# Media Ion Composition Controls Regulatory and Virulence Response of *Salmonella* in Space Flight

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Microbial pathogens cultured during space flight appear to cause disease differently than those cultured using traditional methods. This difference offers a key insight into not only fundamental cellular and molecular response mechanisms but also the potential identification of previously undiscovered classes of genes and proteins that are important for the infectious disease process. Historically, the study of microbial responses in extreme environments (e.g., severe shifts in pH, osmotic concentration, or temperature) has provided tremendous insight into our understanding of how pathogens adapt and respond during the infection process in vivo. For pathogens, many of these conditions reflect the environment they encounter during the normal infection process. In this regard, the conditions that microorganisms encounter in the microgravity environment of space flight are relevant to the low-fluid-shear areas encountered by pathogens during infection of a human host, such as in areas of the intestinal tract. This latter environment holds importance for enteric pathogens such as *Salmonella typhimurium*.

In early NASA-supported studies by Cheryl A. Nickerson of Arizona State University, cultures of *S. typhimurium* grown in space-flight-analog bioreactors in rich Lennox broth (LB) media displayed increased virulence and global changes in gene expression, including a large number of genes encoding ion response pathways that were differentially regulated in response to this low-fluid-shear growth condition. The potential role for ion-mediated regulation of cellular responses to this environment was observed in additional flight-analog bioreactor experiments, which showed that in the low-nutrient, high-inorganic salt of M9 minimal media, *Salmonella* lag phase and generation time were shortened compared to the lag phase and generation time of controls. This effect was not observed in LB media under identical growth conditions. These analog observations led to flight experiments with *S. typhimurium* aboard space shuttle mission STS-115, which demonstrated that cultures grown in rich LB media in space flight display major alterations in global gene expression (including a large number of genes encoding ion response pathways) and increased virulence. A central role was also identified for the conserved small regulatory ribonucleic acid (RNA)-binding protein Hfq in regulating key aspects of the space flight microgravity responses.

Nickerson and her team wanted to further the understanding of the effect of space flight on bacterial virulence and to test the hypothesis that ion concentrations could be manipulated to prevent the enhanced *Salmonella* virulence imparted during flight. They performed additional analyses on STS-115 with *S. typhimurium* cultured in M9 minimal media, and then on a follow-up shuttle flight opportunity on STS-123 that allowed direct comparisons between *Salmonella* cultured in LB, M9, and LB-M9 salts supplemented with inorganic ions to the same levels as those found in M9 medium (LB-M9 salts). As with space flight growth in LB media, *S. typhimurium* growth in M9 media displayed differential expression of many genes, including those associated with either the regulation of, or regulation by, the Hfq protein and small regulatory RNAs – thus suggesting conserved regulatory mechanisms for

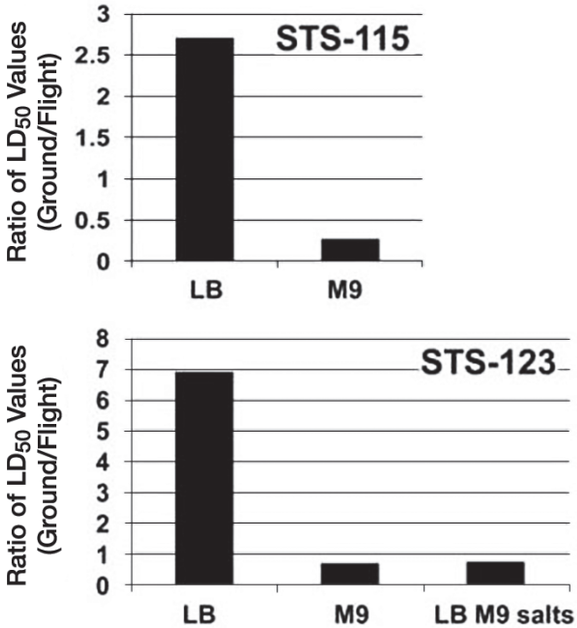


***Fig. 1.*** S. typhimurium *virulence in LB, M9, and LB-M9 salts.*

cellular responses to space flight. *S. typhimurium* grown in various media demonstrated that ion concentrations had a direct effect on the virulence of the cultures – beyond what would be expected in normal ground controls. When *S. typhimurium* was grown in LB medium on board STS-123, an evaluation of the median lethal dose ($LD_{50}$) values and time to death using a murine model of infection confirmed a space-flight-associated increase in virulence (figure 1). However, when *S. typhimurium* was grown in M9 media on this same flight, no increase in virulence was observed. To determine whether key inorganic salts in the M9 media were responsible for virulence changes during flight, additional cultures of *S. typhimurium* were grown in LB medium supplemented with five salts/ions that were in high concentration in the M9 medium (LB-M9 salts). *S. typhimurium* grown in LB-M9 salts had $LD_{50}$ and time-to-death values similar to those of cultures grown in M9 media, indicating no increase in virulence and suggesting that the ions played a key role in the space-flight-associated virulence effect. Subsequent space-flight-analog bioreactor studies indicate that higher concentrations of phosphate ions are a key in the mechanism for altering *S. typhimurium* responses to space flight.

This space flight experiment provides the first evidence of an environmental mechanism behind space-flight-associated virulence changes in *S. typhimurium*, imparts a breakthrough in our understanding of how NASA can mitigate infectious disease risk to a crew during space flight, and may be exploited to better understand microbial pathogenesis as well as to develop innovative therapeutics on Earth.



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