Spacecraft Microbiology of Human Exploration Missions

Current Knowledge and Research Activities

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Adverse Effects of Microorganisms

Timbury,et al, 2004

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

Prevention is Primary Microbial Control

Control using Vehicle Design

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

Control using Operational Activities

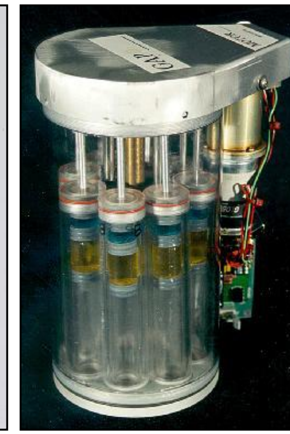
Health Stabilization Program

Mission	Illness (Crew)
Apollo 7	Upper respiratory infection (3)
Apollo 8	Viral gastroenteritis (3)
Apollo 9	Upper respiratory infection (3)
Apollo 10	Upper respiratory infection (2)
Apollo 11	
Apollo 12	Skin infection (2)
Apollo 13	Rubella (1)
Apollo 14	
Apollo 15	
Apollo 16	
Apollo 17	Skin infection (1)
Skylab-2	
Skylab-3	Skin infection (2)
Skylab-4	Skin infection (2)

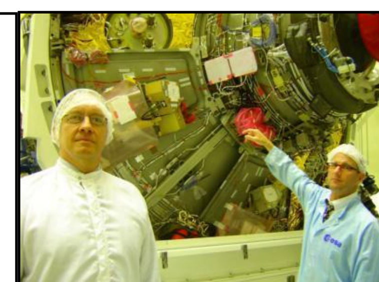
*Billica, Pool, Nicogossian, *Space Physiology and Medicine*, 1994

Preflight Microbiological Monitoring

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



Common microbiological monitoring requirements are employed by all international partners and commercial organizations carrying NASA crewmembers



Microbiological Requirements

Air	
• Total bacteria	1,000 CFU/m^3
• Total fungi	100 CFU/m^3
Surfaces	
• Total bacteria	10,000 CFU/100 cm^2
• Total fungi	100 CFU/100 cm^2
Water	
• Heterotrophic plate count	50 CFU/ml
• Total coliform bacteria	Not detected in 100 ml

Remediation is initiated during spaceflight missions if monitoring indicates microbial concentrations are above these conservatively low levels or if medically significant microorganisms are detected.

Preflight Monitoring Synopsis

- Common environmental flora isolated from vehicle surfaces, air, cargo, and potable water*
  - Occasional opportunistic pathogens
    - *Burkholderia cepacia*
    - *Pseudomonas aeruginosa*
    - *Staphylococcus aureus*
- Isolation of pathogens from the food is uncommon, but if found, does results in disqualification of the lot
  - Previous microbial contaminants include:
    - *Salmonella enterica* serovar Typhimurium
    - *Enterobacter cloacae*
    - *Staphylococcus aureus*

*Castro, *et al, Microb Ecol* 2004

Contamination Potential

Preflight contamination

Complexity of spacecraft environments

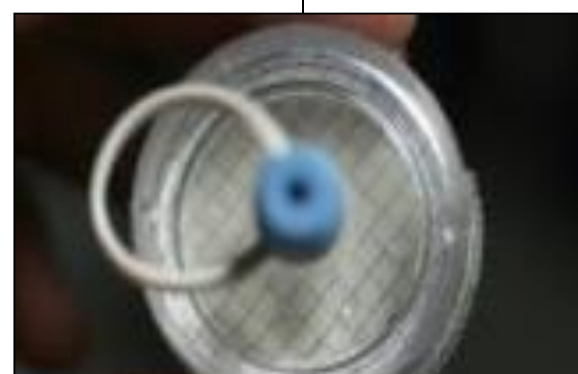
Astronaut activities, such as eating and hygiene

