What is Space Biology Research?

Human Health Emphasis

Human Exploration Emphasis
- Exploration Subsystems
- Humans

Biological Systems Emphasis
- Small Organisms
  - Mice, Rats
- Tissues Organs
- Mammalian Cells

Model Organisms
- Microbes
- Plants

BioMolecules

David Tomko, Ph.D, Program Scientist
Space Biology
April 11, 2016
Space Life Sciences Recommendations for 2010-2020: NRC Decadal Study 2011

• **Plant and Microbial Biology**
  - Multigenerational studies
  - Responses to spaceflight
  - Plants and microbes in closed-loop life support

• **Animal and Human Physiology**
  - Bone and muscle studies
  - Drug/countermeasure evaluations
  - Vascular and interstitial pressure changes during spaceflight
  - Orthostatic intolerance
  - Deposition of aerosols in lung
  - T-cell and immune system studies
  - Multi-generation and early development

• **Cross-Cutting Issues for Humans in Space**
  - Artificial-G as a countermeasure
  - Animal studies to assess radiation risks
  - Cellular studies to define biomarkers for radiation toxicity
  - Understanding gender differences in adaptation to spaceflight

Cell culture and microbial culture incubator system (CGBA)

Micro 8 - Nielsen-Preiss
(Candida albicans – biofilms)

Micro-5 Nickerson
(C. elegans & Salmonella Videography upgrade)
Biological Research in Canisters (BRIC)  
Petri Dish Fixation Unit (PDFU)

- The BRIC-PDFU hardware facilitates Rapid Turn-Around payloads involving in-flight fixation & multiple NRA-selected PIs.
- Provides shorter period of time for PI to obtain flight opportunity and ultimately flight data for analysis and publication.

Injection of fluids into PDFU petri dishes:
A. Actuator Tool prior to attachment to BRIC-PDFU.
B. Actuator partially depressed to Liquid 1.
C. Completion of actuation allows Liquid 2 to be delivered.
Free Flyers

ECamSAT

SporeSat
**Microbial Observatory-1 Investigation**
**PI Grant Selected by SLPS in 2013**

**Investigation Name:** ISS Microbial Observatory – A Genetic Approach  
**PI:** Dr. Kasthuri Venkateswaran, NASA Jet Propulsion Laboratory  
**CoI's:** Duane Pierson (JSC), George Fox (UH), and Douglas Botkin (JSC)

**Objectives:**
1. Collect microbes on the ISS surfaces and in the air to characterize the types of microbial populations on the ISS  
2. Adapt molecular characterization technologies used in the Mars Program to elucidate a broad microbial diversity profile, which allows identification of unculturable microbes.  
3. Deliver the ISS-MO database that will be a compilation of genomic sequences and genetic information for all microorganisms encountered within the ISS habitable areas.

**Relevance/Impact:**
This investigation aims at characterizing the microbial communities in the air and on the surface on board the ISS. This study will provide NASA with tools to 1) quantify crew health and performance risks associated with human spaceflight and 2) develop countermeasures to provide mission planners and system developers with strategies for mitigating crew health and spacecraft performance risks. Finally, one of the aim is to develop a database to catalog all the microbial communities present on ISS. The study applies directly to 2011 Decadal Plant and Microbial Biology section and in particular the microbial observatory program (P1).

**Developer:** NASA Ames Research Center  
**Schedule:** SpaceX-5, -6, and -7
Summary Microbial Observatory-X

**Description and Objectives**

**Objective:** 1) Collect environmental samples onboard the ISS (surface, water, and Air) to characterize microbial communities, 2) Perform omics analyses to assess microbial composition and diversity, and 3) Populate “ISS-MO” database with phylogenetic information.

**Justification:**
This project responds to the 2011 NRC decadal survey highest priority recommendation P1 and to the 2010 Space Biology Science Plan-Cell, Microbial, and Molecular Biology. Previous studies have shown that a large diversity of microorganisms are present on the ISS. Therefore, further understanding of microbial composition and diversity onboard space habitats is critical to 1) better understand how the space environment affects population dynamics and survival, 2) have a better assessment of threats towards the health of immunocompromised astronauts and integrity of spacecraft materials, 3) develop suitable countermeasures to minimize opportunistic and technophilic microorganisms, and 4) maintain database of microbial communities present and the corresponding spatial and temporal variation on ISS.

•  SLPS Priority 1

**Approach**

The project will develop specific kits to collect biological samples onboard the ISS. Swabbing and specimen collection kits have previously flown. However new sampling devices might be tested, validated, and certified for flight. The crew will perform the biological sampling of surface, water, and the air onboard the ISS 4 times a year and return for analysis.

