Spaceflight-Induced Changes in Microbial Virulence and the Impact to the Host Immune Response

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Introduction

- Over the past 50 years, microorganisms have exhibited unexpected responses relevant to infectious disease when grown in spaceflight and spaceflight analogues, including changes in final cell concentration, biofilm production, stress resistance, antibiotic sensitivity, gene expression, and virulence.
- Seminal studies demonstrated that the foodborne pathogen, *Salmonella enterica* serovar Typhimurium, increased its virulence and pathogenesis-related characteristics, and globally altered its transcriptomic and proteomic profiles, in response to both spaceflight and spaceflight analogue culture[1-8].

Introduction

- Since those experiments, alterations in the pathogenesis-related characteristics of other pathogens have been documented in response to growth in these environments[9-13].
- Notably, a recent study demonstrated an increase in virulence of *Serratia marcescens* cultured during spaceflight, documenting that pathogens other than *Salmonella* can also manifest this response[14].
- However, our overall knowledge of which microorganisms will alter their virulence in response to spaceflight and spaceflight analogue culture, and the underlying mechanisms governing this phenotype, remains very limited.

Experimental Design

- Using bacteria that are cultured in both the spaceflight analogue Rotating Wall Vessel bioreactor (Synthecon) and control conditions, this study will:
  - **Aim 1: Characterize the effect of spaceflight analogue culture on microbial pathogenesis-related stress responses and *in vitro* host-pathogen interactions.** Analysis will include microbial stress responses as well as colonization and viability following pathogen challenge of three-dimensional (3-D) tissue co-culture models containing immune cells.
  - **Aim 2: Characterize the effect of spaceflight analogue culture on the virulence potential of pathogenic microorganisms.** Changes in virulence will be assessed using a mouse model of infection.
  - **Aim 3: Characterize the effect of spaceflight analogue culture on the global molecular genetic responses of pathogenic microorganisms.**

Experimental Design

- We selected obligate and opportunistic pathogens that are medically important and have been or are likely to be found aboard spacecraft, including *Streptococcus pneumoniae*, *Salmonella enterica* serovar Enteritidis, *Pseudomonas aeruginosa*, *Burkholderia cepacia,* and enterohemorrhagic *Escherichia coli* (EHEC).

