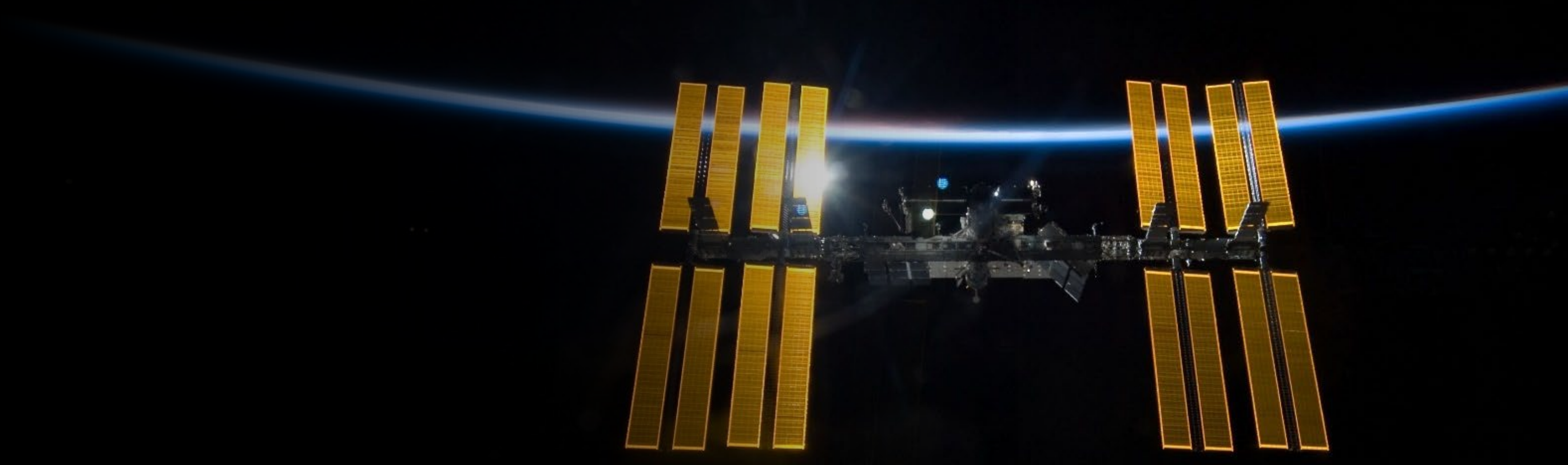
Redefining Spaceflight Microbiology



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JSC Microbiology

NASA Johnson Space Center, Houston, TX

University of Houston 2023


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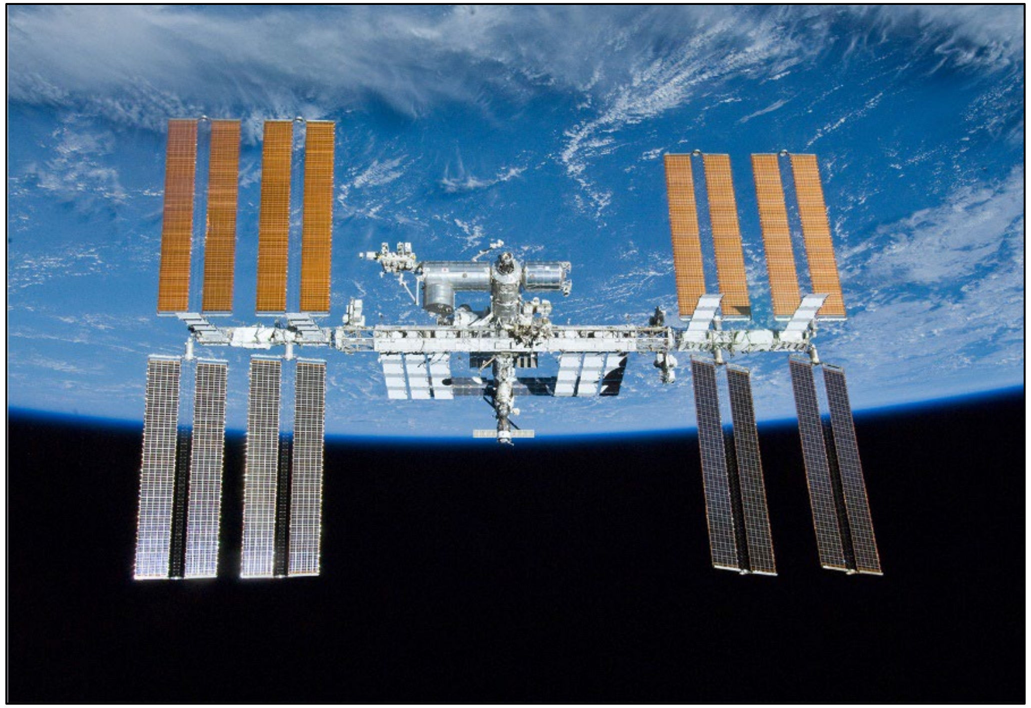
NASA

