Microbial Control During Spaceflight:

Preventing Infectious Disease in Very Remote Locations

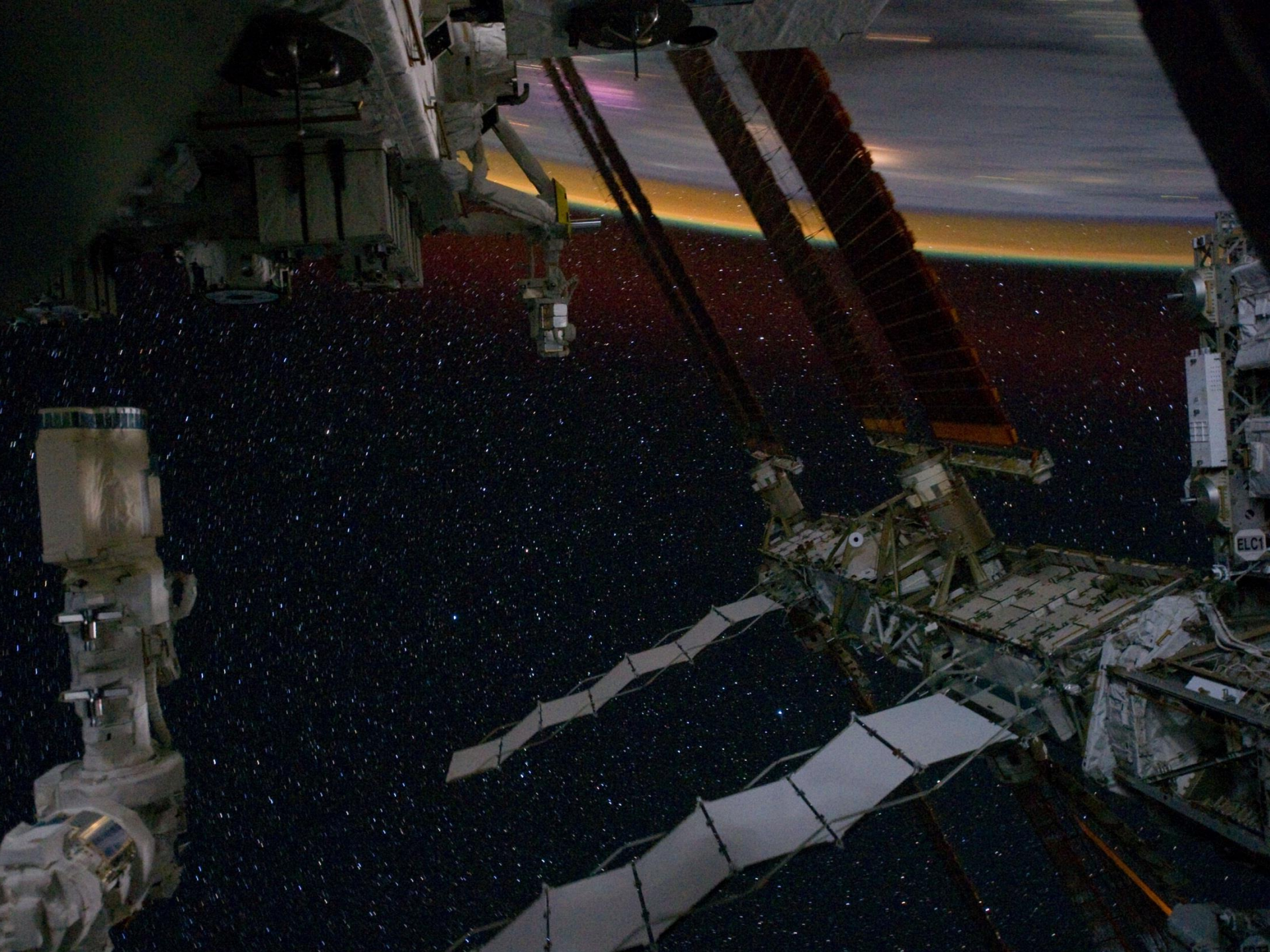


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NASA Johnson Space Center, Houston, TX


NASA/ESA and The Hubble Heritage Team (STScI/AURA)


ELC1

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
  - Astronaut hygiene areas
- Spaceflight foods
  - In addition to packaged food, NASA is developing "pick and eat" vegetable growth systems
- Planetary protection
  - How do we track the microorganisms we are bringing versus what we are finding in our search for life on other planets?