Microbiological Monitoring on the ISS

Surfaces

Air

Water

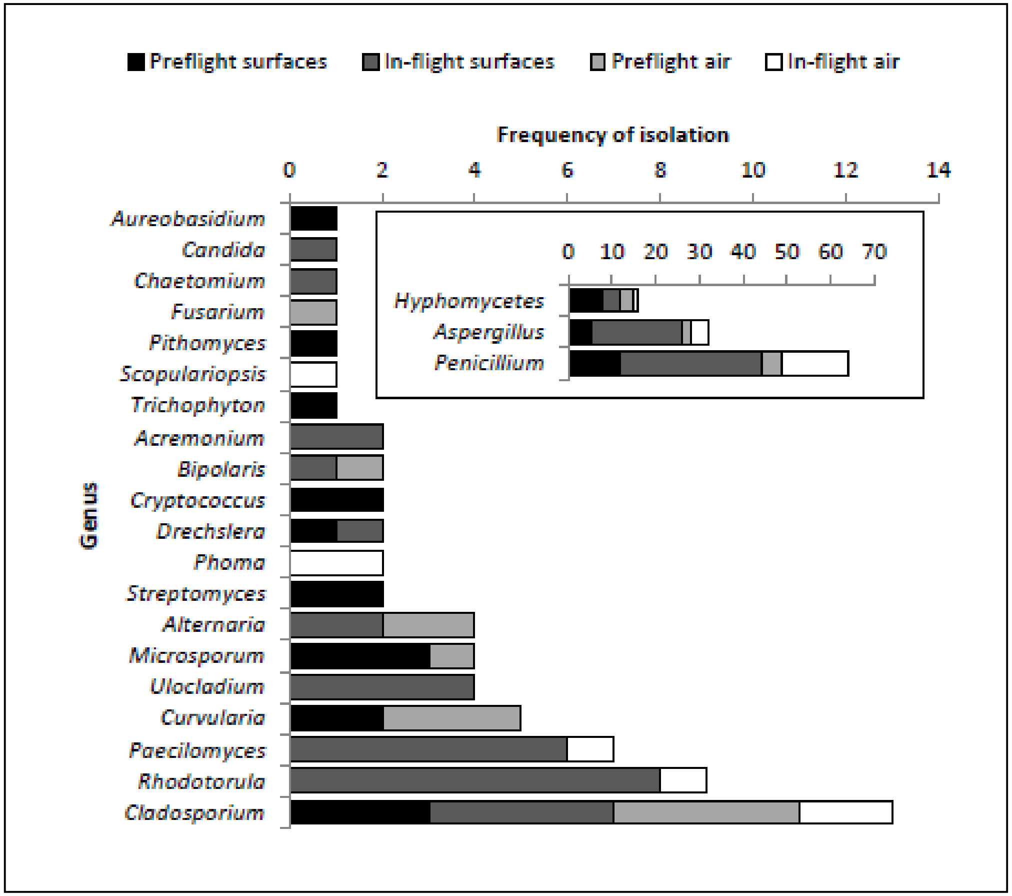


Quantified in-flight and returned to JSC for identification

Microbiological Monitoring of Water

ISS Air and Surface Monitoring

Fungal Isolates over the First 10 Years



These microorganisms reflect a healthy environment similar to a home or office.

