Spaceflight Microbiology

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NASA

Microbiological Areas of Concern

- Astronaut Health
- Vehicle integrity
- Life support and other systems failure
  - Biofilm formation/biofouling
  - Biocorrosion and biodegradation
  - Trash and human waste containment
  - Risk of condensation
  - Astronaut hygiene areas
- Spaceflight foods
  - Impact of "pick and eat" foods on the environment
  - Impact of the environment on "pick and eat" foods
  - Rinsing food with potable water is impractical
- Planetary protection
  - How do we track what we are leaving versus what we are finding in our search for life on other planets?

Astronaut Health

- Preventative measures
  - Preflight medical exams and medical consult throughout a mission
  - Preflight crew quarantine
  - Stringent microbiological monitoring of spacecraft and its cargo
- Generally healthy crew
  - Infectious disease and allergic symptom rate on ISS has been estimated at 3.4 events/flight year (Crucian et al. 2016)
  - Survey of Space Shuttle missions (STS-1 through STS-89) indicated infectious disease accounted for 1.4% of all medical events (not including skin and subcutaneous tissue) (Risin 2009)
- Incidence is usually based on symptomology
  - Rash
  - Dry hacking cough
  - Diarrhea

Astronaut Health

- Examples of diseases during spaceflight missions
  - Upper respiratory infections
  - Urinary tract infections
  - Ear infections
  - Various fungal infections
  - Herpes Zoster
  - Rashes & skin disorders
  - Allergic reactions
  - Gastroenteritis

- Notable factors
  - Clear routes of infection have not been eliminated
  - No current environmental viral monitoring
  - Risks reflect the vehicle design and operations of specific missions

Protecting the Environment

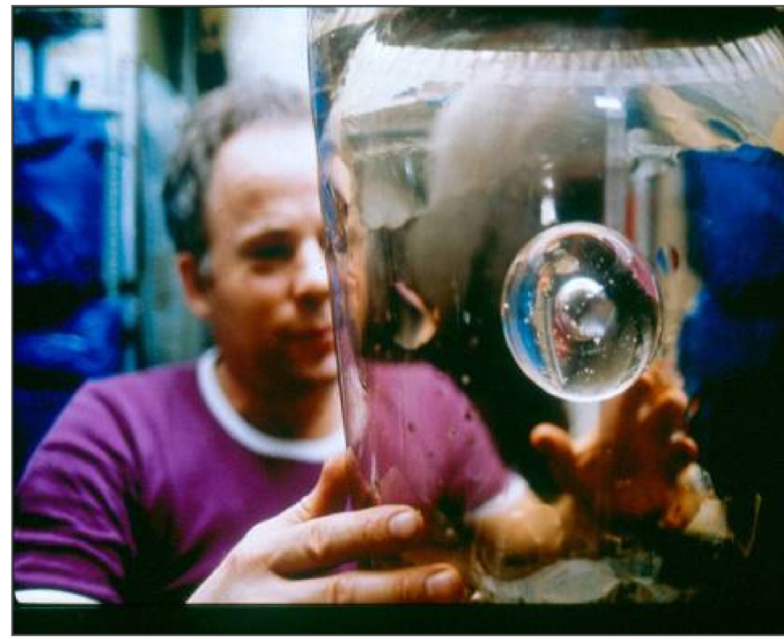
- Prevention - Preflight
  - Extensive microbial monitoring and preflight disinfection
  - Preflight monitoring targets environmental areas that are probable routes of infection, including vehicle air and surfaces, spaceflight food, potable water, cargo, and a biosafety review of experimental payloads.
  - Flight requirements
    - Vehicle air - 100 CFU/m^3 fungi; 1000 CFU/m^3 bacteria
    - Vehicle surfaces - 100 CFU/100 cm^2 fungi; 10,000 CFU/100 cm^2 bacteria
    - Potable water – 50 CFU/ml heterotrophic plate count; no detectable coliforms in 100 ml; no detectable fungi in 100 ml; treatment technique to prevent transmission of parasitic protozoa.