Why Microbiology?

- Astronaut health and performance
- Spaceflight foods
- Vehicle integrity
- Life support and other systems
  - Biofilm formation/biofouling
  - Biocorrosion and biodegradation
  - Hygiene areas
  - Trash and human waste containment
- Planetary protection
  - How do we protect the crew and extraterrestrial environments in our search for life on other planets?



Why is it important to redefine our approach to spaceflight microbiology?

- Over the past 60 years, NASA has gained a wealth of lessons learned about how microorganisms can affect spaceflight missions.
- Currently, NASA is reaching to the moon and Mars, as commercial spaceflights and space stations become common parts of our economy.
- Over the next few years, reevaluating microbiological risks (and associated requirements) will help to optimize the resources needed to maintain human health and habitat sustainability.

Where we began…

- Historically, spaceflight missions have relied on stringent microbiological requirements for vehicle design and operations.
  - Microbiological monitoring targeted astronauts, spaceflight foods, vehicle air/surfaces, potable water, hardware, and cargo bags.
  - Microbial concentrations targeted "essential sterility" in the environment.
  - The Health Stabilization Program (including preflight crew quarantine) began in 1971.

How stringent?

✓ As recently as the late 1990s, NASA potable water requirements limited preflight microbial concentrations to 1 Colony Forming Unit (CFU) per 100 ml.

J. D. MacEwen in *Bioastronautics Data Book* (Scientific and Technical Information Office, National Aeronautics and Space Administration, 1973);
Koenig and Pierson. *Water Sci Technol* 1997;35(11-12):59-64;

What have we learned?

- **We found the best microbiological mitigation technique is <u>preflight prevention</u>.**
- The Health Stabilization Program works, as incidence of infectious disease during Apollo missions dropped dramatically when the program was implemented.
- Environmentally, the International Space Station (ISS) has provided extensive microbiological operations and research data characterizing the complex interface between humans and their spaceflight habitat.
  - In general, current operational activities greatly reduce medically significant microorganisms beyond opportunistic pathogens that have naturally travelled with the crew.

Preflight Prevention - Spacecraft design

- Vehicle surfaces
  - Materials that do not promote microbial growth
- Vehicle air
  - Air flow through HEPA filters
- The water recovery system that recycles urine and cabin humidity relies on multiple microbial mitigation techniques, including:
  - Catalytic oxidizer (267°F for 10 minutes)
  - Iodination/iodine removal
  - 0.2-micron filtration

Preflight Prevention – Food monitoring

- Spaceflight foods that are considered commercially sterile (*e.g.*, thermostabilized, irradiated) do not require microbiological monitoring.
- Non-thermostabilized foods may be microbiologically monitored for total aerobic counts, Enterobacteriaceae, *Salmonella*, and/or Yeast and Molds.
- Preflight monitoring of ISS spaceflight food has indicated the presence of
  - *Salmonella enterica* serovar Typhimurium
  - *Staphylococcus aureus*
  - *Klebsiella pneumoniae*
  - *Enterobacter cloacae*
  - *Cronobacter sakazakii*
  - *Aspergillus flavus*
  - *Aspergillus fumigatus.*