Pierson, *et. al*. *Environmental Monitoring: A Comprehensive Handbook* 2012

ISS Air and Surface Monitoring

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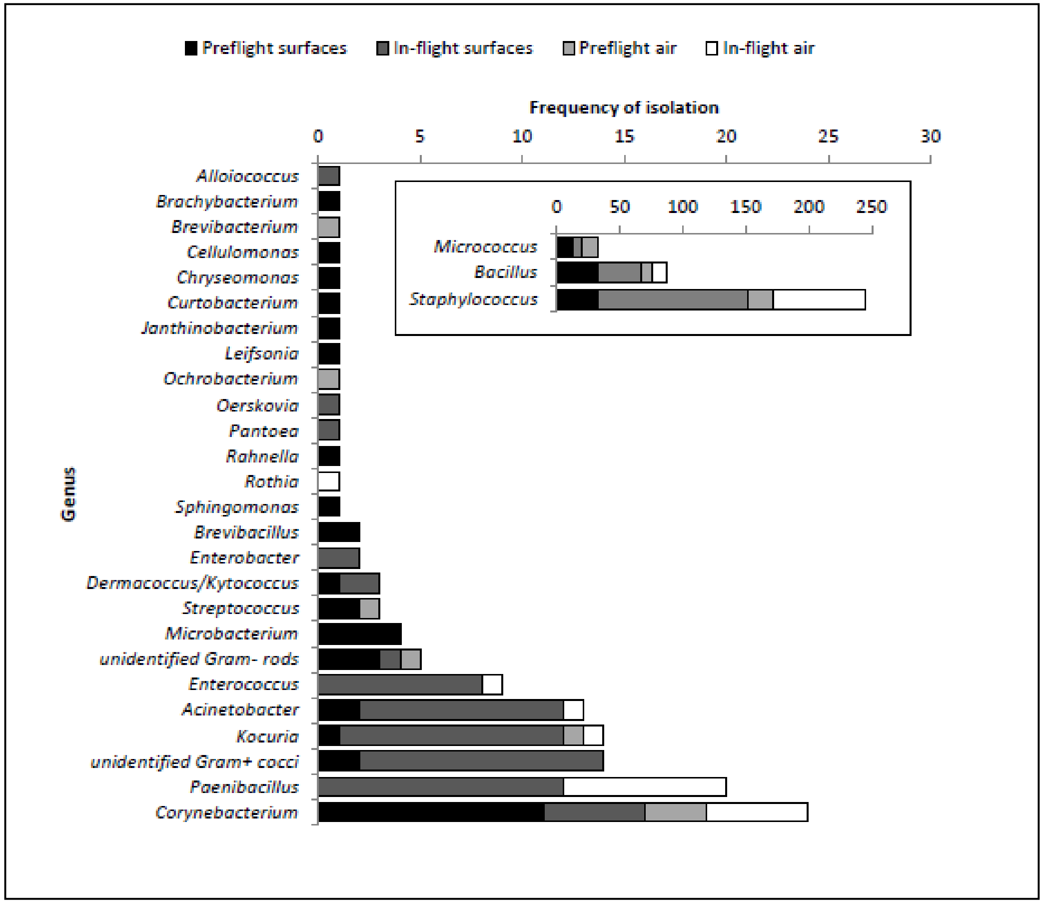

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U. S. Potable Water Dispenser

- Provides "hot" and "ambient" potable water
- Processing includes:
  - Catalytic oxidizer
  - Iodine disinfection
  - In-line filter (0.2 micron)
- No medically significant organisms have been isolated
- Commonly isolated
  - *Ralstonia pickettii*
  - *Burkholderia multivorans*
  - *Sphingomonas sanguinis*
  - *Cupriavidas metallidurans*

Operational Monitoring Summary

- Current preventative controls generally maintain microorganisms at relatively low levels providing a healthy environment aboard ISS after 15 years of habitation.
- The microorganisms isolated from ISS are common environmental organisms similar to those found in terrestrial homes.
- Contamination events do occur, often from uncontrolled water sources, such as condensate formation and crew hygiene activities.

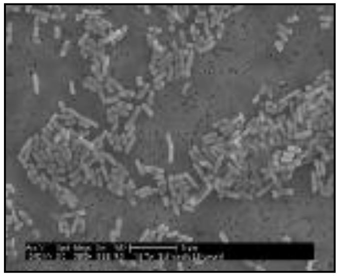
“Establish a “microbial observatory” program on the ISS”
– National Research Council

Microbiological Spaceflight Research

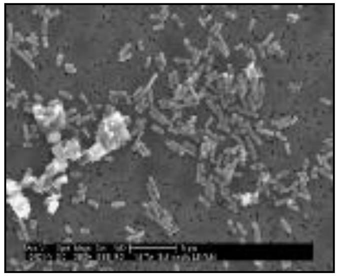
- The vast majority of the historic data describing microorganisms in the environment and from the crew is based on media-based analyses.
- Multiple experiments over the past 50 years indicate unique microbial responses when cultured during spaceflight.
- The environmental stimulus/stimuli initiating the response mechanisms are unclear.

Salmonella Typhimurim

- The virulence, gene expression, and morphology of *S*. Typhimurium were compared between spaceflight cultures and otherwise identical ground controls
  - *PI: Dr. Cheryl Nickerson, Arizona State University*
- Virulence
  - Virulence of spaceflight grown *S*. Typhimurium increased 2.7 fold (STS-115) and 6.9 fold (STS-123)
  - Addition of inorganic ions to the medium negated this effect
- Gene Expression
  - 167 globally distributed genes were differentially regulated
  - Many genes traditionally associated with virulence were down-regulated
  - The global gene regulator, Hfq, was associated with the transcription of many of the identified genes. Notably, *hfq* was down-regulated
- Biofilm Formation
  - *S*. Typhimurium cultured in the spaceflight environment displayed greater levels of extracellular polymeric substances reminiscent of biofilm formation