Prevention - Spacecraft Design

- Vehicle surfaces
  - Use of materials that are not conducive to microbial growth
  - Remediation: Disinfectant wipes
- Vehicle air
  - Air flow through HEPA filters
- 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
  - Remediation: High concentration iodine solution



Prevention - Operations



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

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, 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.



Contamination Potential



Preflight contamination



Spacecraft are complex



Astronaut activities, such as eating and hygiene

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

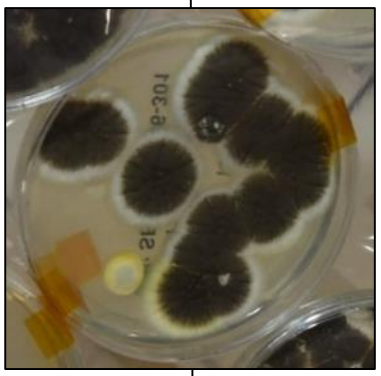
Surfaces





Air





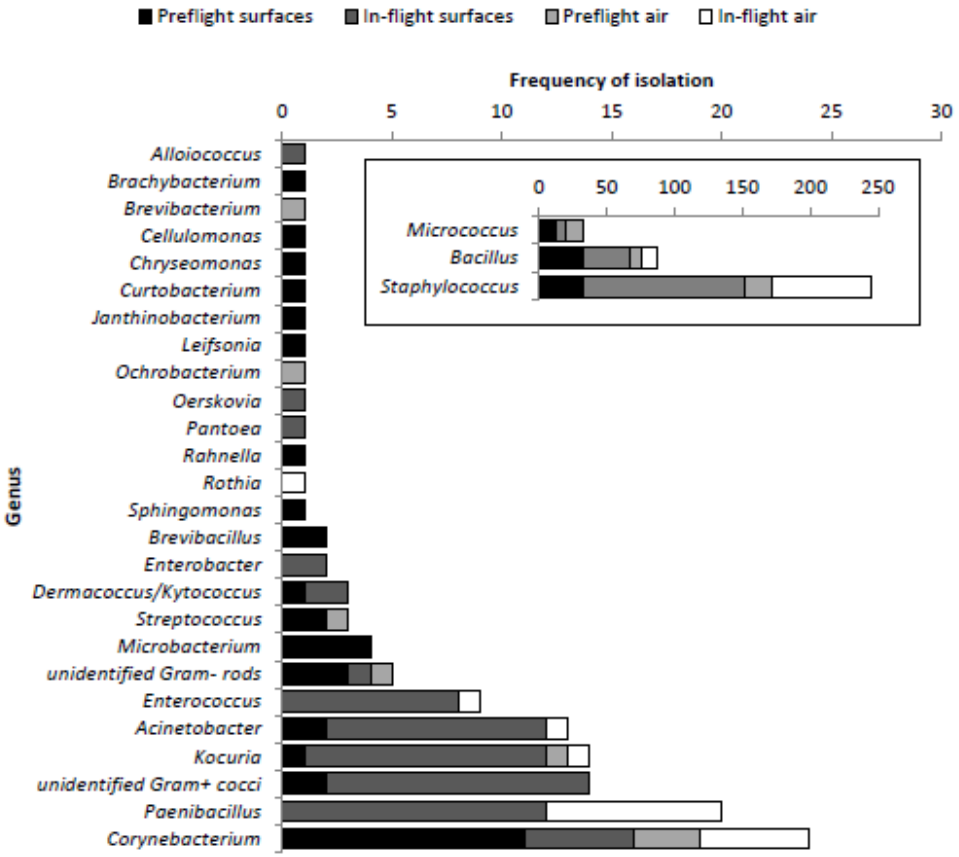
Water





Quantified in-flight and returned to JSC for identification

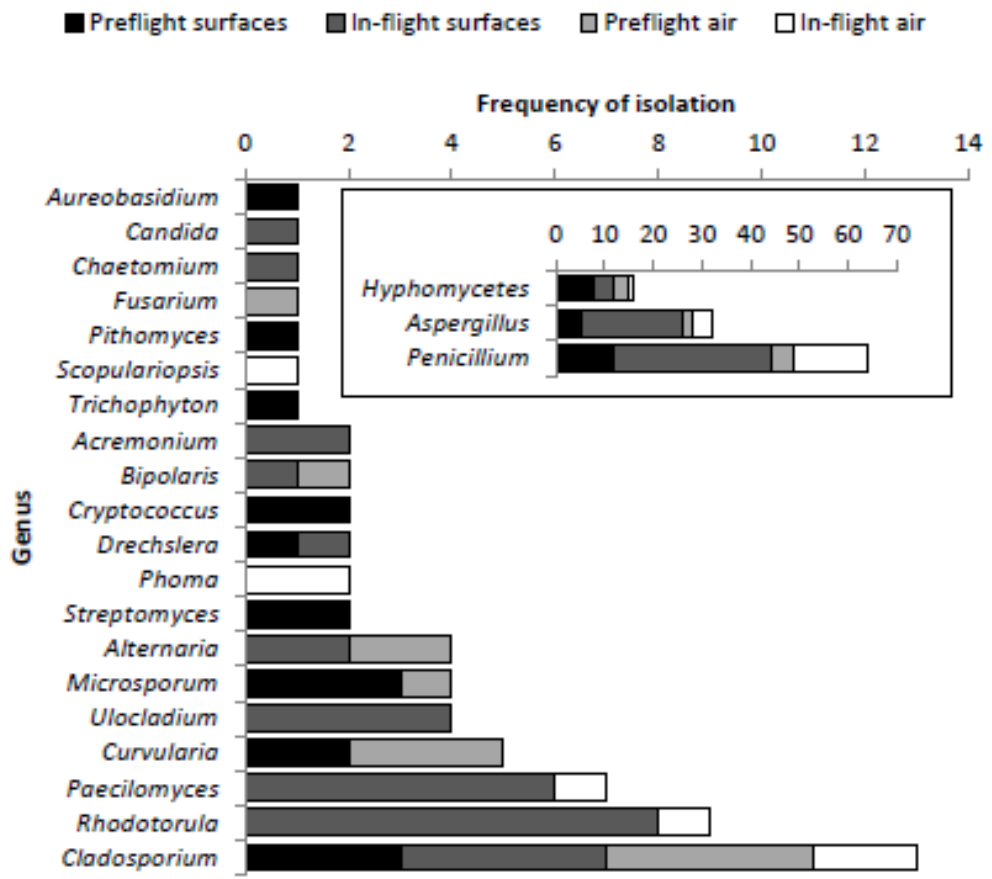
ISS Air and Surface Monitoring
Bacterial Isolates