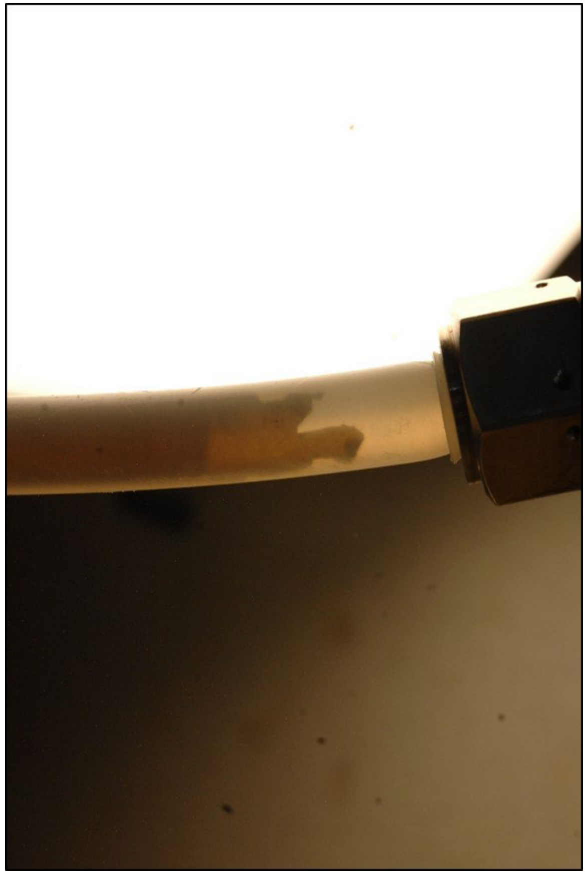
Preflight Prevention – Environmental monitoring

- Extensive microbial monitoring and preflight disinfection
- Preflight monitoring targets environmental areas that are probable routes of infection, including vehicle air and surfaces, potable water, and cargo.
- Environmental microbiological requirements (Missions over 30 days)
  - 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.
- Spaceflight experimental payloads also receive a biosafety review to support the determination of appropriate levels of containment and control.

Does contamination still happen?

Trash and waste control

Astronaut activities, such as eating and hygiene



Biofilm formation in life support system water lines

Constraints of spaceflight hardware development

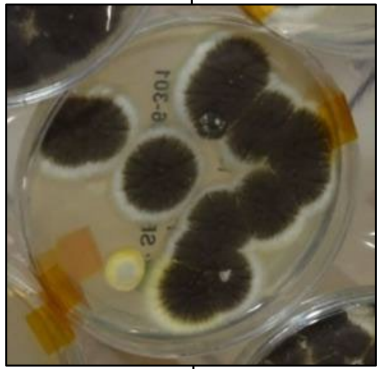
- When considering spaceflight hardware (e.g., monitoring equipment) and cargo (e.g., disinfection wipes, clothing, food), the benefit of the item is balanced against the need for:
  - Safe operation
  - Minimal power
  - Minimal mass
  - Minimal volume
  - Minimal crew time