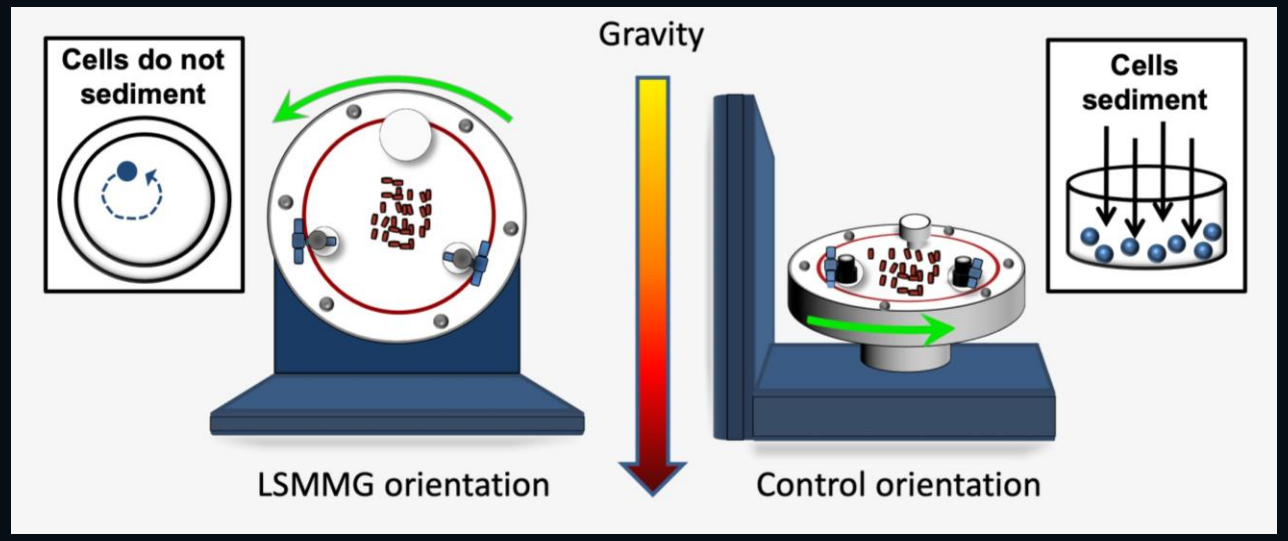
Progress

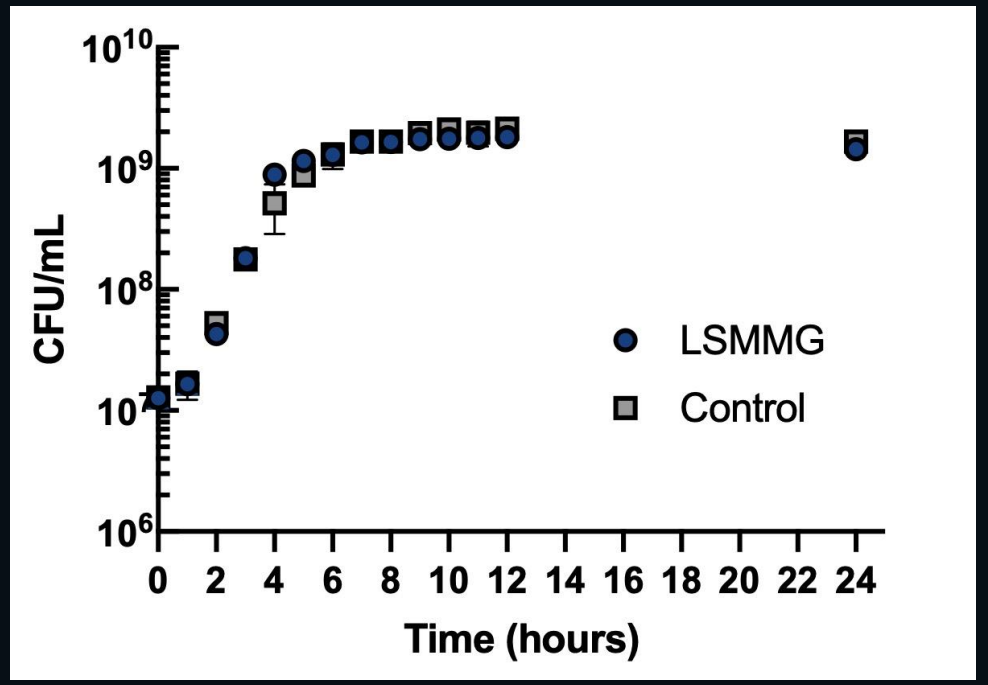
- ***S.* Enteritidis**: Growth kinetics, stress responses, infection of 3-D tissue culture models have been completed. Virulence studies are nearly complete and transcriptomic studies are ongoing.
- **EHEC**: Growth kinetics, stress responses and 3-D tissue culture infections are completed; virulence studies are nearly complete and transcriptomic studies are ongoing.
- ***P. aeruginosa***: Growth kinetics and stress responses have been completed. 3-D tissue culture infections and transcriptomic studies are ongoing. Data is being analyzed to determine the need for animal virulence testing.
- ***B. cepacia***: Growth kinetics and several stress responses have been completed. 3-D tissue culture infections and transcriptomic studies are ongoing. Data is being analyzed to determine the need for animal virulence testing.
- ***S. pneumoniae***: Growth conditions and media requirements have been optimized. Growth kinetics and stress responses are ongoing.

