Spaceflight Microbiology:

From small things…

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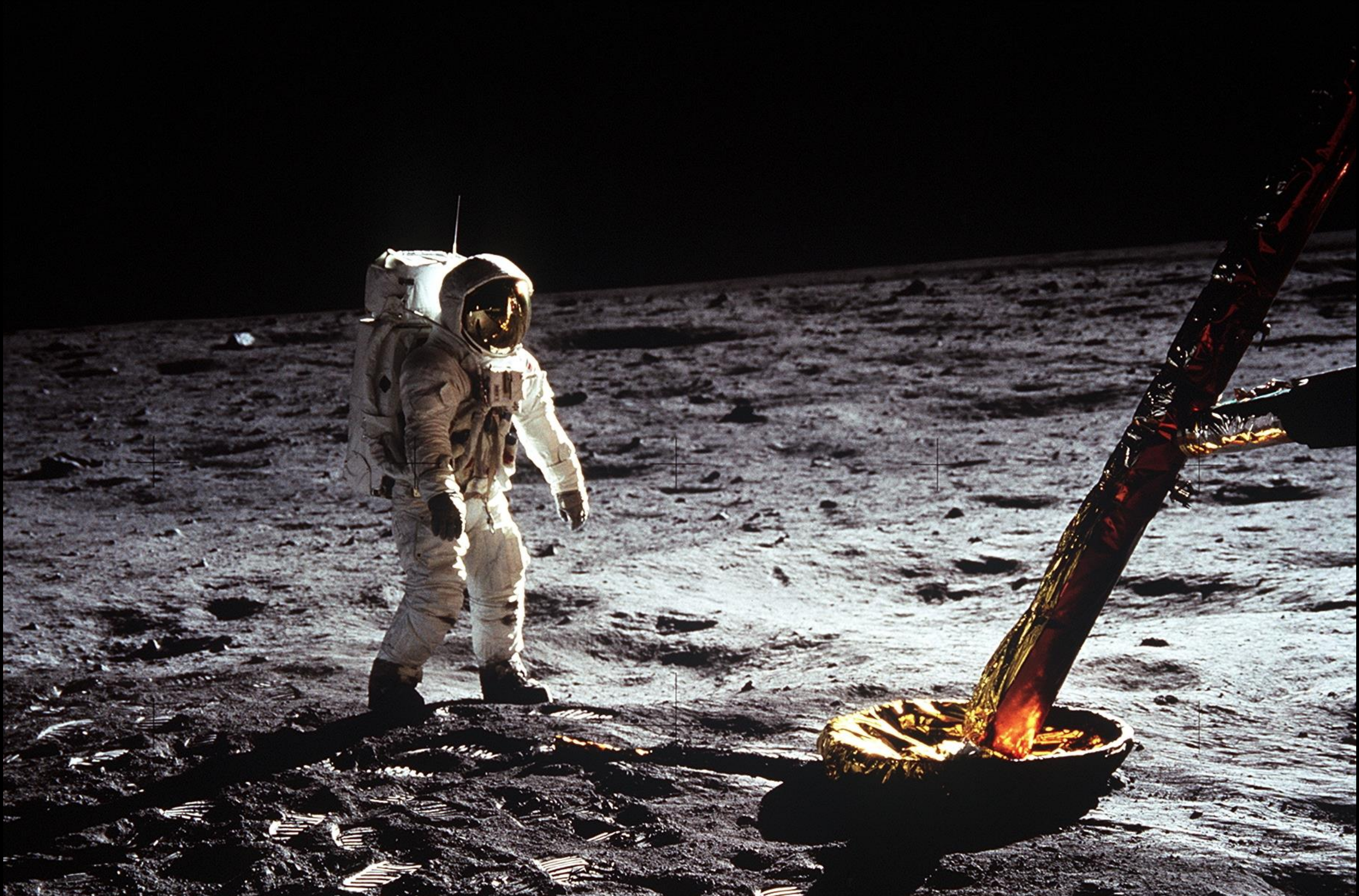


NASA

Commercial Spaceflight

- Commercial Cargo
    - SpaceX
    - Northrup Grumman
    - Sierra Nevada
- Commercial Crew
    - SpaceX
    - Boeing
- Axiom Segment/Axiom Space Station
- Commercial Low Earth Orbit Destinations (CLD) Program
    - Starlab Space Station
    - Orbital Reef
    - Northrup Grumman