Protecting the Environment

- Prevention – Vehicle design
  - Potable water recovery system
    - Recovers humidity condensate and urine
    - Final water recycling steps of the NASA water system include catalytic oxidizer (267 °F for 10 minutes); iodination; iodine removal; 0.22 micron filtration
  - Vehicle air
    - Air flow through HEPA filters
  - Vehicle surfaces
    - Use of materials that are not conducive to microbial growth

Constraints of Spaceflight

- When considering spaceflight hardware (*e.g.*, monitoring equipment) and cargo (*e.g.*, disinfection wipes, clothing, food), the benefit of the item is balanced against the need for:
  - Safe operation
  - Minimal power
  - Minimal mass
  - Minimal volume
  - Minimal crew time



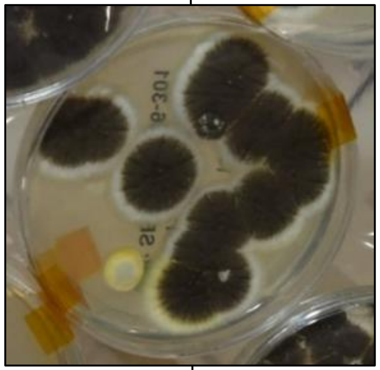
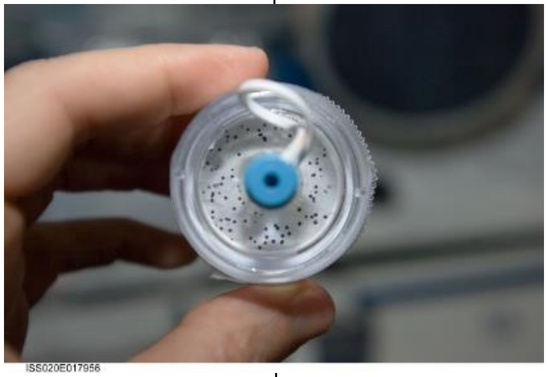
No phase separation

Microbiological Monitoring on the ISS

Surfaces | **Air** | **Water**





Quantified in-flight and returned to JSC for identification

Environmental Health

- Microbial monitoring by JSC and others indicate **ISS environmental flora is similar to a home** (Pierson, *et al.* 2011; Lang, *et al.* 2017; Blaustein, *et al.* 2019).
- The bacteria in this environment reflect human-associated microorganisms.

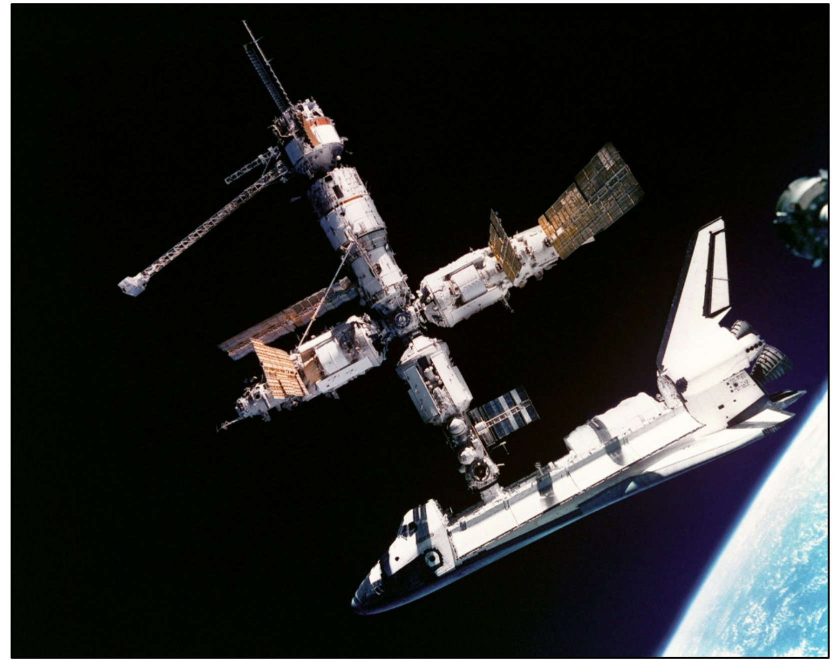
- Media-based monitoring occasionally identifies opportunistic bacterial pathogens, such as ***Staphylococcus aureus*** and ***Bacillus cereus***. No methicillin resistant *S. aureus* has been identified. ***Enterobacter*** and ***Enterococcus*** **species** are occasionally identified throughout the ISS.

