Microbiology and the International Space Station

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Microbiological Monitoring on the ISS

Surfaces

Air

Water

Quantified in-flight and returned to JSC for identification
ISS Air and Surface Monitoring
Fungal Isolates

ISS Air and Surface Monitoring
Bacterial Isolates

Adverse Effects of Microorganisms

- Biodegradation
- Systems failure
- Food spoilage
- Release of volatiles

“(fungi) feeding behind control panels, slowly digesting the ship’s air conditioner, communications unit, and myriad other surfaces.”

Gareth Cook, Boston Globe Staff (10-1-00)
Microbial Mutation and Evolution

• Craig Everroad, NASA Ames Research Center
  – Experimental Evolution of *Bacillus subtilis* Populations in Space; Mutation, Selection and Population Dynamics

• Wayne Nicholson, University of Florida
  – Global Transcriptome Profiling to Identify Cellular Stress Mechanisms Responsible for Spaceflight-Induced Antibiotic Resistance

• 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
Biofilm Studies

• Robert McLean, Texas State
  – Polymicrobial Biofilm Growth and Control during Spaceflight

• Luis Zea, University Colorado, Boulder
  – Characterization of Biofilm Formation, Growth, and Gene Expression on Different Materials and Environmental Conditions in Microgravity
Human Health

- Cheryl Nickerson, Arizona State University
  - Investigation of Host-Pathogen Interactions, Conserved Cellular Responses, and Countermeasure Efficacy During Spaceflight using the Human Surrogate Model *Caenorhabditis elegans*

- Clay Wang, University of Southern California
  - Influence of Microgravity on the Production of *Aspergillus* Secondary Metabolites (IMPAS) - a Novel Drug Discovery Approach with Potential Benefits to Astronauts’ Health

- Sheila Nielsen, Montana State University
  - Genotypic and phenotypic responses of *Candida albicans* to spaceflight

- 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
Human and Environmental Microbiomes

- Hernan Lorenzi, J. Craig Venter Institute
  - Study of the Impact of Long-Term Space Travel on the Astronauts' Microbiome
- Fred Turek, Northwestern
  - Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms and Impact on Multi-System Physiology
- Crystal Jiang, Lawrence Livermore National Laboratory
  - International Space Station, Microbial Observatory of Pathogenic Virus, Bacteria, and Fungi (ISS-MOP) Project
- Kasturi Venkateswaran, NASA Jet Propulsion Laboratory
  - ISS Microbial Observatory - a Genetic Approach
  - Bacterial, Archaeal, & Fungal Diversity of the ISS--HEPA Filter System
The International Space Station: A Research Platform to Understand Environmental and Human Microbiomes

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Debbie Aldape
Audry Almengor, Ph.D.
Bekki Bruce
Victoria Castro
Christian Castro
Brandon Dunbar
Todd Elliott
Tanner Hamilton

Jane McCourt
Cherie Oubre, Ph.D.
Duane Pierson, Ph.D.
Joan Robertson
Melanie Smith
Sarah Stahl
Sarah Wallace, Ph.D.
Prevention
Vehicle Design Controls

- HEPA air filters
- In-line water filters
- Contamination resistant surfaces
- Water biocides
- Water pasteurization systems
- Minimize condensation
- Contain trash and human waste
## Operational Controls

### Health Stabilization Program

<table>
<thead>
<tr>
<th>Mission</th>
<th>Illness (Crew)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apollo 7</td>
<td>Upper respiratory infection (3)</td>
</tr>
<tr>
<td>Apollo 8</td>
<td>Viral gastroenteritis (3)</td>
</tr>
<tr>
<td>Apollo 9</td>
<td>Upper respiratory infection (3)</td>
</tr>
<tr>
<td>Apollo 10</td>
<td>Upper respiratory infection (2)</td>
</tr>
<tr>
<td>Apollo 11</td>
<td></td>
</tr>
<tr>
<td>Apollo 12</td>
<td>Skin infection (2)</td>
</tr>
<tr>
<td>Apollo 13</td>
<td>Rubella (1)</td>
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<tr>
<td>Apollo 14</td>
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<tr>
<td>Apollo 15</td>
<td></td>
</tr>
<tr>
<td>Apollo 16</td>
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</tr>
<tr>
<td>Apollo 17</td>
<td>Skin infection (1)</td>
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<tr>
<td>Skylab-2</td>
<td></td>
</tr>
<tr>
<td>Skylab-3</td>
<td>Skin infection (2)</td>
</tr>
<tr>
<td>Skylab-4</td>
<td>Skin infection (2)</td>
</tr>
</tbody>
</table>