**Mission Timeline**

<table>
<thead>
<tr>
<th>Kickoff</th>
<th>On-Orbit Ops</th>
<th>Final Sp Rtn</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCR</td>
<td>SRR EVT FRR/FHA L</td>
<td>L-12 L-11 L-6 L-2 L L+12</td>
</tr>
</tbody>
</table>

**Collaborators & Roles:**

Space Biology, HRP, and International Partners

**Assumptions for Future Payloads:**

• One PI selected per Year
• One or multiple sessions on SpaceX
• Assumes 9 to 12 month development timeline depending on complexity
• Every other year includes a modified sampling device that would require testing, validation, and certification (12 month project)
High dimensional biology to understand functional response of Salmonella to long-term multigenerational growth in chronic stress of microgravity, PI C. Nickerson, AZ State U

**Science Objective/Goal:** Investigator team from ASU, JSC, Harvard and University of Minnesota will use Salmonella and observe its mutation rate and adaptation to spaceflight conditions over the course if ~300 generations. Team will evaluate transcriptomic, genomic, proteomic, and virulence changes over time. They will also compare wild type and hfq mutants. Samples will be serially diluted with an aliquot taken & saved every ~30 generation to get a nice longitudinal study.

**Expected Outcomes / Products of Research:**
- Characterization of the effects of space flight on long duration culturing of S. Typhimurium (WT and Δhfq strains) over multiple generations (0, ~10, ~150, and ~300 generations)
- Comprehensive system biology-based characterization of the generational specimens for genomic, epigenetic, transcriptomic, virulence, and pathogenesis-related characteristics.

**Implementation Challenges:**
- Multigenerational growth requires multiple manual subculturing sessions and 3 sampling sessions on-orbit
  - 30 subculture sessions
  - Sampling following 2\(^{nd}\), 15\(^{th}\), and 30\(^{th}\) subculturing sessions into RNAlater, PFA with wash, or glycerol
- Access to ISS and crew time availability

**Implementation Approach:**
Organism: *Salmonella Typhimurium* (WT and Δhfq strains)
H/W: BioServe FEP bags and Monovette syringes
No. Flights: 1
Duration: 30 days
GeneLab: Data Submission

**NRC 2011 Decadal Survey:**
- P1 & P2

**Experiment Status:**
Flight Definition Kick-off
Genelab – Understanding how Spaceflight Affects the Building Blocks of Life

- Maximize use of ISS biological research resources
  - Collect genomic, transcriptomic, proteomic, and metabolomics data (known as “omics”)
  - Enable exploration of the molecular network responses of terrestrial biology to the space environment (Translational Research)
  - Make all the data available to a worldwide network of researchers in an open-access database.

- The GeneLab project provides
  - Public bioinformatics repository for spaceflight omics data
  - Collaborative analysis platform for spaceflight omics data
  - Omics data focused on spaceflight and ground simulations of microgravity and radiation experiments
GeneLab Studies Omics To Support Open Science

Wu, RD et al. JDR 2011; 90:561-572

Genelab Database Enables Systems Biology Approach To Translate Spaceflight Science Results
## Genelab “Legacy” Database Sample Content

### 3 search results for "microbe"

**Candida albicans response to spaceflight (NASA STS-115)**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Factors</th>
<th>Assay Types</th>
<th>Release Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>gravity</td>
<td>transcription profiling</td>
<td>Nov-01-2013</td>
<td>This study presents the first global transcriptional profiling and phenotypic characterization of the major human opportunistic fungal pathogen, Candida albicans, grown in spaceflight conditions. Microarray analysis revealed that C. albicans...</td>
</tr>
</tbody>
</table>

**Transcriptional and proteomic response of Pseudomonas aeruginosa PAO1 to spaceflight conditions involves Hfq regulation and reveals a role for oxygen**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Factors</th>
<th>Assay Types</th>
<th>Release Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>simulated microgravity</td>
<td>transcription profiling</td>
<td>Jun-28-2011</td>
<td>Characterization of bacterial behavior in the microgravity environment of spaceflight is of importance towards risk assessment and prevention of infectious disease during long-term missions. Further, this research field reveals new insights...</td>
</tr>
</tbody>
</table>

**Salmonella Typhimurium transcription profiles in space flight**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Factors</th>
<th>Assay Types</th>
<th>Release Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enterica</td>
<td>gravity</td>
<td>transcription profiling</td>
<td>Sep-13-2007</td>
<td>Salmonella transcription profiles were obtained from samples flown on space shuttle mission STS-115, and compared to profiles from Salmonella grown under identical conditions on the ground. Keywords: stress response, transcriptional profile...</td>
</tr>
</tbody>
</table>