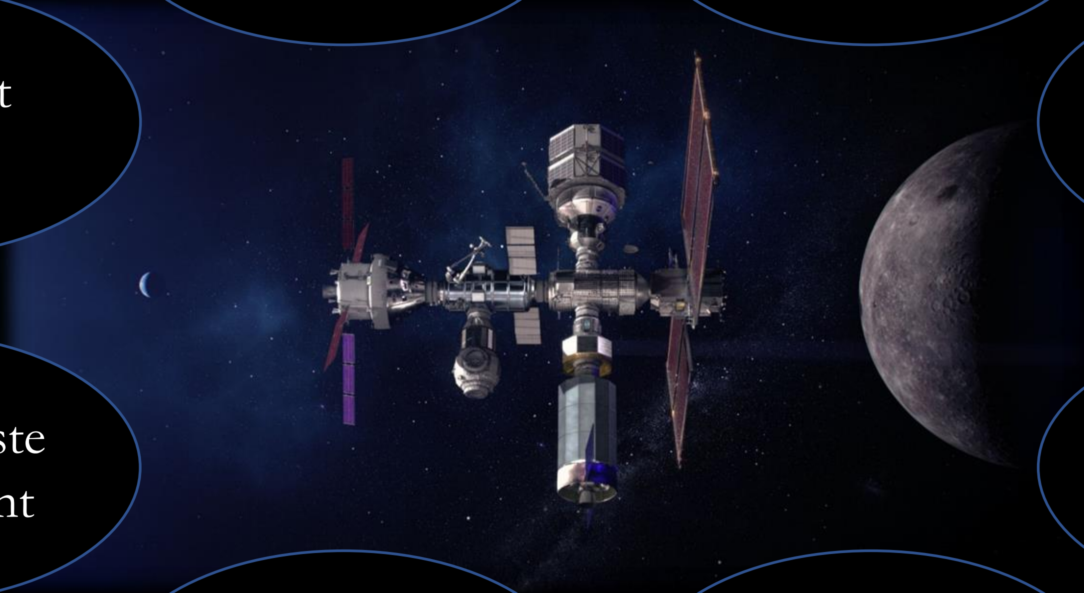
Microbiology and Space Missions

Astronaut health

Spaceflight foods

Spacecraft integrity

Life support systems



Human waste containment

Trash containment

Hygiene activities

Planetary protection

Approach to Contamination Control

- Historically, spaceflight missions and their crew have relied on stringent microbiological requirements for vehicle design and spaceflight operations.
  - The Health Stabilization Program (including preflight crew quarantine) began in 1971.
  - Spacecraft design includes elements to minimize microbial growth and crew exposure.
  - Extensive microbiological monitoring is performed, focusing on astronauts, spaceflight foods, vehicle air/surfaces, potable water, hardware, and cargo bags.

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.

D. Koenig and D. Pierson. *Water Sci Technol* 1997;35(11-12):59-64;

Astronaut Health

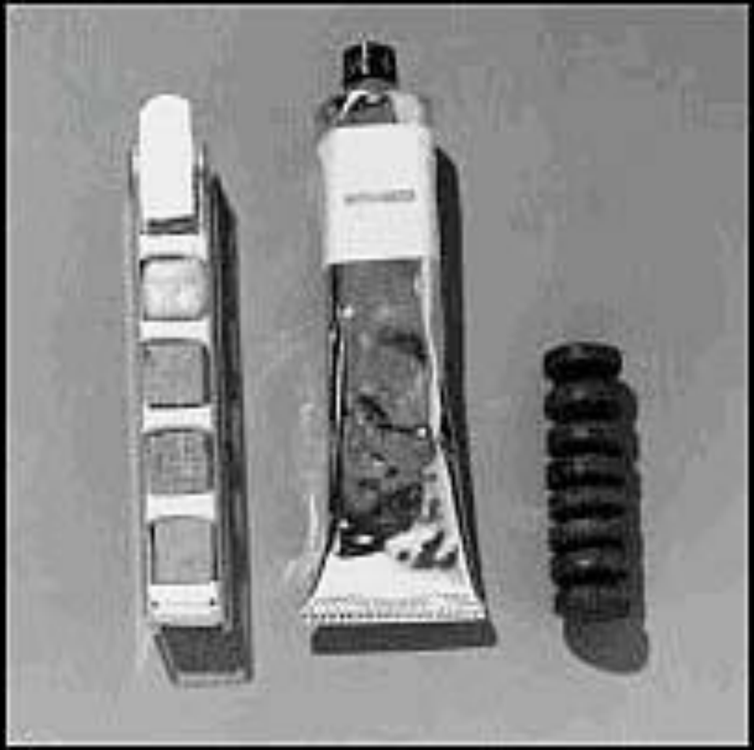
- Diagnosis is often based on symptomology (e.g., headache, rash, dry hacking cough, diarrhea)
- Examples of infectious diseases during spaceflight missions include upper respiratory infections, urinary tract infections, ear infections, herpes zoster, rashes and skin disorders, and gastroenteritis.
- 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)
- Astronaut immune system dysfunction
- Alterations in microbial virulence