Salmonella Enteritidis

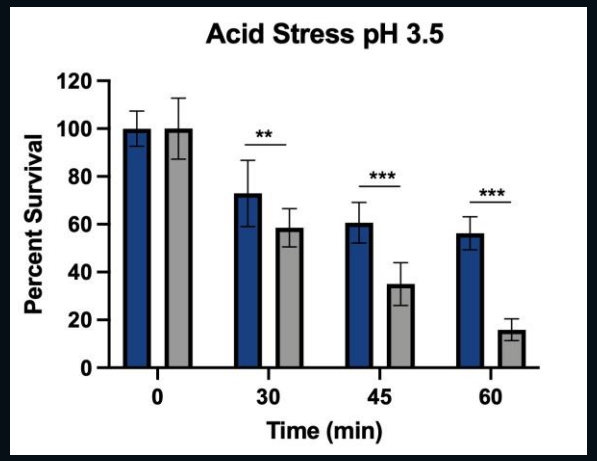
- Major cause of food- and water-borne illnesses and one of the the leading cause of human salmonellosis outbreaks worldwide since the 1980s [15]
- Like *S*. Typhimurium, represents a potential health threat for Low Earth Orbit (LEO) and deep space missions.
- Limited knowledge about how spaceflight could influence the disease-causing characteristics of *S*. Enteritidis

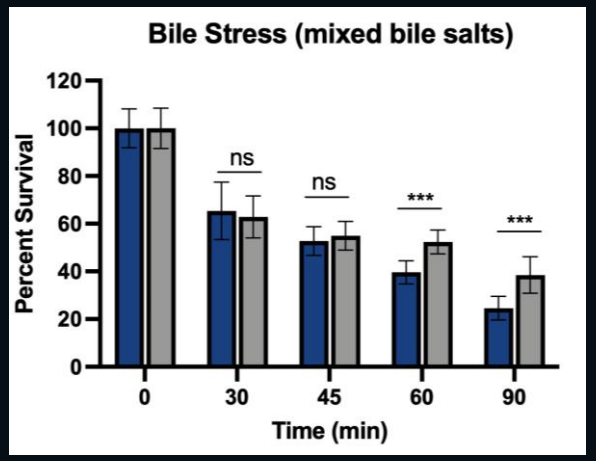
S. Enteritidis displayed similar growth kinetics and final densities between LSMMG and control conditions

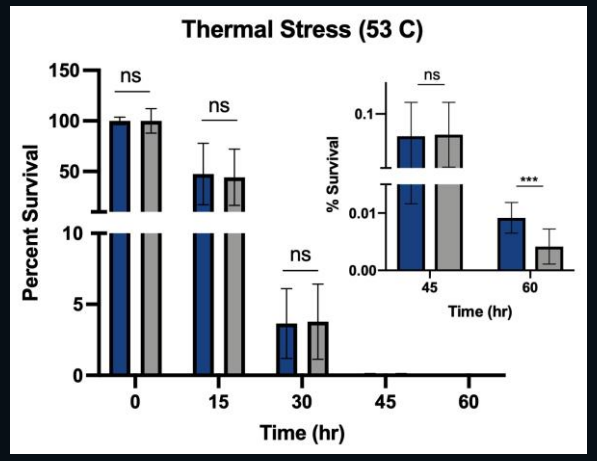


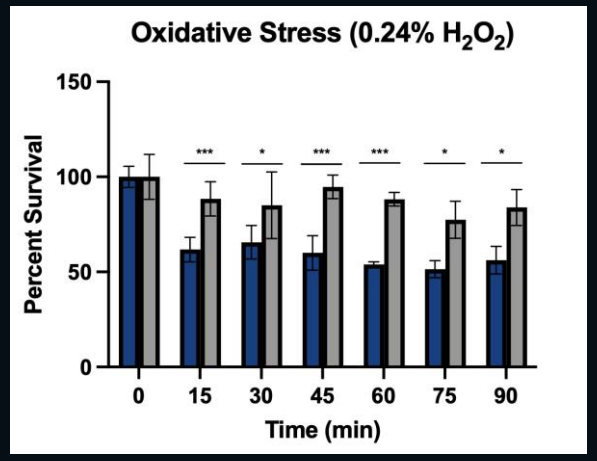


LSMMG culture selectively alters the resistance of *S.* Enteritidis to pathogenesis-related stressors