Environmental Health

- The most prevalent fungal genera are *Aspergillus* and *Penicillium*. ***A. flavus, A. niger,*** and ***A. fumigatus*** have been identified from spaceflight samples. ***Stachybotrys chartarum*** has been isolated preflight.
- Potable water reflects common non-pathogenic bacterial species. The 0.22 micron filter upstream of the water ports have limited increases in diversity.
- The ISS is only semi-closed and "new" bacterial and fungal isolates are regularly identified.

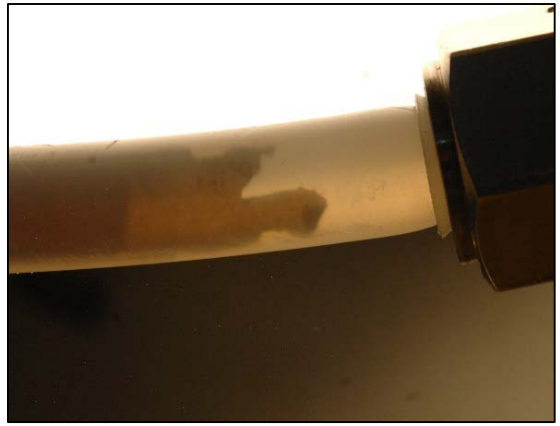
Environmental Anomalies

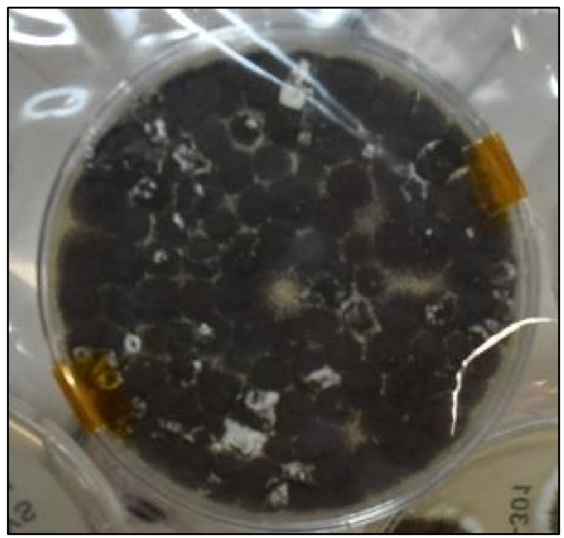
- Mir Space Station
  - Power failures and problems with temperature control eventually caused large amounts of free-floating condensate
  - Samples of condensate revealed the presence of high diversity, including ***E. coli, Serratia marcescens, Legionella*** species, and **protozoa** (Ott *et al.*, 2005)



- ISS anomalies
  - Surface contamination is often associated with uncontrolled water
  - Air contamination and unpleasant odors do occur; however, the sources are often unclear. Luckily, these events have been transient and/or handled by the HEPA filtration system