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

D. Risin. Human Health and Performance Risks of Space Exploration Missions, (NASA SP-2009-3405, 2009); *Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells: Novel Insights into Disease Mechanisms* (CA Nickerson, CM Ott, N Pellis, eds., Springer Publishing, 2016)..

Spaceflight Food

Mercury Program



ISS Program

Deep Space Exploration?

Spaceflight Food

- Several categories
  - Fresh foods
  - Irradiated foods
  - Thermostabilized foods
  - Non-thermostabilized foods
- Non-thermostabilized foods
  - Includes dehydrated foods, tea, food bars, and cheese
  - Samples from each production lot are tested
  - Lots have been disqualified after detection of microbes, including the bacteria *Salmonella enterica* Typhimurium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and fungi *Aspergillus fumigatus*, and *Aspergillus flavus*.

The Future of Spaceflight Food

- The goal is to provide nutrient rich, tasty, and safe food that is stable for long-duration exploration missions is challenging.
- Several new concepts are being investigated on the International Space Station (ISS) to enable and enhance future spaceflight missions.
  - "Pick and eat crops", such as lettuce and peppers, are being grown and consumed by the ISS astronauts.
  - The concept of preparing and cooking food on the ISS is being evaluated for future missions.
- New "Categories" of foods are being provided, especially through commercial space providers.

How do we continue to keep space travelers safe as these new food concepts are implemented?

Design of Spacecraft Habitats

- Prevention is the best approach to minimize microbiological problems during spaceflight missions.
- When designing a new spacecraft/habitat, our experience with ISS indicates the risk of microbial contamination can be reduced using:
  - Habitat surfaces that are not conducive to microbial growth
  - High Efficiency Particulate Air (HEPA) filters to maintain good air quality
  - Temperature and humidity controls that minimize condensate formation
- Microbial control of potable water depends on the source and processing of the water (*e.g.*, recycled humidity and/or urine may require more treatment)

On ISS, recycled water experiences a catalytic oxidizer (267°F for 10 minutes), iodine disinfection, and passes through a 0.2-micron filtration

Operations of Spacecraft Habitats

- Health Stabilization Program
  - Preflight quarantine to decrease the risk of infectious disease
  - Employs other prevention techniques, such as raising infectious disease awareness
  - Preflight microbiological monitoring and disinfection of the vehicle and its cargo
- Inflight cleaning and remediation
  - Regular housekeeping
  - Disinfectant wipes for contaminated surfaces
  - High concentration iodine solution to disinfect potable water systems

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)