[https://genelab-data.ndc.nasa.gov/genelab/search_studies/?q=microbe](https://genelab-data.ndc.nasa.gov/genelab/search_studies/?q=microbe)
<table>
<thead>
<tr>
<th>Focus of Project</th>
<th>Number of Grants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inventory + Surveillance + tools</td>
<td>13</td>
</tr>
<tr>
<td>Microbial Adaptations to Spaceflight (Non Virulence Studies)</td>
<td>16</td>
</tr>
<tr>
<td>Microbial Adaptations to Spaceflight (Pathogenisis/Ab resistance)</td>
<td>17</td>
</tr>
<tr>
<td>Host/Microbe Relationships (Animals-commensals or protocooperatives)</td>
<td>7</td>
</tr>
<tr>
<td>Plant/Microbe Relationships</td>
<td>2</td>
</tr>
<tr>
<td>Viral Research</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>62</strong></td>
</tr>
<tr>
<td><strong>Total number of Pis</strong></td>
<td><strong>38</strong></td>
</tr>
</tbody>
</table>
Contact information and Internet Addresses

NASA Space Life and Physical Sciences Division of HEOMD
https://www.nasa.gov/directorates/heo/slpsra

Space Biology Program
https://www.nasa.gov/content/space-biology-program

Human Research Program
https://www.nasa.gov/hrp

Genelab Strategic Plan
http://genelab.nasa.gov/discovery-genelab-strategic-plan.html

NASA Taskbook
https://taskbook.nasaprs.com/Publication/welcome.cfm

David Tomko, 202-358-2211 dtomko@nasa.gov
BACK UP SLIDES
Recent Space Biology Solicitations

• Request for Information (RFI) on Developing a “NASA Space Life and Physical Sciences (SLPS) GeneLab Designed ISS Reference Mission” Released Nov 5, 2015; 36 Responses received by Feb 5, 2016 deadline
• Space Biology Omnibus NRA – “Research Opportunities in Space Biology” – Released March 24, 2016
• Appendix A – “GeneLab Innovation Awards for Systems Biology Informatics Research Using the GeneLab Data System” - Target Release March 24, 2016
• NASA Research Announcement - “Soliciting Research Proposals for Post-doctoral Fellowships to Study the Microbiome of the ISS as a Built Environment” – Target Release May 1, 2016
• Other Appendices during the year
2014 ILSRA Grant Awards: New ISS Research

- 10 proposals were selected by SLPS from the NRA, "Research Opportunities for Flight Experiments in Space Biology (ILSRA)."

- 3 of these grants are jointly-funded by Space Biology and HRP (blue highlight)

- 1 additional grant was awarded by HRP and is jointly-funded by Space Biology (green highlight)
  
  - Dr. Cheryl Nickerson, Arizona State University, "High Dimensional Biology to Understand the Functional Response of Salmonella to Long-Term Multigenerational Growth in the Chronic Stress of Microgravity"
  
  - Dr. Crystal Jaing, Lawrence Livermore National Security, LLC, "International Space Station Microbial Observatory of Pathogenic Virus, Bacteria, and Fungi (ISS-MOP) Project"
  
  - Dr. Gioia Massa, NASA Kennedy Space Center, "Pick-and-Eat Salad-Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System"
  
  - Dr. Fred Turek, Northwestern University-Evanston, "Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms and Impact on Multi-System Physiology"
  
  - Dr. Alexander Robling, Indiana University, "Foundational in-vivo Experiments on Osteocyte Biology in Space"
  
  - Dr. Scot Wolverton, Ohio Wesleyan University, "Characterizing Plant Gravity Perception Systems"
  
  - Dr. Russell Turner, Oregon State University, "Spaceflight-Induced Changes in Non-Shivering Thermogenesis and Effects on Bone in Mice"
  
  - Dr. Siva Vanapalli, Texas Tech University-Lubbock, "Determining Muscle Strength in Space-Flown Caenorhabditis elegans"
  
  - Prof. Norman Lewis, Washington State University-Pullman, "An Integrated Omics Guided Approach to Lignification and Gravitational Responses: The Final Frontier"
  
  - Dr. Grace Douglas, NASA Johnson Space Center, "The Integrated Impact of Diet on Human Immune Response, the Gut Microbiota, and Nutritional Status during Adaptation to Spaceflight"
Global Transcriptome Profiling to Identify Cellular Stress Mechanisms Responsible for Spaceflight-Induced Antibiotic Resistance (BRIC 21)

PI: Wayne Nicholson, University of Florida

Science

- **Hypothesis:** The underlying stress response mechanisms leading to alterations in cells’ antibiotic susceptibility can be identified using transcriptome profiling of model organisms.
- **Goals:** Study spaceflight stress response at the genomic level by 1) exposing model bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* to spaceflight, 2) analyzing cultivated cells using transcriptome and phenotypic methods, 3) extracting total RNA and conducting “RNA-Seq” to identify the suite of stress responses induced by exposure to spaceflight.