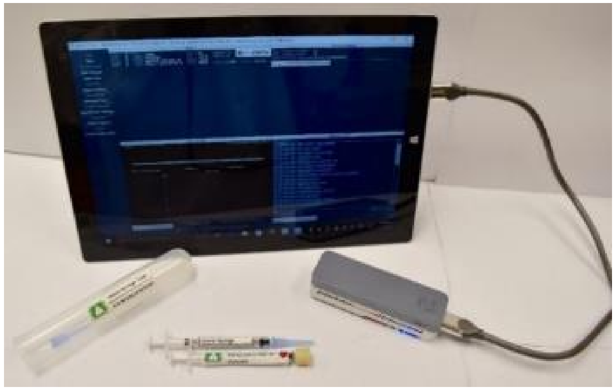
ISS Environmental Anomalies

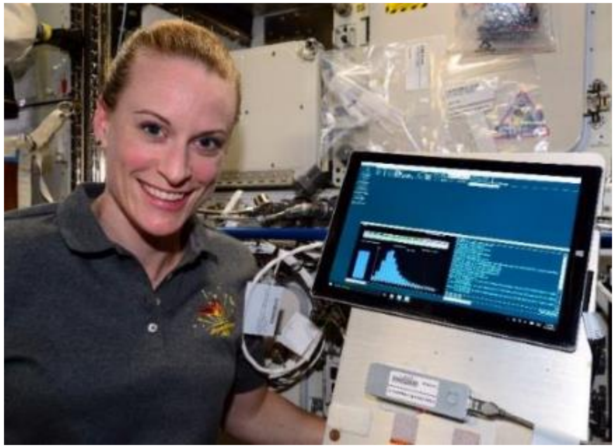




Advances in Environmental Monitoring

- DNA sequencing in space was first performed with the Biomolecular Sequencer in 2016 using Oxford Nanopore Minion (Castro-Wallace *et al*. 2017).
  - No decrease in sequencing performance
  - Demonstrated flow cell reuse and shelf-life stability to at least 6 months in space
- Microbial cells from a media plate used for surface sampling were processed and sequenced on ISS.
  - The Minion accurately identified the three isolates that were selected.





Advances in Environmental Monitoring

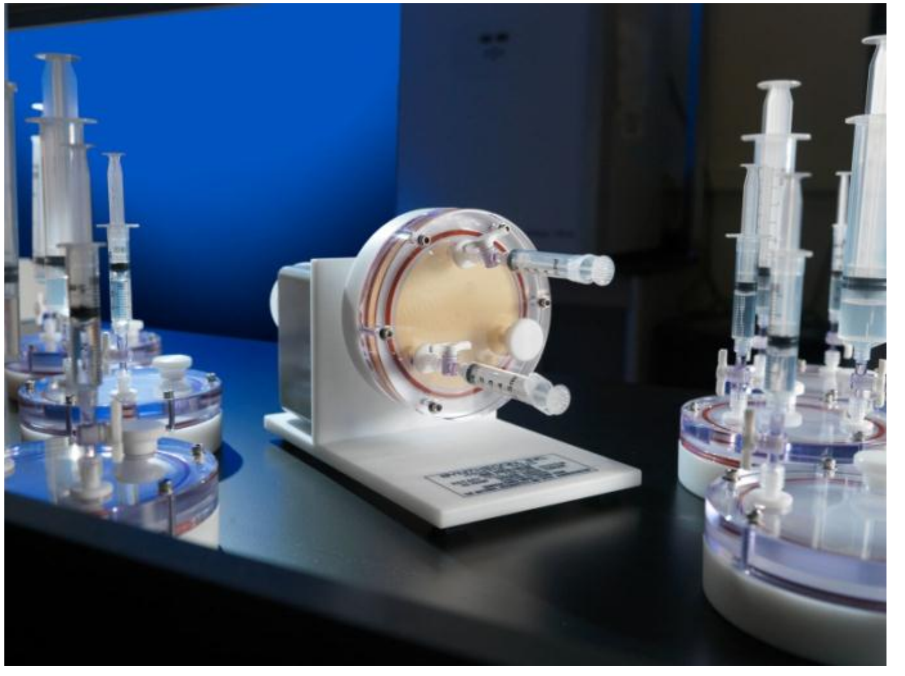
- Spaceflight experiments on ISS are currently being performed focusing on:
  - Swab-to-sequencer (non-culture based identification)
  - Cellular evolution
  - Direct RNA sequencing
- Earth-based investigations comparing sequencing and media-based results are being performed to provide insight into how to translate sequencing results for crew health purposes.

Spaceflight Microbiology Research

- 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.