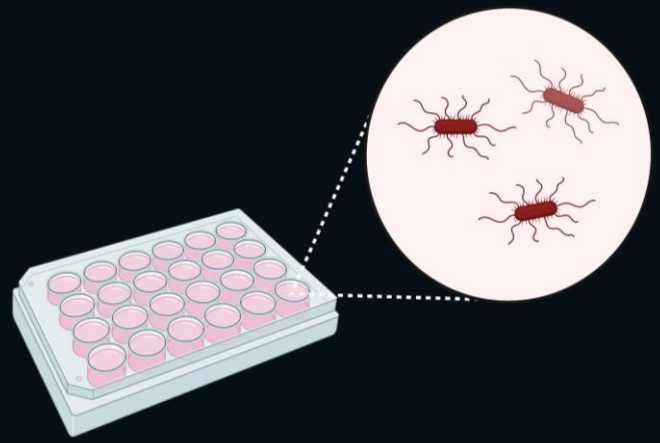


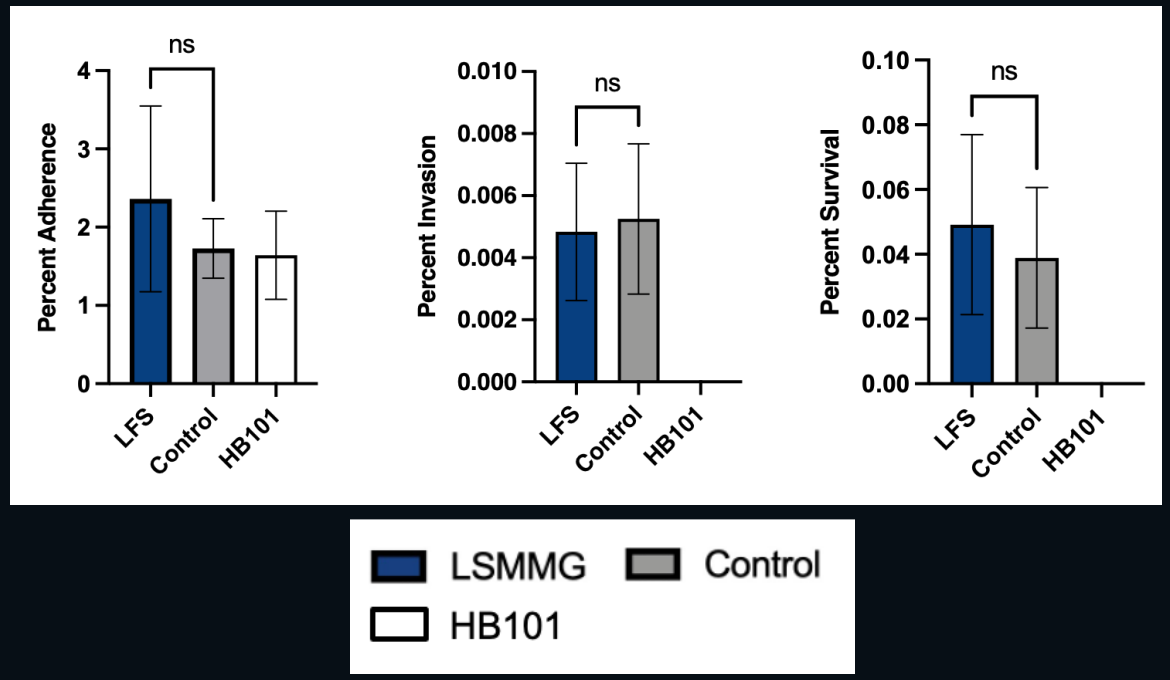




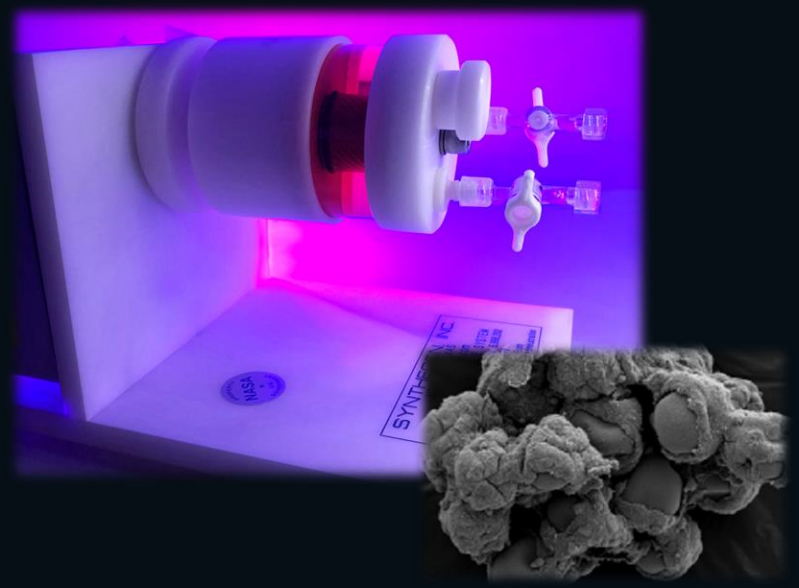


No differences in colonization observed in 2-D monolayers of human intestinal epithelium between LSMMG and control cultures

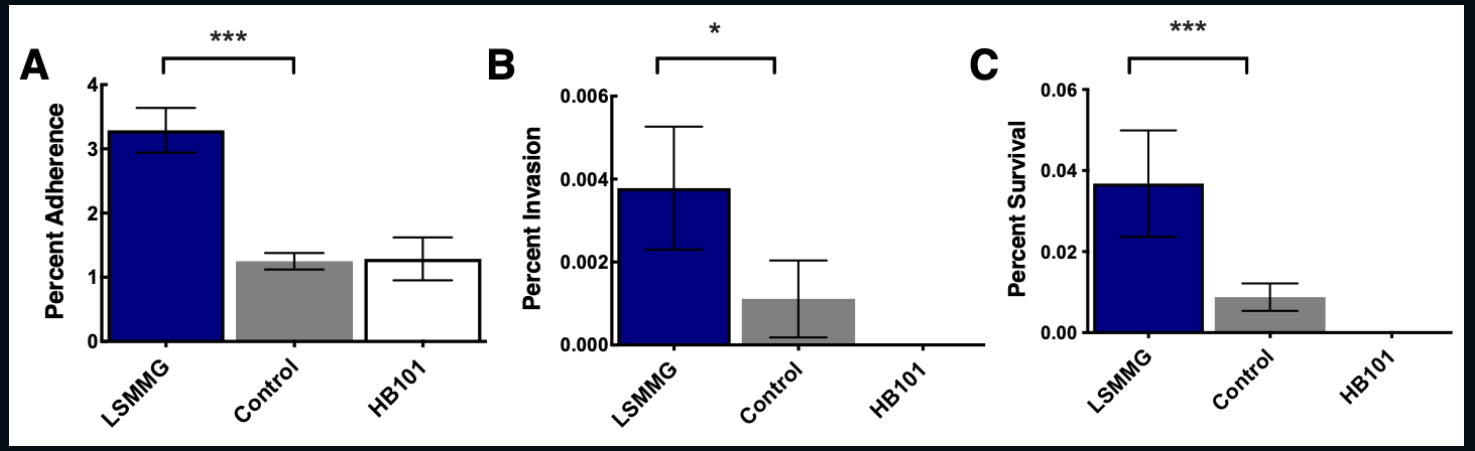


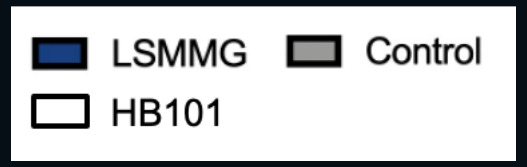


LSMMG-cultured *S.* Enteritidis displayed enhanced colonization in a 3-D human intestinal co-culture model



Adherence Invasion Intracellular survival





Significance

- This information will provide critical understanding into the impact of microgravity on potential alterations in microbial virulence and associated infectious disease risk to crew health during spaceflight missions.[16]

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