*Billica, Pool, Nicogossian, *Space Physiology and Medicine*, 1994
Preflight Microbiological Monitoring

- Crewmembers
- Food
- Potable water
- Vehicle surfaces
- Vehicle air
- Cargo
- Biosafety review of payloads
Acceptability Limits

**Air**
- Total bacteria: 1,000 CFU/m³
- Total fungi: 100 CFU/m³

**Surfaces**
- Total bacteria: 10,000 CFU/100 cm²
- Total fungi: 100 CFU/100 cm²

**Water**
- Heterotrophic plate count: 50 CFU/ml
- Total coliform bacteria: Not detected in 100 ml
Preflight Monitoring Synopsis

- Few reported clinical infections
  - Dermatitis
  - Urinary tract infection
  - Upper respiratory infection
- Common environmental flora*
- Opportunistic pathogens*
  - *Burkholderia cepacia*
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus*

Disqualified Food Samples
International Space Station (ISS)

Freeze dried shrimp  
Salmonella enterica serovar Typhimurium

Oatmeal with raisins  
Aspergillus flavus

Miso soup  
Staphylococcus aureus

Berry medley  
Total aerobic (TNTC) - Bacillus species

Chicken Pineapple salad  
Enterobacter cloacae

Freeze dried chopped pecans  
Aspergillus fumigatus, Penicillium species

Freeze dried corn  
Klebsiella pneumoniae, Enterobacter cloacae

San Francisco seasoning  
Total aerobic (TNTC) - Bacillus species

Onion medley seasoning  
Total aerobic (TNTC) - Bacillus species

Almond M&Ms  
Yeast species

Japanese sugar candy  
Yeast species

Trail mix  
Aspergillus niger, Aspergillus fumigatus

Chicken salad  
Enterobacter cloacae, Enterobacter intermedius, Pantoea agglomerans
Contamination Potential

Preflight contamination

Spacecraft are complex (cluttered)

Astronaut activities, such as eating and hygiene
Microbial Monitoring during Spaceflight

• Safety concerns
• Minimal
  – Power
  – Weight
  – Volume
  – Crew Time
• No phase separation
Microbiological Monitoring of Water
U. S. Potable Water Dispenser

- Provides “hot” and “ambient” potable water
- Processing includes:
  - Catalytic oxidizer
  - Iodine disinfection
  - In-line filter (0.2 micron)
- Common isolates
  - Ralstonia pickettii
  - Burkholderia multivorans
  - Sphingomonas sanguinis
  - Cupriavidas metallidurans
Staphylococcus aureus

- No Methicillin Resistant *S. aureus* (MRSA) have been recovered from ISS
- 48% of coagulase negative staphylococci were methicillin resistant
- **Blue** - isolated from the crew of ISS-5, the crew of ISS-4, and in-flight environmental isolates
- **Green** - isolated from the crews of ISS-1, ISS-4, and ISS-5
- **Red** - isolated from the crew of ISS-1 and ISS-4 and from an in-flight environmental surface
Wolf spent several hours working with Vinogradov to mop up a basketball-size drop of water…“I didn’t realize I bought myself anywhere from two to six hours per day doing this for the rest of the mission.”

*From DRAGONFLY by Bryan Burrough*
MIR Condensate Samples

NASA 6 “Slimy”
- **Fungi**
  - Acremonium species
  - Candida guilliermondii
  - Candida krusei
  - Cladosporium species
  - Fusarium species
  - Penicillium species
  - Rhodotorula rubra

- **Bacteria**
  - Alcaligenes eutrophus
  - Alcaligenes latus

**Escherichia coli**
- Enterobacter agglomerans
- Escherichia coli
- Hydrogenophaga flava
- Kingella denitrificans
- Methylbacterium species
- Pseudomonas vesicularis
- Serratia liquefaciens
- Stentrophomonas maltophilia


NASA 7 “Slimy”
- **Fungi**
  - Acremonium species
  - Candida guilliermondii
  - Candida krusei
  - Cladosporium species
  - Fusarium species
  - Penicillium species
  - Rhodotorula rubra