R. D. Billica, *et al.* in *Space Physiology and Medicine,* (Lea & Febiger, 1994)

Contamination Potential

Spacecraft are complex

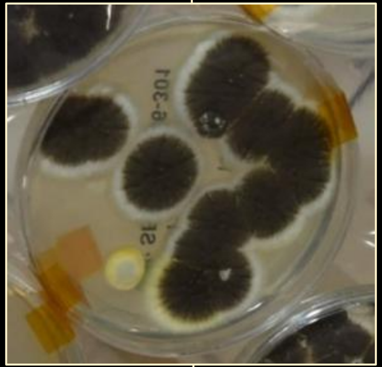
Trash and waste control

Astronaut activities, such as eating and hygiene

Microbial Monitoring on the ISS

Surfaces

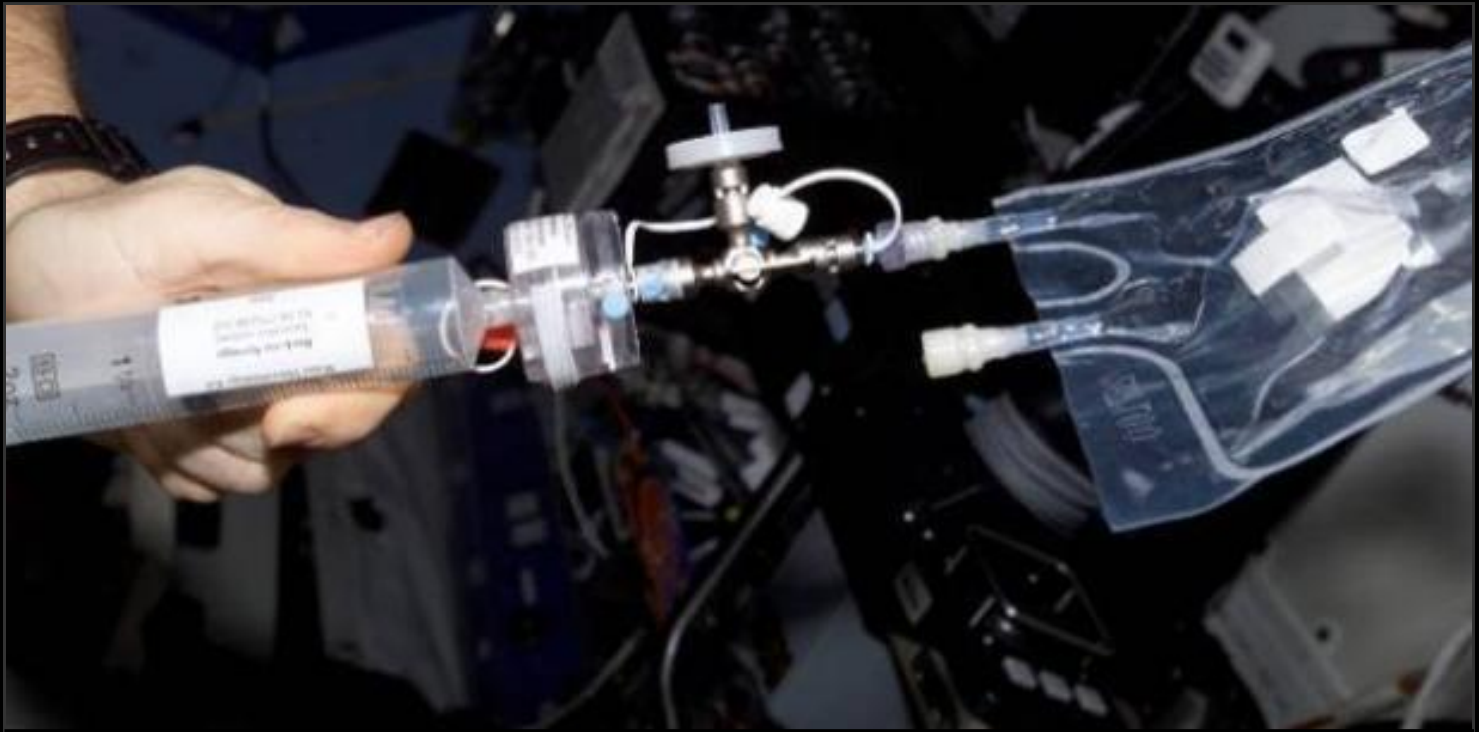
Air

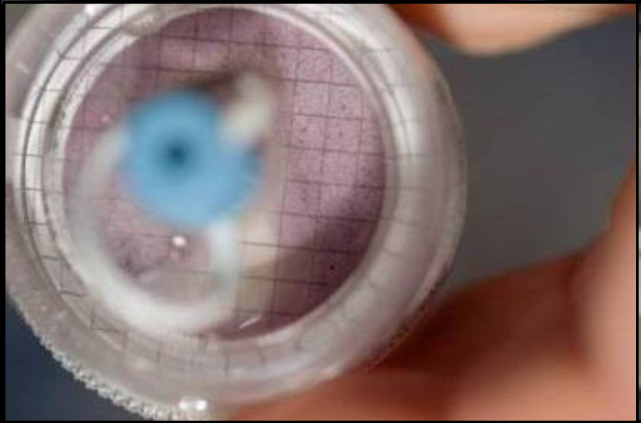


Water

Quantified in-flight and returned to Earth for identification

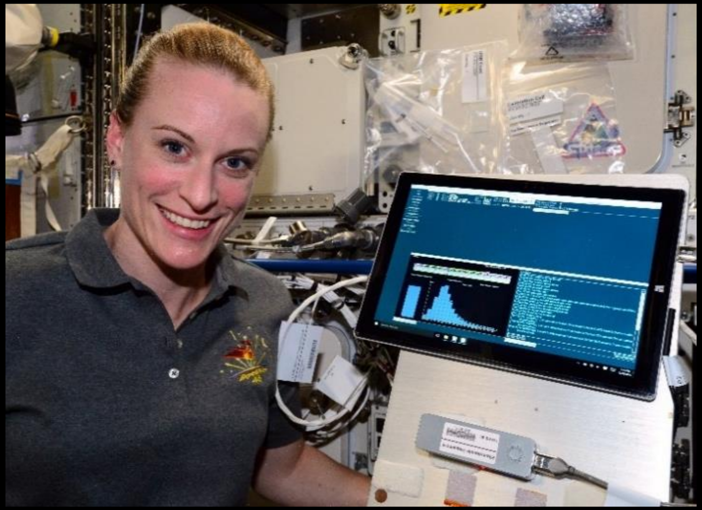
Microbial Monitoring of Water





Advances in Microbial Monitoring

- 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



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S. Stahl-Rommel, *et al. Genes* **12**, 106 (2021).

Environmental Health

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

- Microbiological monitoring occasionally identifies opportunistic bacterial pathogens, such as *S. aureus* and *Bacillus cereus*. No methicillin resistant *S. aureus* (MRSA) has been identified.
- *Klebsiella, Enterobacter* and *Enterococcus* species are occasionally identified throughout the ISS.

D. Pierson, *et al.* in *Environmental Monitoring: A Comprehensive Handbook* (DHI Publishing, 2012)