Phase separation during spaceflight

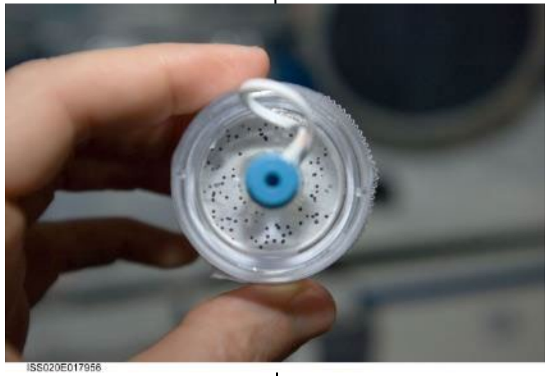
Microbiological monitoring on the ISS

Surfaces

Air

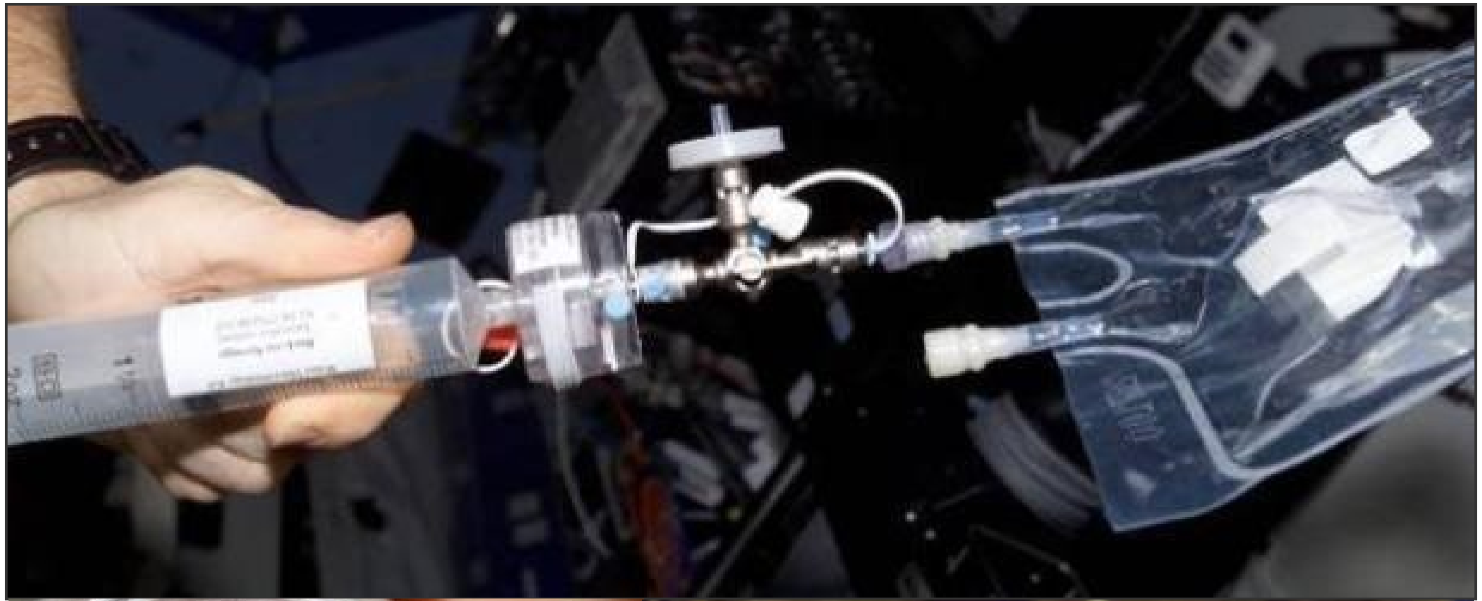


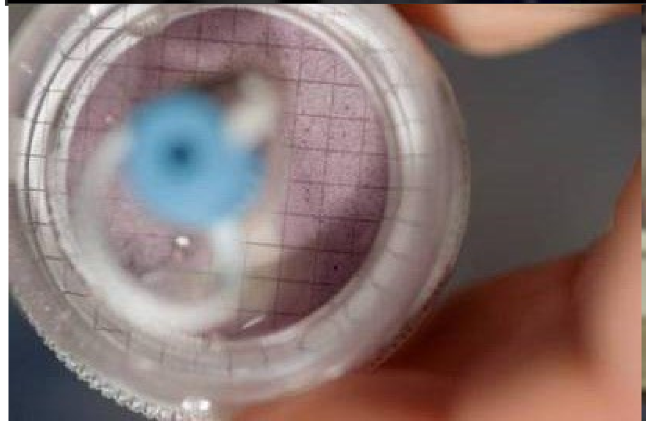
Water



Quantified in-flight and returned to JSC for identification

Microbiological monitoring of water





What have we found in the environment?

Microbial monitoring by NASA and others indicate ISS environmental flora reflect human-associated microorganisms commonly found in terrestrial homes.

- 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.