- **Bacteria**
  - Alcaligenes faecalis
  - Bacillus species
  - Bacillus circulan
  - Bacillus coagulans
  - Bacillus licheniformis
  - Bacillus pumilis
  - Citrobacter braackii
  - Citrobacter freundii
  - Comamonas acidovorans
  - Corynebacterium species
  - Flavobacterium meningosepticum

Legionella species
- Enterobacteria科
- Legionella species
- Pseudomonas species
- Rhodococcus species
- Serratia liquefaciens
- Serratia marcescens
- Sphingobacterium thalpophilum
- Yersinia frederiksenii
- Yersinia intermedia

NASA 7 “Fresh”
- **Fungi**
  - Acremonium species
  - Candida guilliermondii
  - Candida krusei
  - Cladosporium species
  - Fusarium species
  - Penicillium species
  - Rhodotorula rubra

- **Bacteria**
  - Bacillus coagulans
  - Bacillus licheniformis
  - Bacillus pumilus
MIR Condensate Residents

Spirochetes

Dust Mites

Ciliated Protozoa

“Establish a “microbial observatory” program on the ISS”
– National Research Council
Multiple experiments over the past 50 years indicate unique microbial responses when cultured during spaceflight. The environmental stimulus/stimuli initiating the response mechanisms are unclear. The vast majority of microbial ecology data is based on media-based analysis. The impact of radiation on microbial responses/mutational rates is not known.
Ground-based Analogue
The Rotating Wall Vessel (RWV)

- Solid body rotation in the reactor simulates several aspects of culture in microgravity
- Enables relatively high throughput
- Provides good indicators for spaceflight experiments
- Capability to follow up spaceflight findings without the delays associated with true spaceflight experiments

The low shear culture conditions has initiated the term Low Shear Modeled Microgravity (LSMMG) environment
Microgravity Analogue Model Control

(A) Analogue

Gravity

(B) Control

Rotation
Microgravity Analogue Model Results

Rotating Wall Vessel bioreactor

Salmonella typhimurium

Virulence

Gene expression

Stress Resistance
(Macrophage, acid, thermal, osmotic, oxidative)

Unique Microbial Responses

• Investigations by Dr. Cheryl Nickerson at Arizona State University evaluating microbial gene expression, morphology, and virulence

MICROBE
Shuttle Atlantis, STS-115, launch September 2006
Salmonella Typhimurium experiment design

* Synchronous ground controls maintained under identical conditions as those on-board Shuttle - ground and in-flight hardware loaded with same sample.

Wilson et al. Proc Natl Acad Sci USA 2007
Salmonella Typhimurium Response to Spaceflight Culture

- In-flight grown S. Typhimurium showed the presence of an extracellular material not seen in ground control
- In-flight grown S. Typhimurium grown in LB broth killed mice faster and killed mice at lower doses than identical bacterial cultures grown on the ground
- LD<sub>50</sub> was decreased 2.7 fold

Wilson et al. Proc Natl Acad Sci USA 2007
Spaceflight Globally Alters S. Typhimurium Gene Expression

Microarray Analysis identified 167 genes differentially regulated by spaceflight
- Protein secretion
- Outer membrane proteins
- Iron metabolism and storage
- **Ion response pathways**
- Plasmid transfer functions
- Energy and metabolism
- Ribosomal proteins
- Small regulatory RNAs
- Biofilm formation
- Transcriptional regulators
- Unknown function

Global Proteomic Profiling (MudPIT) identified 73 proteins differentially regulated by spaceflight

**Hfq - Master molecular regulator identified**

*Wilson et al. Proc Natl Acad Sci USA 2007*
* Synchronous ground controls maintained under identical conditions as those on-board Shuttle - ground and in-flight hardware loaded with same sample.

MDRV
Shuttle Endeavour, STS-123, launch March 2008
Salmonella Typhimurium experiment design

In-flight hardware

Disease potential

Independent validation of the STS-115 results

Media composition

Wilson et al. PLOS One 2008
Using Lennox Broth, the $LD_{50}$ in the second spaceflight experiment again decreased (6.9 fold).

This trend did not occur when M9 media was used or when the Lennox Broth media was supplemented with the inorganic ions used in M9 media.
## Is there a Spaceflight Contribution?