R. A. Blaustein, *et al.* *mSystems* **4**, doi:10.1128/mSystems.00281-18 (2019)

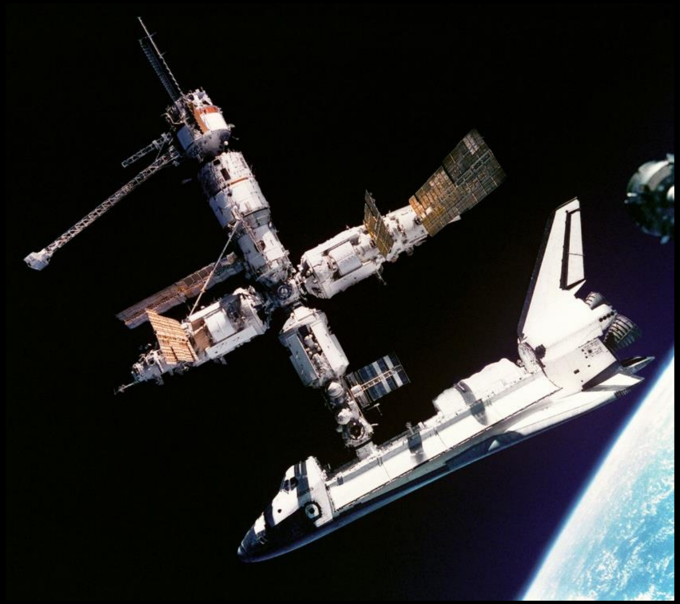
Environmental Health

- 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.
- Samples from potable water (for drinking and hygiene) indicate common water-borne bacterial species, including several species from the genera *Ralstonia*, *Burkholderia*, and *Cupriavidus*.

As ISS has many visiting vehicles with astronauts, "new" bacterial and fungal isolates are regularly identified.

Environmental Anomalies

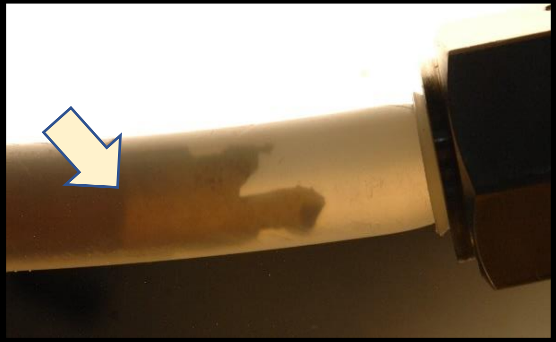
- Russian Mir Space Station (1986-2001)
  - Samples of free-floating condensate caused by power failures and problems with temperature control revealed high microbial diversity, including *Escherichia coli*, *Serratia marcescens*, *Legionella* species, and protozoa
- International Space Station (1998-present)
  - 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)

ISS Environmental Anomalies

Fungal Contamination
in ISS Module



Bacterial Biofilm in ISS
Drinking Water System

Fungal Contamination
in ISS Cabin Air

Fungal Plant Contamination

???