Ground Control

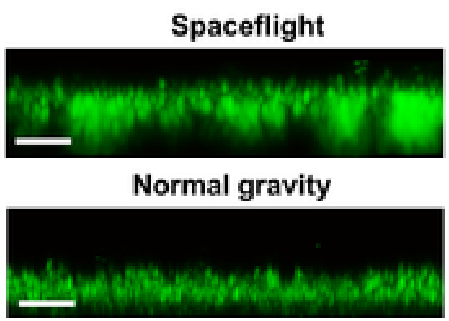


Flight Sample

Wilson *et al. Proc Natl Acad Sci USA* 2007; Wilson *et al. PLOS One* 2008

Pseudomonas aeruginosa

- A NASA experiment aboard STS-115 compared gene expression of *P. aeruginosa* between spaceflight cultures and otherwise identical ground controls.
  - *PI: Cheryl Nickerson Ph.D., Arizona State University*
  - 167 globally distributed genes were differentially regulated
  - The global gene regulator, Hfq, was again associated with the transcription of many of the identified genes. Notably, as with *S*. Typhimurium, *hfq* was down-regulated
- NASA spaceflight experiments aboard STS-132 and STS-135 evaluated biofilm formation by *P. aeruginosa*.
  - *PI: Cynthia Collins Ph.D., Rensselaer Polytechnic Institute*
  - Biofilms formed during spaceflight exhibited a previously unseen architecture described as "column-and-canopy"
  - This biofilm formation was independent of carbon source and phosphate concentrations; however, it was dependent on flagella-driven motility



Crabbe *et al. Appl Environ Microbial* 2011; Kim *et al. PLOS One* 2013; Kim *et al. BMC Microbial* 2013

Microorganisms in the Environment

- Bacterial, Archaeal, & Fungal Diversity of the ISS HEPA Filter System
  - *PI: Kasthuri Venkateswaran, NASA Jet Propulsion Laboratory*
  - This study identifies microorganisms and genetic biosignatures from HEPA filters collected from the ISS and compares them to similar systems on Earth
  - Early results strongly support the culture-based evidence that human skin-associated microorganisms make a substantial contribution to the ISS microbiome.
  - The ISS findings differ from analysis of similar filters of cleanrooms, where the impact of human skin flora is not as prominent.
- Microbial Tracking – 1
  - *PI: Kasthuri Venkateswaran, NASA Jet Propulsion Laboratory*
  - The microbial diversity of the ISS environment (air and surfaces) is being analyzed using next-generation technology, including16S metagenomic analysis, and compared to traditional culture-based methodologies.
  - This study should provide genomic sequences and genetic information for many microorganisms that have not been previously identified aboard ISS.

Checinska *et al. Microbiome* 2015

Microorganisms in the Environment

- Microbial Tracking – 2
  - *PI: Crystal Jiang, Lawrence Livermore National Lab*
  - This study compares traditional methodologies and state-of-the art molecular techniques to measure viral, bacterial, and fungal diversity associated with ISS environmental and associated crew samples.
  - By incorporating a novel Virulence and Antibiotic Resistance gene array, this experiment serves to both better understand the spacecraft environment and identify potential threats to the crew.
- Russian Joint Research (RJR)
  - *Co-PI: C. Mark Ott, NASA Johnson Space Center; Co-PI: Natalia Novikova, Russian Institute of Biomedical Problems*
  - Current ISS microbiological monitoring generally relies on sampling defined targets and standard protocols, unless contamination events warrants different instructions to the crew.
  - The RJR is allowing flexibility in the collection process to include potential areas of microbial buildup that may not otherwise be evaluated.

Microorganisms in the Environment

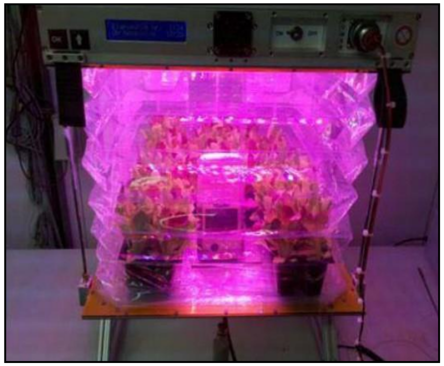
- Utilizing historic microbial isolates for microbiological research
  - The JSC Microbiology Laboratory has been isolating and archiving microorganisms from the environments of spacecraft as a part of crew health monitoring efforts.
  - NASA is currently developing a system to make these environmental isolates available to scientists for research that will improve our knowledge of the spacecraft environment and translate to our overall knowledge of habitats on Earth.
  - NASA has recently signed a Space Act Agreement with the Alfred P. Sloan Foundation to use these isolates in grants for their "Microbiomes of the Built Environment" Program Area.