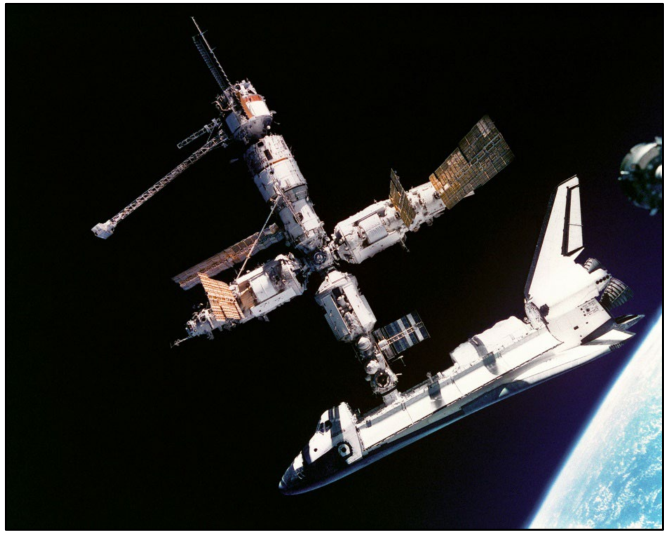
R. A. Blaustein *et al.* *mSystems* 4, (2019); J. M. Lang *et al.* *PeerJ* 5, e4029 (2017);
D. Pierson *et al.* in *Environmental Monitoring: A Comprehensive Handbook* (DHI Publishing, 2012)

What have we found in the environment?

- 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 a limited diversity of common environmental bacterial species, including
  - *Ralstonia pickettii*
  - *Burkholderia multivorans*
  - *Sphingomonas sanguinis*
  - *Cupriavidas metallidurans*

Have we found anything unexpected?

- Mir Space Station
  - Samples of free-floating condensate caused by power failures and problems with temperature control revealed high microbial diversity, including ***Escherichia coli***, ***Serratia marcescens***, ***Legionella*** species, and protozoa.
- International Space Station
  - Surface contamination is often associated with uncontrolled water
  - Air contamination and unpleasant odors do occur; however, the sources are often unknown. These events have been transient and/or handled by the HEPA filtration system.
  - Biofilm formation in ISS water lines have caused clogged lines and pump failure.



C. M. Ott, *et al. Microb Ecol* 47, 133-136 (2004)

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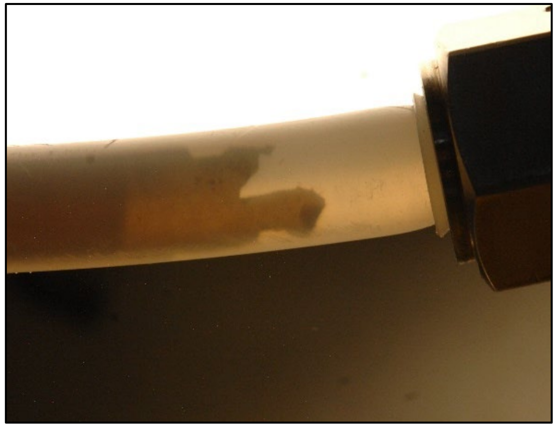


Do the astronauts get infectious diseases?

- Preventative measures
  - Preflight medical exams and medical consult throughout a mission
  - Preflight crew quarantine
  - Stringent microbiological monitoring of spacecraft and its cargo
- Diagnosis is often based on symptomology (e.g., headache, rash, dry hacking cough, diarrhea).
- Infectious diseases do occur during spaceflight missions, examples include:
  - Upper respiratory infections
  - Urinary tract infections
  - Ear infections
  - Herpes Zoster
  - Rashes & skin disorders
  - Gastroenteritis

Do the astronauts get infectious diseases?

- A survey of Space Shuttle missions indicated infectious disease accounted for 1.4% of all medical events (not including skin and subcutaneous tissue).

Why do exceptionally health astronauts in a pristine environment still contract infectious diseases?

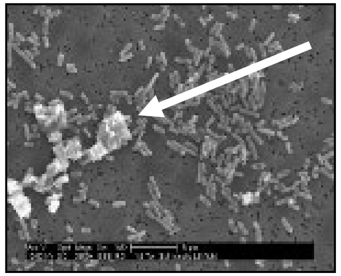
- Microbial phenotypes, including virulence and pathogenesis characteristics, have been observed to change in the spaceflight environment.
- Immune system evidence, including reduced function and distribution of some immune cells, reactivation of latent viruses, and altered cytokine production during long duration spaceflight

D. Risin, in Human Health and Performance Risks of Space Exploration Missions, (NASA SP-2009-3405, 2009).

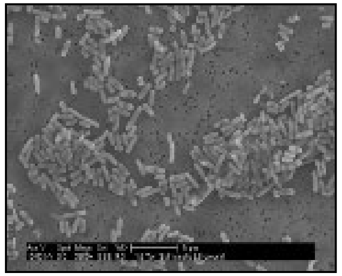
What type of evidence indicates changes in microbial responses to spaceflight?