Hardware Performance

- BRIC-PDFU canisters and standard PDFUs performed nominally on ISS.

Status/Results

- All BRICs currently frozen in MELFI awaiting return for PI analysis.

Actuator Tool + Rod

Rod Kit

Exploded view of BRIC Canister.

BRIC 21 Canister being actuated on ISS.
**PI:** Cheryl Nickerson, Arizona State University  
**Co-Is:** John Alverdy, University of Chicago; C. Mark Ott, NASA, JSC; Catherine Conley, NASA, HQ.  
**PS:** Macarena Parra  
**PM:** Jake Freeman (BioServe Technologies)  
**Engineering Team:** BioServe Technologies

**Objective:**
1) Determine the effect of spaceflight on the host-pathogen interaction in real time as a function of media ion composition when both *C. elegans* (host) and *S. typhimurium* (pathogen) are simultaneously exposed to spaceflight.
2) Determine the evolutionarily conserved role for spaceflight-responsive RNA binding proteins in both *C. elegans* and *S. typhimurium* as a function of media ion composition before and after infection when both the host and the pathogen are simultaneously exposed to spaceflight.
3) Evaluate the use of phosphate and Polyethylene Glycol as a nutritional countermeasures to protect *C. elegans* against *S. typhimurium* induced lethality as a function of media ion composition when both host and pathogen are simultaneously exposed to spaceflight.

**Relevance/Impact:**
This experiment responds to the 2011 NRC Decadal Survey highest priority recommendation P2 and the 2010 Space Biosciences Roadmap – Cell, Microbial, and Molecular Biology. Previous studies show that pathogens are more virulent in microgravity while host immune systems are compromised. Therefore, further understanding the host-pathogen interactions in the spaceflight environment are critical to our ability to treat infections during long term spaceflight. This is the first study to examine host pathogen interactions during an on-orbit infection.

**Development Approach:**
1) Experiment development and biocompatibility testing in the hardware are complete
2) Testing to define and verify optimal storage conditions for samples
3) Integrated tests using the flight hardware
4) Experiment Verification Test to verify procedures and hardware settings (risk mitigation)
5) Facility Trail Run at KSC to verify supplied space and equipment will support the pre-flight operations

**Instrumentation & Experiment Summary**
1) Pre-flight, bacteria and nematodes are loaded in separate hardware compartments in stasis.
2) On orbit, both organisms are activated with growth media and allowed to grow/recover.
3) The Commercial Generic Bioprocessing Apparatus (CGBA) is used to provide temperature control and video capabilities.
4) Bacteria (control and Salmonella) are used to infect the nematodes and viability is tracked using video from a scanning camera system.
5) At predetermined time points the hardware is removed and samples are withdrawn for microscopy (fixed with Paraformaldehyde) and RNA studies (fixed with RNALater).

<table>
<thead>
<tr>
<th>Accommodation (carrier)</th>
<th>CGBA on ISS</th>
</tr>
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<tbody>
<tr>
<td><strong>Upmass (kg)</strong></td>
<td>25 kg</td>
</tr>
<tr>
<td>(w/o packing factor)</td>
<td></td>
</tr>
<tr>
<td><strong>Volume (m²)</strong></td>
<td>1 MLE</td>
</tr>
<tr>
<td>(w/o packing factor)</td>
<td></td>
</tr>
<tr>
<td><strong>Power (kw)</strong></td>
<td>0.090 kW (ascent)</td>
</tr>
<tr>
<td>(peak)</td>
<td></td>
</tr>
<tr>
<td><strong>Crew Time (hrs)</strong></td>
<td>10 hrs</td>
</tr>
<tr>
<td><strong>Autonomous Ops (hrs)</strong></td>
<td>Approximately 24 hr</td>
</tr>
<tr>
<td><strong>Launch/Increment</strong></td>
<td>SpX-3; Nov. 28, 2013 (returned on SpX-3)</td>
</tr>
</tbody>
</table>