Spaceflight Microbiological Research

- Multiple spaceflight experiments over the past six decades have demonstrated unexpected microbial responses when microorganisms are cultured during spaceflight, including alterations in:
    - Antibiotic resistance
    - Biofilm formation
    - Growth rates
    - Virulence
    - Gene expression
- Rotating Wall Vessel (RWV) bioreactor
    - Fluid movement in the reactor simulates several aspects of the microgravity environment
    - Provides predictive and follow-up studies for spaceflight experiments

Fluid Processing Apparatus (FPA), an example of in-flight hardware

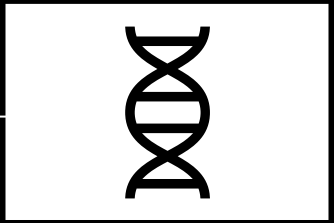
Low fluid shear culture conditions in the RWV prompted the term Low Shear Modeled Microgravity (LSMMG) environment

MICROBE

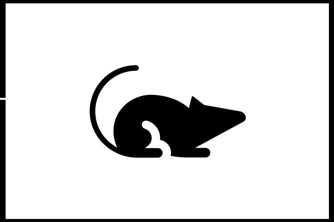
Shuttle Atlantis, STS-115, launch September 2006

***Salmonella* Typhimurium experiment design**

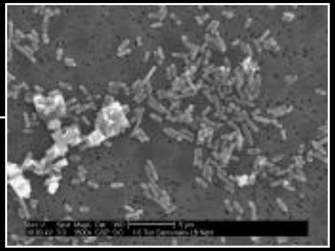
In-flight hardware



Gene expression



Disease potential



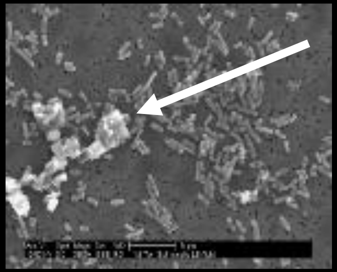
Cellular morphology

**** Synchronous ground controls maintained under identical conditions as those on-board Shuttle***

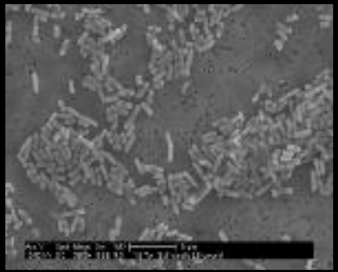
- ground and in-flight hardware loaded with same sample.

Wilson *et al. Proc Natl Acad Sci USA* 2007

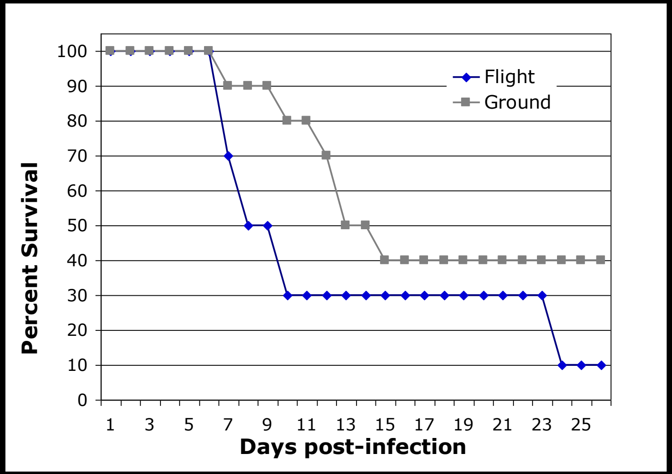
Salmonella Typhimurium Response to Spaceflight Culture



