

tryptic digestion of the protein followed by peptide mapping using SELDI-TOFMS analysis of the peptides. The peptide map is then compared to an NIH-provided peptide map database for previously reported proteins.

Facilities

The Laboratory for Integrated Physiology (LIP), housed in the Department of Health and Human Performance at the University of Houston has a fully functional biochemistry laboratory in which the ISSO Post-Doctoral Aerospace Fellow will carry out specific elements of this study includ-

ing biochemical analysis. The Muscle Research Laboratory at NASA-Johnson Space Center is also a fully equipped biochemistry laboratory. Core facilities at NASA-JSC available for use in this project are a fully staffed animal facility (currently the home of the DFS-HLS rat model of muscle atrophy) and a proteomics core facility that is equipped with a SELDI-TOFMS analysis system and robotic work station to ease the task of preparing samples.

PRINCIPAL INVESTIGATORS

Mark S. Clarke, UH / Daniel L. Feedback, NASA-JSC

MICROORGANISMS ARE inevitable companions in human space exploration with the consequent risk of human disease. This risk increases if astronauts in space have suppressed immune systems, making them more susceptible to bacterial infection (Nefedov, Nickerson 1997).

Bacterial populations can impact manned missions in other ways. For example, the buildup of biofilms may damage or interfere with the performance of hardware, and changes in bacterial populations in advanced life support systems may interfere with biodegradation of waste or food production. The background radiation levels encountered on the International Space Station are approximately 70-100 times those seen on the Earth and the gravity vector approaches zero. A key risk is that such a doubly stressed environment may select for significant changes in the microorganisms themselves over the lifetime of an extended mission.

To begin to define the microbial risk associated with human space missions, efforts have been under way for some time to directly characterize the populations encountered in space. This characterization was first attempted on the U.S. Space Shuttle and the Russian Mir Space Station using culture-based techniques (LaRocco). More recently, researchers undertook detailed identification of culturable bacteria isolated from air, water, and surfaces on the International Space Station using 16S rRNA sequencing (Castro). The general observation is that Gram-positive bacteria such as *Staphylococcus*, *Micrococcus*, and *Bacillus* are most common in air and surface samples, whereas Gram negative bacteria are dominant in water samples. The surveys illustrate that the organisms most likely to be present are those that are routinely associated with humans and those which are found in spacecraft assembly environments. This is perhaps expected, but not comforting; as these are the organisms most like-

The Effects of Simulated Microgravity and Radiation on Microbial Gene Expression

ly to be able infect a human host if they develop novel virulent properties.

Preliminary Results

We have successfully employed a high aspect rotating vessel (HARV) bioreactor in the Pierson lab to study the response of *Escherichia coli* to modeled microgravity. The device minimizes fluid motion while maintaining culture aeration through a gas permeable membrane. The rotation also has the effect of randomizing the gravity vector, by rotating in the plane of gravity, producing a low shear modeled microgravity (LSMMG) environment. To obtain this environment, the HARV device is rotated at a speed sufficient to maintain cell suspension in the media and must be completely filled so that gas bubbles cannot cause solution turbulence (i.e., shear).

Our expression studies to date have revealed a substantial number of genes that are either up-regulated or down-regulated relative to controls in replicate experiments. While many of these genes are currently of unknown function, some of the genes with increased transcription in response to LSMMG are involved in the *E. coli* acid tolerance response system (transcriptional gene regulators [yhiE, yhiF] and the chaperones [hdeA, hdeB and hdeD), or are involved in cell motility (many flg and fli genes) or are chemotaxis regulating genes (cheZ and tar). The induction of acid tolerance response genes could indicate their involvement in a general *E. coli* stress response pathway. Increased transcription of the flagellar and chemotaxis genes in LSMMG, increasing cell mobility, may indicate that zones of low nutrients and high waste are occurring in the LSMMG HARV similar to those theorized to occur in space. These identified changes in bacterial LSMMG gene expression could lead to increased cell survival, virulence, and antibiotic resistances, indicating serious potential problems during long-term space flight.

Specific Aim

We hypothesize that long term simultaneous exposure to microgravity and elevated background radiation will lead to changes in gene and protein expression patterns in common bacteria. We will seek to determine in ground-based studies the extent to which these changes will occur and what genes are affected in a representative Gram-negative and Gram-positive bacteria and whether or not the changes are likely to be consequential with regard to other cellular activities.