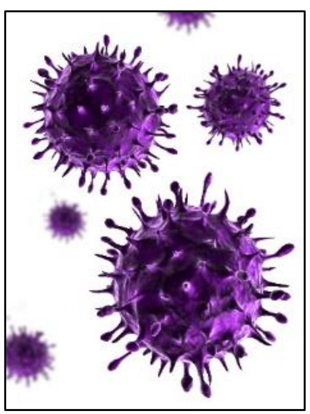
Ground Based Spaceflight Analogues

- Rotating Wall Vessel (RWV)
  - Solid body rotation in the reactor simulates several aspects of culture in microgravity
  - Enables relatively high throughput
  - Provides predictive trends for spaceflight experiments
  - Capability to follow up spaceflight findings without the delays associated with true spaceflight experiments



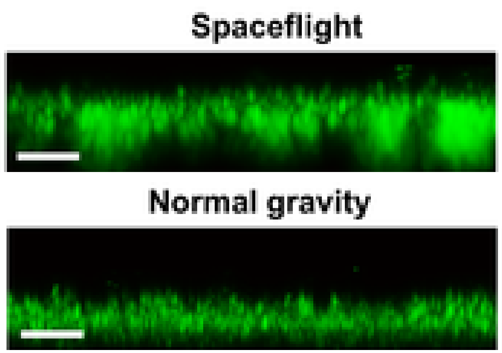
Microbial Responses to Spaceflight

- **PI: Cheryl A. Nickerson, Arizona State University**
- Seminal studies evaluating the response of the enteric pathogen, *Salmonella enterica* serovar Typhimurium
- First studies to effectively study **microbial virulence and global molecular genetic responses of microorganisms** when cultured in the spaceflight and spaceflight analogue environment.



- **PI: Duane Pierson, NASA (Emeritus)**
- 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. <u>VZV subclinically reactivates in healthy individuals after acute stress.</u>

Biofilm Development during Spaceflight



- **PI: Cynthia Collins, Rensselaer Polytechnic Institute**
- Identified a unique biofilm architecture produced by *Pseudomonas aeruginosa* in response to spaceflight culture, described by the authors as "a column-and-canopy structure".

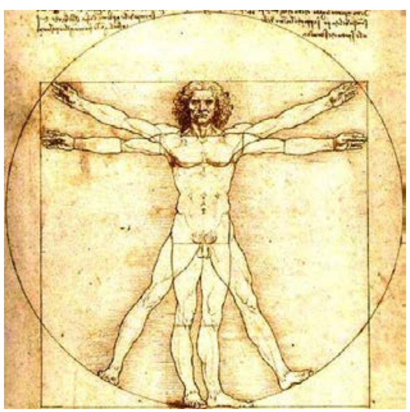
- **PI: Bob McLean, Texas State University**
- Investigation into polymicrobial biofilm development during spaceflight by *P. aeruginosa* and *E. coli.*
- The study focuses on biofilm architecture, disinfection, and corrosion potential during spaceflight.

Astronaut Microbiome

- *PI: Hernan Lorenzi, J. Craig Venter Institute*
- Baseline evaluation of the microbiome of from two skin sites, nostrils, and fecal samples from 9 astronauts during spaceflight missions (preflight, inflight, post-flight). Blood and saliva were also collected for immunological assays.
- The composition of the intestinal microbiota became more similar across astronauts in space, mostly due to a drop in the abundance of a few bacterial taxa.
- Alterations in the skin microbiome that might contribute to the high frequency of skin rashes/hypersensitivity episodes experienced by astronauts in space were also observed.

- ***Sloan***
- Investigation into polymicrobial biofilm development during spaceflight by *P. aeruginosa* and *E. coli*.
- The study focuses on biofilm architecture, disinfection, and corrosion potential during spaceflight.

Astronaut Microbiome



- **PI: Hernan Lorenzi, J. Craig Venter Institute**
- **<u>Baseline</u>** study of changes in the microbiome of 9 astronauts during spaceflight missions
  - Samples were collected preflight, in-flight, and post-flight samples and included two skin sites, nostrils, fecal samples, potable water, as well as blood and saliva for immunological evaluations.
  - Tightly monitored metadata (*e.g.*, temperature, humidity, crew diet, medications).
- The composition of the intestinal microbiota became more similar across astronauts in space, mostly due to a drop in the abundance of a few bacterial taxa.
- Alterations in the skin microbiome were observed that might contribute to the high frequency of skin rashes/hypersensitivity episodes experienced by astronauts in space.