<table>
<thead>
<tr>
<th>Media</th>
<th>Growth Location</th>
<th>LD$_{50}$ (CFU)</th>
<th>Fold Increase Relative to LB Media - Flight</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB media</td>
<td>Flight</td>
<td>5.81 x 10$^4$</td>
<td>1.0</td>
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<tr>
<td>LB-M9 salts media</td>
<td>Flight</td>
<td>7.45 x 10$^5$</td>
<td>12.8</td>
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<tr>
<td>M9 media</td>
<td>Flight</td>
<td>3.30 x 10$^6$</td>
<td>56.8</td>
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</table>

<table>
<thead>
<tr>
<th>Media</th>
<th>Growth Location</th>
<th>LD$_{50}$ (CFU)</th>
<th>Fold Increase Relative to LB Media - Ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB Media</td>
<td>Ground</td>
<td>4.02 x 10$^5$</td>
<td>1.0</td>
</tr>
<tr>
<td>LB-M9 salts media</td>
<td>Ground</td>
<td>5.73 x 10$^5$</td>
<td>1.4</td>
</tr>
<tr>
<td>M9 media</td>
<td>Ground</td>
<td>2.30 x 10$^6$</td>
<td>5.7</td>
</tr>
</tbody>
</table>

*Wilson et al. PLOS One 2008*
Which component of the media?

Increased phosphate ion concentration prevents altered *S. typhimurium* acid tolerance in analogue culture.

Spaceflight data supplemented with ground-based model

Wilson *et al.* PLOS One 2008
Summary *Salmonella* Typhimurium

- **Virulence**
- **Stress Resistance and Biofilm formation**
- **Gene Expression**
  - 167 genes
  - Virulence genes down
  - *Hfq* - evolutionarily conserved low fluid shear response regulator found in other microorganisms

**Modulate ion levels**
- Phosphate controls low fluid shear-associated pathogenesis response

**RWV Bioreactor**
- Spaceflight

**Summary Pseudomonas aeruginosa**

- **Stress Resistance**
- **Biofilm production**
- **Gene Expression**
- **Production virulence factors**

**Pseudomonas aeruginosa**

- **RWV Bioreactor**
- **Spaceflight**

**Hfq** - evolutionarily conserved low fluid shear response regulator in other pathogens

Summary *Staphylococcus aureus*

- **Gene Expression**
- **Stress Resistance**
- **EPS production**
- **Carotenoid Production (Virulence Factor)**
- **Gene Expression**

*Hfq* - evolutionarily conserved low fluid shear response regulator in other pathogens


*Staphylococcus aureus*
Current Studies in Microbiology

- **Astronaut Microbiome**
  - *Hernan Lorenzi, J. Craig Venter Institute*
  - Designed to gather information on changes in the crew microbiome during a spaceflight mission.
  - Investigation will include preflight, in-flight, and post-flight samples from 9 astronauts.
  - Tightly monitored conditions (e.g., temperature, humidity, diet)
Current Studies in Microbiology

• Latent viral reactivation in crewmembers
  – Dr. Duane Pierson, NASA
  – A series of experiments investigating the reactivation of Epstein Barr Virus (EBV), Cytomegalovirus, and Varicella Zoster Virus (VZV) in crewmembers during a mission
  – Increased concentrations of EBV and VZV in astronaut saliva during a mission
  – VZV can reactivate subclinically in healthy individuals after acute stress.
The ISS as a Microbial Observatory

- The ISS is a semi-closed, well controlled research platform advancing our ability to mitigate microbiological risk to the crew and their vehicle enabling space exploration.

- The unique research enabled by access to space provides novel insight into our scientific understanding of life on Earth.
## The Risk of Astronaut Infection

### Positives
- Preflight medical exams
- Preflight crew quarantine
- Stringent microbiological monitoring
- Limited exposure to many public health pathogens
- Healthy, well-conditioned crew
- Medical consult throughout a mission

### Negatives
- Small enclosed environment
- Recycled air/water
- Stressful conditions
- Dysfunctional aspects of the immune system
- Altered microbial characteristics, including virulence
- Limited diagnostics and treatment on board
- Limited remediation capabilities
Infectious Disease during Spaceflight

- Upper respiratory infections
- Ear infections
- Various fungal infections
- Herpes Zoster
- Gastroenteritis
- Stye
- Allergic reactions
- Rashes & skin disorders