- In 2006, the MICROBE Experiment (PI: Cheryl Nickerson, Arizona State University) identified **alterations in microbial virulence** in spaceflight grown cultures of *Salmonella enterica* serovar Typhimurium.
  - Proteomic profiling identified **73 differentially regulated proteins**, and microarray analysis identified **167 differentially regulated, globally distributed genes** compared to ground controls
  - Common molecular regulatory protein (Hfq) associated with 32% of the differentially regulated genes
  - In-flight grown *S*. Typhimurium showed the presence of an extracellular material not seen in ground control.

In-flight hardware

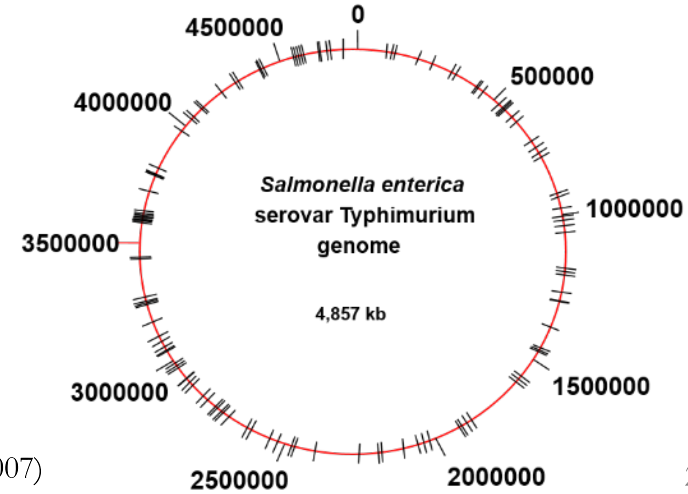


Flight Sample



Ground Control

J. W. Wilson *et al. Proc Natl Acad Sci U S A* 104, 16299-16304 (2007)



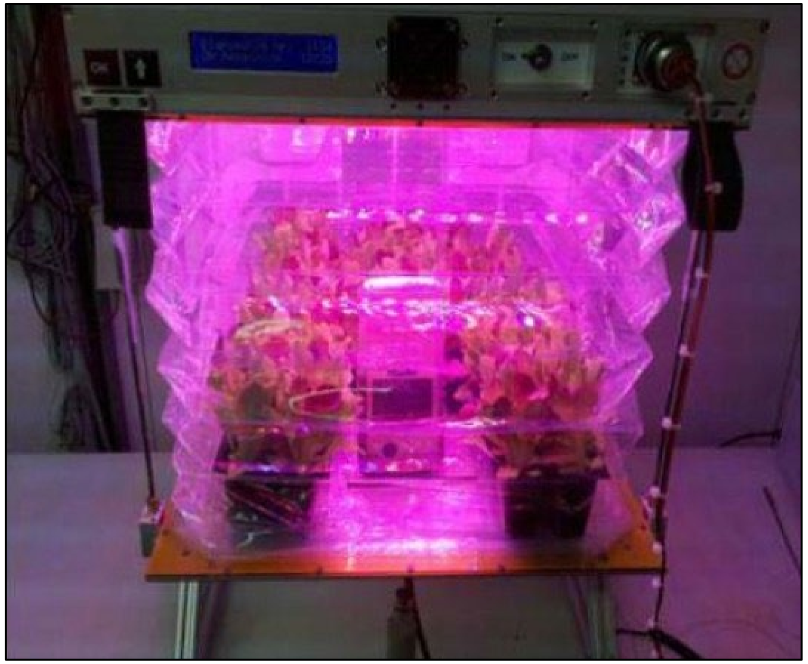
What type of evidence indicates changes in microbial responses to spaceflight?