NASA + Alfred P. Sloan Foundation

- **Postdoctoral Fellowships** focusing on the **Microbiology of the Built Environment** using bacterial from the ISS archives
  - **Fellow: Michael Lee; PI-Advisor: Craig Everroad**, NASA Ames Research Center Microbial evolution and transmission aboard the ISS: inferring mutation rates, assessing pangenomes, and tracking microbiome transmission between astronauts and the space-based built environment. [NASA Space Biology Program]
  - **Fellow: Aubrie O'Rourke; PI-Advisor: William Nierman**, J. Craig Venter Institute Virulence and drug resistance of *Burkholderia* species isolated from ISS potable water systems. [NASA Space Biology Program]
  - **Fellow: Noelle Bryan; PI-Advisor: Maria Zuber**, Massachusetts Institute of Technology
  - Genomic and functional analysis of biofilm morphotypes of International Space Station isolated *Staphylococcus epidermidis* and their pathogenicity in *Caenorhabditis elegans*. [NASA Space Biology Program]
  - **Fellow: Blake Stamps; PI-Advisor: John Spear**, Colorado School of Mines Biodeterioration and Biocorrosion in Spaceflight Ecosystems: Implications for Material/ Microbiome Interactions on the International Space Station. [Sloan Foundation]
  - **Fellow: Jiseon Yang; PI-Advisor: Cheryl Nickerson**, Arizona State University Developing predictive model systems of polymicrobial biofilm formation and susceptibility to chemical disinfectant: A longitudinal study with implications for spaceflight systems integrity and health risks. [Sloan Foundation]

Considerations for Future Research

- Historically, spaceflight research has often been burdened by sensationalized or unsubstantiated conclusions.
  - Highly visible field
  - Limitations in experimental reproducibility
  - Misconceptions from these reports can last for years and actually be damaging to our progress
- Our current resources and ability to perform science on ISS enables solid hypothesis driven science.
- Recommendations and reviews of future space research should be sensitive to the need for the same levels of evidence and credibility that terrestrial studies require.
  - Proper controls
  - Conclusions that reflect the evidence

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Sarah Wallace, Ph.D.