Flight Sample



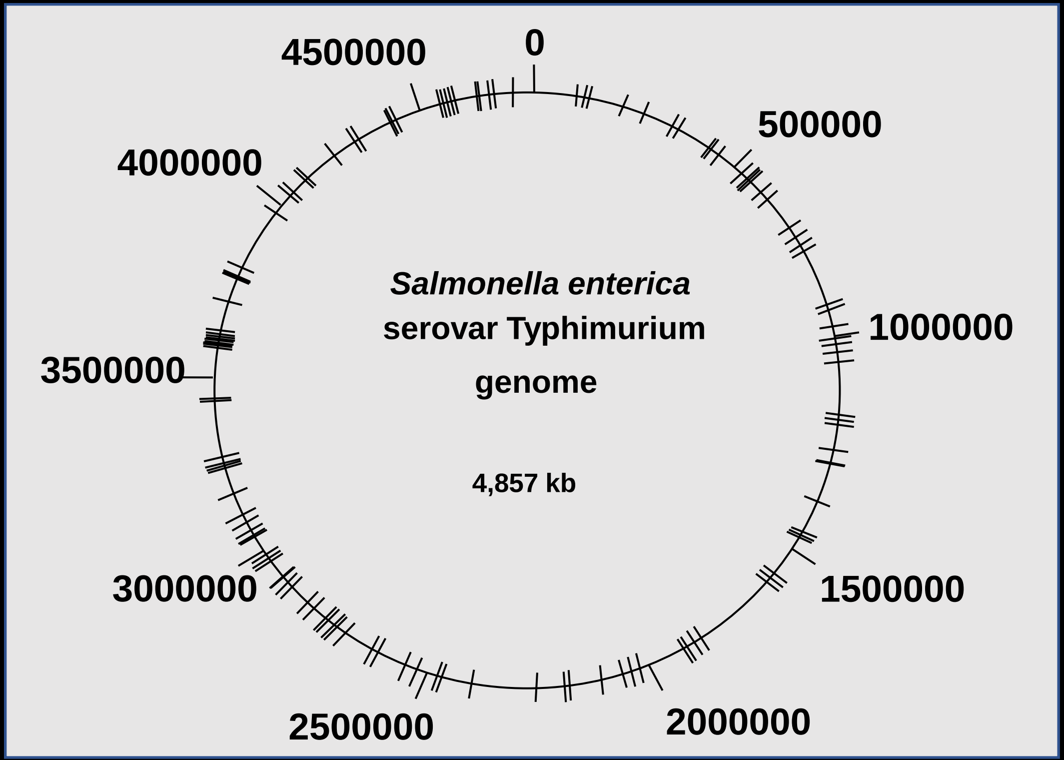
Ground Control



- 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_{50} was decreased 2.7 fold

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

Spaceflight Globally Alters *S.* Typhimurium Gene Expression



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

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

Hfq - Master molecular regulator identified

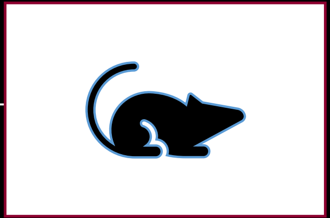
J. W. Wilson *et al. Proc Natl Acad Sci U S A* 104, 16299-16304 (2007)

MDRV

Shuttle Endeavour, STS-123, launch March 2008

***Salmonella* Typhimurium experiment design**

In-flight hardware



Disease potential

Independent validation of the STS-115 results

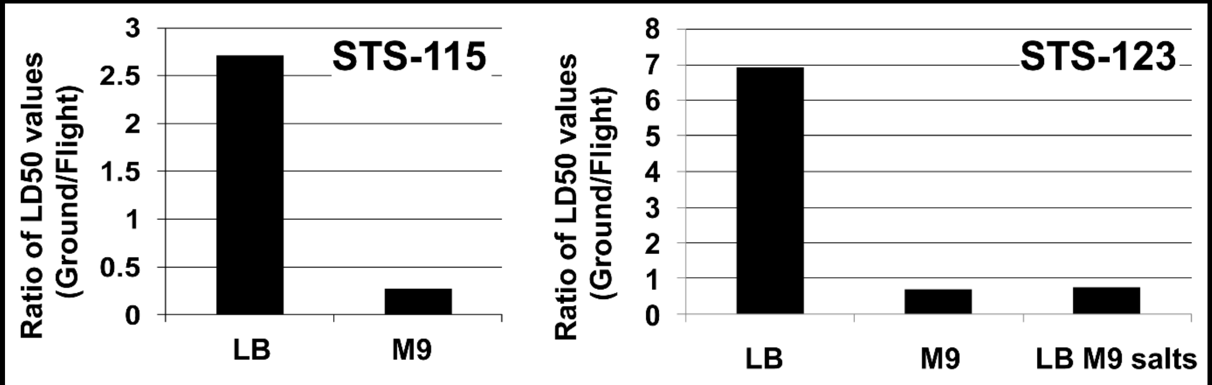
KH_2PO_4 NaH_2PO_4 NH_4Cl $MgSO_4$ $NaCl$