- A follow-up study of ***S.* Typhimurium** confirmed the spaceflight-associated increase in virulence and found the changes were affected by media type to a greater extent than terrestrial controls.
- ***Serratia marcescens*** cultured during spaceflight also demonstrated an increase in virulence using a *Drosophila melanogaster* infection model.
- Increased resistance to multiple antibiotics was observed with spaceflight-grown ***S. aureus*** (oxacillin, chloramphenicol, and erythromycin)and ***E. coli*** (colistin and kanamycin).
- ***Pseudomonas aeruginosa*** also responded to spaceflight conditions through differential regulation of 167 genes and 28 proteins, with Hfq as a global transcriptional regulator.
- Microbial biofilms of ***P. aeruginosa*** grown in space were altered into a novel "column and canopy" structure.
- ***Candida albicans*** cultured in space displayed 452 differentially regulated genes and random budding patterns (as opposed to bipolar budding normally expected on earth).

J. W. Wilson *et al. PLoS One* 3, e3923 (2008); R. Gilbert *et al. npj Microgravity* 6, 4 (2020); R. Tixador, *et al. Aviat Space Environ Med* 56, 748 (1985); A. Crabbe, *et al. Appl Environ Microbiol* 77, 1221 (2011); Crabbé, A. *et al. PLoS ONE* 8, e80677 (2013); W. Kim *et al. PLoS One* 8, e62437 (2013)

With so many findings…

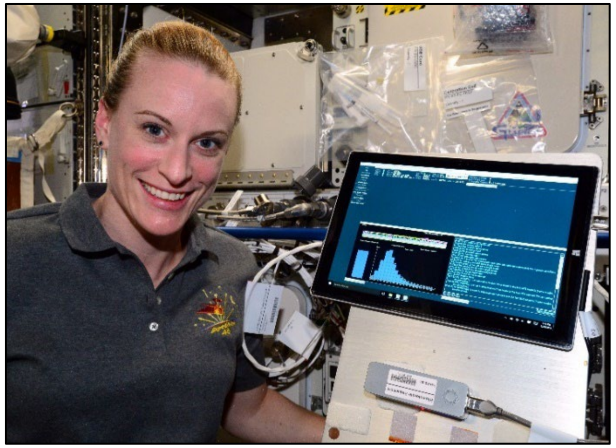
- What microbiological requirements are needed for future space missions?
  - Do we continue to repeat what we have done in the past?
  - Do we change our monitoring targets and/or sampling frequency?
  - Do we add or change requirements considering new operational activities, such as pick and eat crops and other bioregenerative systems?
- If we aren't sure, what new research is needed to help make these decisions?



The Veggie plant growth system provides crops for crew consumption.

Can we use advanced technology inflight?

- DNA sequencing in space
  - First performed in 2016 using Oxford Nanopore's MinION technology
- In-flight identification of environmental samples
  - Three microbial colonies from a media plate used for ISS surface sampling were processed and sequenced on ISS.
  - The MinION accurately identified the three isolates that were selected.
- Following up with "Swab to Sequencer" direct analysis
  - Technology Demonstration using the flight hardware and process was successful for vehicle surface monitoring.
  - Feasibility of water monitoring is currently being evaluated.

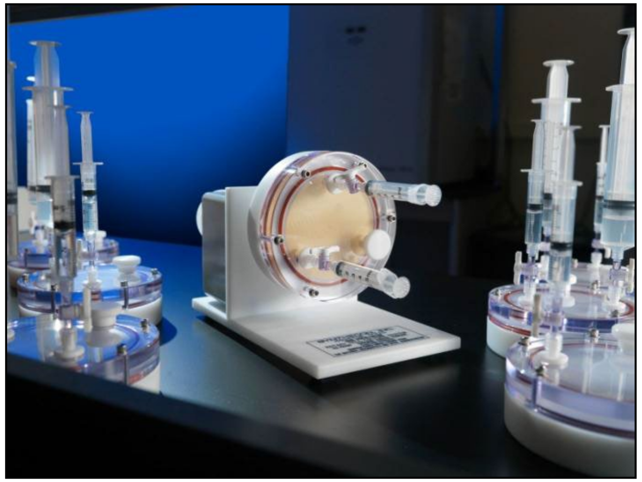


When do we use advanced molecular techniques versus simple assays?