Astronaut Microbiome

- Evaluation of changes in the crew microbiome during spaceflight missions
  - *PI: Hernan Lorenzi, Ph.D., J. Craig Venter Institute*
  - Samples are collected preflight, in-flight, and post-flight samples from 9 astronauts
  - Samples include two skin sites, nostrils, fecal samples, potable water, as well as blood and saliva for immunological evaluations
  - Tightly monitored metadata (*e.g.*, temperature, humidity, diet) is available
- Future NASA research plans further microbiome investigations

Other Pertinent Studies

- The VEGGIE spaceflight experiment
  - *PI: Gioia Massa, Ph.D, NASA Kennedy Space Center*
  - The VEGGIE experiment grew lettuce on ISS for consumption.
  - NASA is addressing how best to monitor in-flight produce and its impact on the microbiological environment.
- Microbial Mutation Rate
  - *PI: Cheryl Nickerson, Ph.D.*
  - Changes in the mutation rate of microorganisms during spaceflight is not known.
  - NASA is performing experiments culturing a microorganism targeting 300 generations of growth.
  - Whole genome sequencing of these organisms during this process should provide an understanding of changes in mutation rate and heritable changes that could impact their destination or return to Earth.

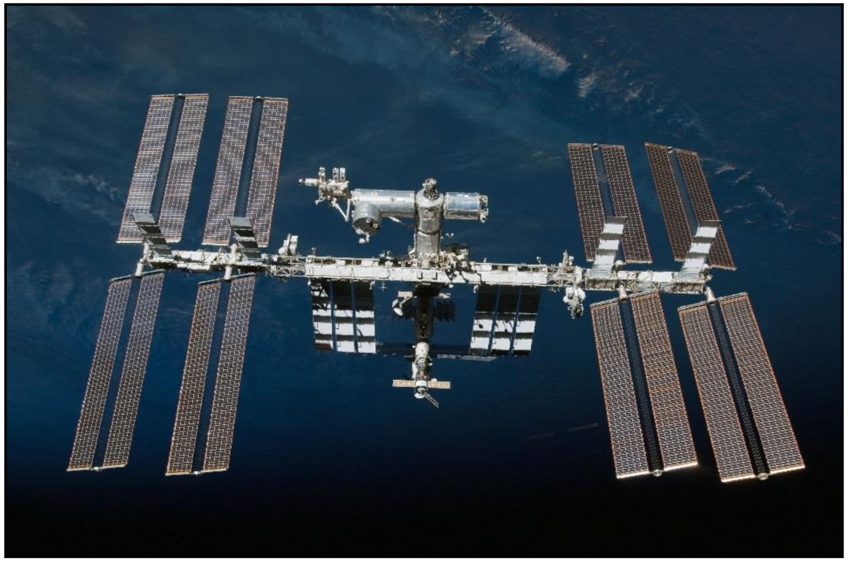


Next Generation Spaceflight Monitoring

- Spaceflight technology demonstrations
  - Razor system
    - QPCR technology – Targeted probes
    - Designed for and used by the military
    - Dry chemistry for easier sample prep
    - Limited number of sample wells
  - MinION system
    - Nanopore technology
    - Sequences all organisms in the sample
    - Requires sample prep
  - Scheduled to fly aboard SpaceX 9 (July 2016)

Understanding Spacecraft Microbiomes for Planetary Protection

- Overall, NASA maintains microbial concentrations in spacecraft environments at low levels.
- Microorganisms isolated from spacecraft air and surface samples strongly reflect the types of microorganisms found in and on the crew.
- Microbiological contamination events do occur; however, current design and remediation approaches have been successful.



Understanding Spacecraft Microbiomes for Planetary Protection

- NASA is leveraging recent technological advances to enable sophisticated research into the true concentration and diversity of microorganisms that can be found in spacecraft.
- These tools are also providing novel insight into the unique responses of microorganisms to the spaceflight environment that could impact their characteristics, such as virulence, stress resistance, and biofilm formation, that could threaten mission success or disrupt our current planetary protection efforts.