Media composition

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

J. W. Wilson *et al.* *PLoS One* 3, e3923 (2008)

The Impact of Media on Spaceflight Changes in *S*. Typhimurium Virulence



LB – Lennox Broth
M9 – Minimal salts media
LB M9 Media – LB media supplemented with inorganic salts (NaH_2PO_4, KH_2PO_4, NH_4Cl, $NaCl$, and $MgSO_4$)

Media	**Growth Location**	**LD_{50} (CFU)**	**Fold Increase Relative to LB Media - Flight**
LB media	Ground	4.02×10^5	1.0
LB-M9 salts media	Ground	5.73×10^5	1.4
M9 media	Ground	2.30×10^6	5.7

Media	**Growth Location**	**LD_{50} (CFU)**	**Fold Increase Relative to LB Media – Ground**
LB Media	Flight	5.81×10^4	1.0
LB-M9 salts media	Flight	7.45×10^5	12.8
M9 media	Flight	3.30×10^6	56.8

J. W. Wilson *et al. PLoS One* 3, e3923 (2008)

Mechanisms behind Unexpected Microbial Spaceflight Responses

- There is a general consensus that unexpected microbial responses during spaceflight are not a direct response to gravity, but rather an indirect response to the environment (*e.g.*, decreased hydrodynamic, convective forces)
- Comparison of data between spaceflight experiments is challenging due to differences in flight hardware, media types, aeration levels, and microbial species.

Possible Mechanisms

- Mechanotransductive
  - Lack of gravity and associated fluid forces create a quiescent environment without fluid shear forces inducing altered microbial responses
    - Fang, *et al. Appl Env Microbiol* 1997; Nickerson, *et al. MMBR* 2004; Nauman, *et al. Appl Env Microbiol* 2007
- Extracellular mass diffusion
  - The quiescent spaceflight environment suppresses the natural flow of nutrients into and waste out of the cell, altering microbial response
    - Klaus, *et al. Microbiology* 1997

Mechanisms behind Unexpected Microbial Spaceflight Responses

- Hfq Spaceflight and/or Spaceflight Analogue Regulation Identified in a Variety of Microorganisms
  - *Salmonella enterica* Serovar Typhimurium
    - Wilson, *et al. PNAS* 2007 (spaceflight); Wilson, *et al. PNAS* 2002 (spaceflight analogue)
  - *Pseudomonas aeruginosa*
    - Crabbé, *et al. Appl Environ Microbiol* 2011 (spaceflight); Crabbé, *et al. Envion Microbiol* 2010 (spaceflight analogue)
  - *Staphylococcus aureus*
    - Castro, *et al. Appl Environ Microbiol* 2011 (spaceflight analogue)
  - *Vibrio fischeri* (as part of a symbiotic squid model)
    - Grant, *et al. Int J Astrobiol* 2013 (spaceflight analogue)

Selected Recent Studies

- PI: Dr. Mark Ott, NASA
  - Co-PI: Dr. Cheryl Nickerson, Arizona State University
  - Determine if growth in the RWV spaceflight analogue (RWV) environment increases virulence in other bacteria, five pathogenic species are being investigated to characterize changes in stress response, adhesion/invasion of 3-D tissue culture models, and animal testing if warranted.

- PI: Dr. Bob McLean, Texas State University
  - Co-PI: Dr. Cheryl Nickerson, Arizona 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.

Selected Recent Studies

- PI: Dr. Cheryl Nickerson, Arizona State University
  - Investigate the combined effect of spaceflight analogue (RWV) culture with low dose radiation to determine if the combination synergistically increases the potential infection risk.
- PI: Dr. Hernan Lorenzi, J Craig Venter Institute
  - First comprehensive study of the microbiomes of nine astronauts on ISS, including monitoring of skin sites, nostrils, fecal samples collected preflight, in-flight, and post-flight
  - Identified changes in the astronaut intestinal and skin microbiomes

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

Selected Recent Studies

- PI: Dr. Janet Jansson, Pacific Northwest National Laboratory
  - Investigate how microgravity affects metabolic interactions in communities of soil microbiomes.

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

- PI: Dr. Cheryl Nickerson, Arizona State University
  - Evaluate the effect of spaceflight on the host-pathogen interaction between human intestinal epithelial cells and *S*. Typhimurium

J. Barrila , *et al. npj Microgravity* (2021)

Questions?