S. L. Castro-Wallace *et al.* *Sci Rep* 7, 18022 (2017); S. Stahl-Rommel *et al.* *Genes* 12, 106 (2021).

Ground Based Spaceflight Analogues

- Rotating Wall Vessel (RWV) bioreactor
  - Solid body rotation in the reactor simulates several aspects of culture in microgravity
  - Provides predictive trends for spaceflight experiments
  - Enables follow up to spaceflight findings without the delays associated with true spaceflight experiments
- Earth based habitats
  - Controlled habitats that mimic certain aspects of spaceflight conditions
  - Examples of study topics include human behavior, life support system development, immune system function, environmental microbiome



The low shear culture conditions has prompted the term Low Shear Modeled Microgravity (LSMMG) environment

Concordia Station (Antarctica)

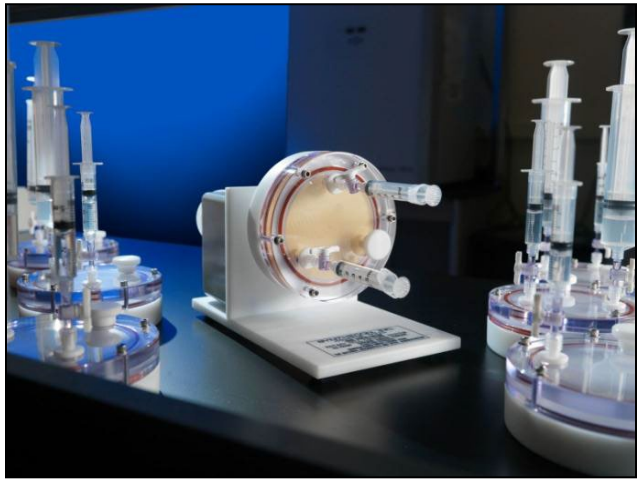
What other questions are we answering?

Do other microorganisms display an increase in virulence?

- PI: Mark Ott, NASA
  - Determine if growth in a spaceflight analogue environment changes stress response, adhesion/invasion of 3-D tissue co-culture models, and virulence of five bacterial species.

Does low dose irradiation of host cells synergistically exacerbate the increased virulence observed with spaceflight analogue culture?

- PI: Cheryl Nickerson, Arizona State University
  - Investigate the compound effect of spaceflight analogue culture with low dose radiation to determine if the combination synergistically increases the potential infection risk.



What other questions are we answering?

How do bacterial biofilms change when cultured in the spaceflight environment?

- PI: Bob McLean, Texas State University
  - Investigate polymicrobial biofilm development by *P. aeruginosa* and *E. coli* during **spaceflight** to identify changes in biofilm architecture, disinfection, and corrosion potential during spaceflight.

Are we fully utilizing the data that we have?

- PI: Kristyn Hoffman, KBR/NASA
  - Employ machine learning techniques to compare historical environmental monitoring data and astronaut clinical data to determine if there is any correlation.

Do microorganisms build up a tolerance to inflight disinfectants?

- PI: Erica Hartmann, Northwestern University
  - Characterize benzalkonium chloride (active agent in ISS disinfection wipes) resistance in 73 *Staphylococcus* isolates collected from a variety of surfaces inside the ISS between 2006 and 2015.

What other questions are we answering?

What do we know about astronaut microbiomes?

- PI: Hernan Lorenzi, J. Craig Venter Institute
  - Baseline study of skin sites, nostrils, fecal samples from **nine** astronauts collected preflight, in-flight, and post-flight.
  - The composition of the intestinal microbiota became more similar across astronauts in space
  - Alterations in the skin microbiome were observed that might contribute to the high frequency of skin rashes/hypersensitivity episodes.
  - Spaceflight studies inherently have a very low sample number but extensive environmental, medical, and dietary information.
  - This study provided a foundation for multiple astronaut microbiome experiments that are currently underway.

A. A. Voorhies *et al.* *Sci Rep* 9, 9911 (2019)

How do scientists get involved?

- Does NASA fund research?
  - Announcements can be found at NSPIRES (https://nspires.nasaprs.com/external/)
- What topics are being funded by NASA?
  - HRP Human Research Roadmap (https://humanresearchroadmap.nasa.gov/)
  - Biological and Physical Sciences (https://science.nasa.gov/biological-physical)
  - Plus many more…
- Can you participate without NASA personnel?
- Does anyone else fund spaceflight research?
- What can we expect in the future?

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