.05	Involved in biogenesis of flagella
nudE	2.00	Enzyme superfamily that helps remove potentially toxic metabolites and stress-induced signaling molecules from the bacterial host
lpxD	2.04	Lipopolysaccharide biosynthesis gene, expression essential for biofilm formation, and decreased expression reduced bacterial attachment to cultured airway epithelial cells
tatB	2.08	Reduced expression results in slowed growth, impaired cytochrome oxidase c activity, and increased susceptibility to intracellular infection.
hslU	2.31	Heat shock protein (ATPase) that is expressed in response to cell stress
dnaK	2.00	Involved in the heat shock response, deletion decreases bacteria cell survival

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), melanosarcoma, 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