Proposed Studies

We will monitor the immediate gene expression response to LSMMG in two additional cases. (1.) The first will be *E. coli* cells grown under anaerobic conditions using nitrate as the terminal electron acceptor. An initial study has previously been run but further replicates are needed to allow statistical verification of the results. The second will focus on the Gram positive model organism, *Bacillus subtilis*. This spore-forming organism is the best characterized Gram positive bacterium. Its complete genome sequence is available as are genomic expression arrays. These studies will allow us to compare the genes affected in three organisms (e.g., *Salmonella* (Wilson), in four distinct environments.

(2.) Gene expression response of LSMMG grown *E. coli* populations (aerobic and anaerobic) to a variety of stresses (acid, base, heat, antibiotic, H_2O_2 , $NaCl$, and Sucrose) will be examined and compared to $1 \times g$ HARV controls to gain insight as to whether the types of changes induced by LSMMG are likely to affect other cellular responses; e.g., pathogenesis.

(3.) We will conduct long-term studies (three-six months) to determine if *E. coli* cells continuously exposed to LSMMG—but not continuously growing—evolve a different gene expression pattern. The *E. coli* will suffer regular periods of nutrient depletion to allow a range of selection conditions. At the end of three months and six months, we will reexamine the response to acid and temperature shock.

(4.) A parallel set of long term experiments (three-six months) will be conducted using flask cultures continuously exposed to low levels of ionizing radiation. Bacteria will again be grown intermittently, with periods of nutrient depletion. At the three-month and six-month time points, the global patterns of gene expression will be determined and the responses to acid and temperature shock monitored.

(5.) Finally the experiments in (4) will be conducted with the simultaneous presence of LSMMG and radiation. The experiments in (4) and (5) will likely be run simultaneously.

Timeline

In the first six months, anaerobic studies on *E. coli* will be completed. The second six months will focus on *B. subtilis* and pilot studies of procedures to be used in radiation studies. Year Two will focus on simultaneous exposure to the stress of LSMMG and radiation and the effect of adaptation to such a double stressed environment on cellular response to common stimuli such as heat shock, acid tolerance, etc.

JSC Facilities

A Ph.D. appointed to a Post-Doctoral Aerospace Fellowship (PDAF) will work in the laboratory of Dr. Duane Pierson at JSC and will utilize HARV bioreactors which are routinely utilized in that laboratory. A JSC Cesium-137 source will be utilized in the initial radiation studies.

List of Works Cited

- Castro, V. A., A. N. Thrasher, M. Healy, C. M. Ott, and D. L. Pierson. "Microbial Diversity Aboard Space Craft: Evaluation of the Intern. Space Station," *Micro Ecol* (2004). (In press.)
- LaRocco, M. T. and D. L. Pierson. "Deep Space Exploration: Will We Be Ready?" *ASM News* 65 (1999): 817-82.
- Nefedov, Y. U. G., A. V. Yeregin, V. I. Drozdova, A. S. Skryabin, O. A. Guseva, and N. N. Mukhina. "Immunological Reactivity and Prediction of Allergic Complications in the Crew of the Second Expedition of Salyut 4," *Kosm Biol I Avikosm Med* 12 (1978): 15-29.
- Nickerson, C. A. and R. Curtis, III. "Role of Sigma Factor RpoS in Initial Stages of *Salmonella typhimurium* Infection," *Infect Immun* 65 (1997): 1814-23.
- Nickerson, C. A., C. M. Ott, S. J. Mister, B. J. Morrow, L. Burns-Keliher, and D. L. Pierson. "Microgravity as a Novel Environmental Signal Affecting *Salmonella enterica* serovar *typhimurium* virulence," *Infect Immun* 68 (2000): 3147-52.
- Wilson, J. W., R. Ramamurthy, S. Porwollik, M. McClelland, T. Hammond, P. Allen, C. M. Ott, D. L. Pierson, and C. A. Nickerson. "Microarray Analysis Identifies *Salmonella* Genes Belonging to the Low-Shear Modeled Microgravity Regulon," *Proc., Natl. Acad. Sci. U. S. A.* 99 (2002): 13807-12.

PRINCIPAL INVESTIGATORS

George E. Fox, UH / Richard C. Willson, UH / Duane L. Pierson, NASA-JSC