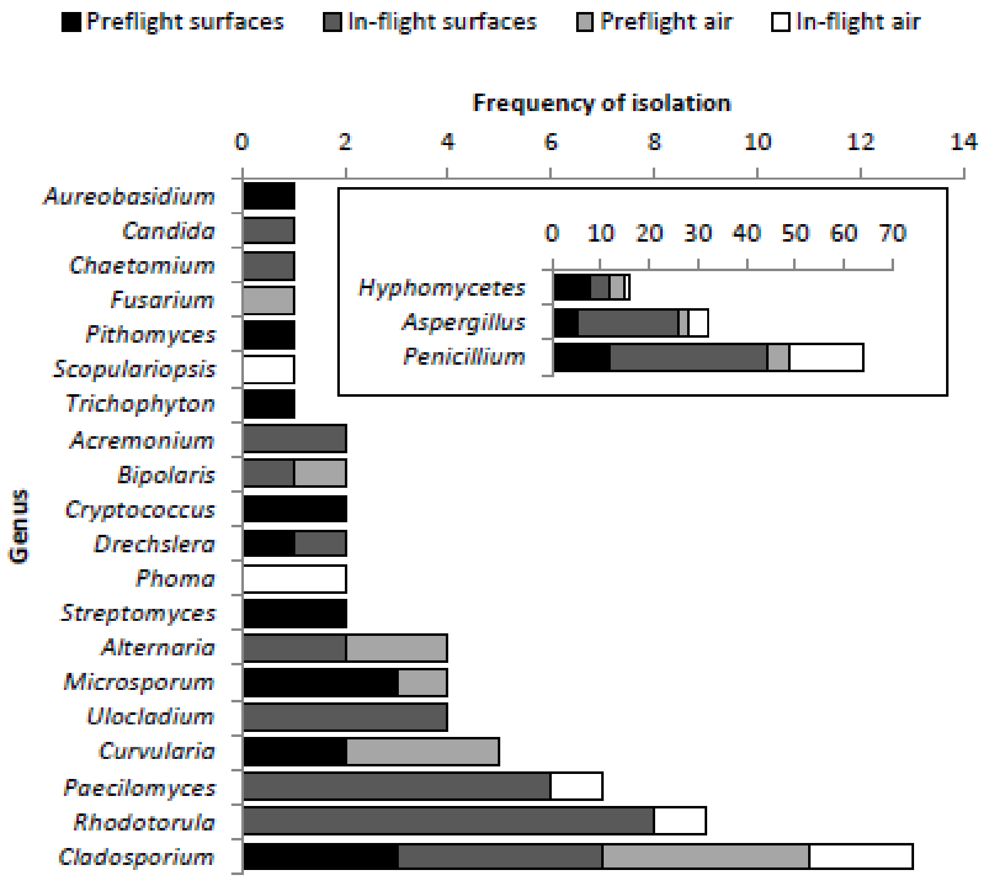
Microbiology Research aboard ISS

- NASA NSPIRES System
  - Solicitation information
  - https://nspires.nasaprs.com/external/
- NASA Space Biology
  - Microbiology is one of five science Elements in the Space Biology Science Plan
  - https://www.nasa.gov/sites/default/files/atoms/files/16-05-11_sb_plan.pdf
- NASA Human Research Program
  - Microbiology information is under the "Risk of Adverse Health Effects Due to Host-Microorganism Interactions" in the HRP Research Roadmap
  - https://humanresearchroadmap.nasa.gov/

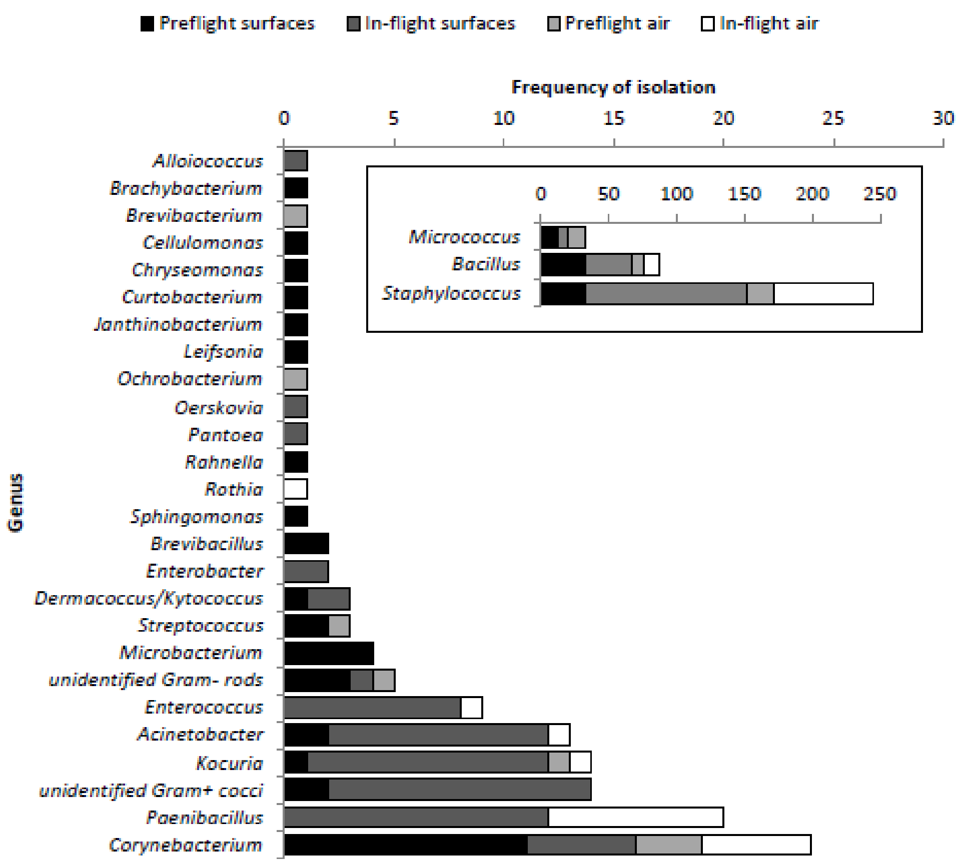
BACKUP SLIDES

ISS Air and Surface Monitoring

Fungal Isolates



ISS Air and Surface Monitoring
Bacterial Isolates



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*