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

ISS Air and Surface Monitoring
Fungal Isolates

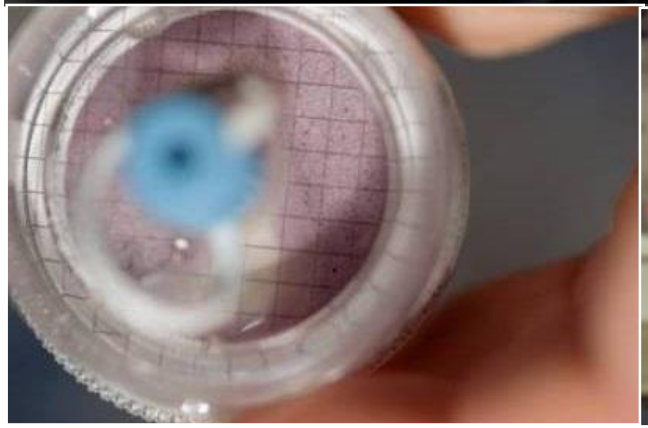




Pierson, *et al.* Environmental Monitoring: A Comprehensive Handbook. 2012

Microbiological Monitoring of Water







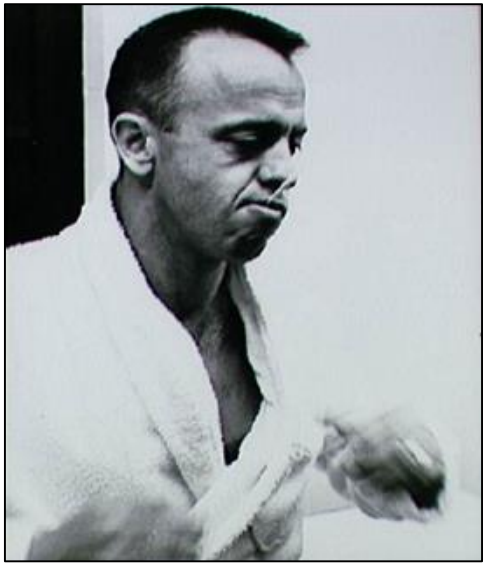
U. S. Potable Water Dispenser

- 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*



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

Astronaut Health

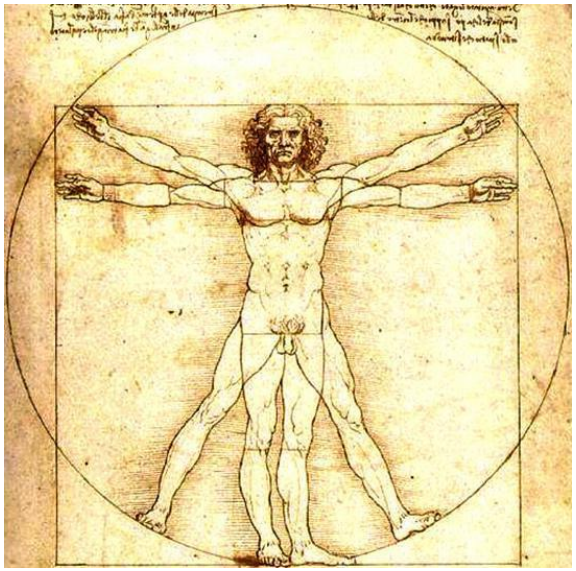


- Diagnosis is often based on symptomology
  - Headache
  - Rash
  - Dry hacking cough
  - Diarrhea
- Examples of diseases during spaceflight missions
  - Upper respiratory infections
  - Urinary tract infections
  - Ear infections
  - Herpes Zoster
  - Rashes & skin disorders
  - Gastroenteritis



Astronaut Health

- Generally healthy crew
  - 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)
  - Infectious disease and allergic symptom rate on the International Space Station (ISS) has been estimated at 3.4 events/flight year
- Immune system evidence for long duration spaceflight
  - Reduced function and distribution of some immune cells (e.g., natural killer (NK) cells, T cells, monocytes, and neutrophils)
  - Altered cytokine production patterns
  - Reactivation of latent viruses



Risin. Human Health and Performance Risks of Space Exploration Missions, 2012;
Crucian, *et al.* Int J Gen Med, 2016

Environmental Health



- Microbial monitoring by NASA and others indicate **ISS environmental flora** reflect human-associated microorganisms **similar to a home**.
- 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.

Pierson, *et al.* Environmental Monitoring: A Comprehensive Handbook. 2012;
Lang, *et al.* PeerJ, 2017; Blaustein, *et al.* mSystems, 2019.

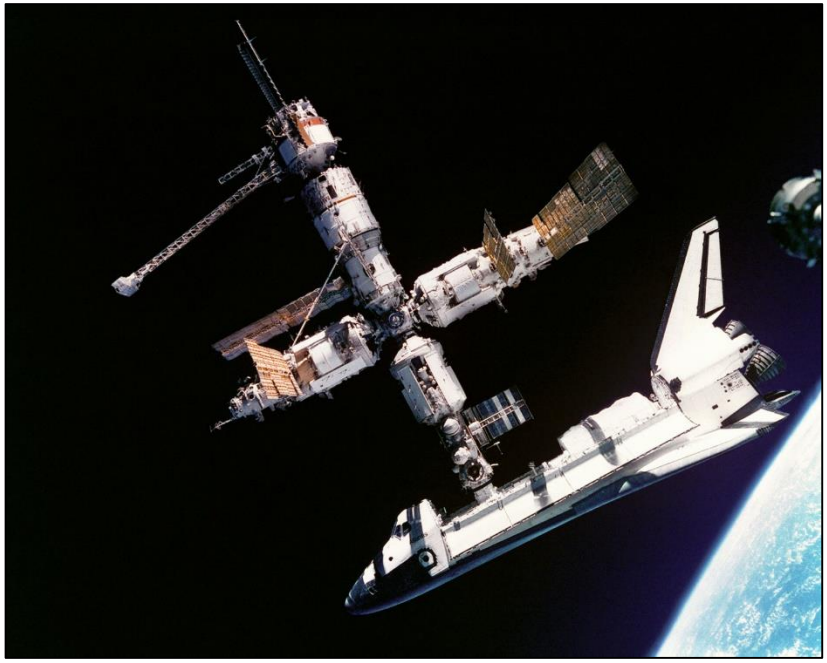
Environmental Health

- 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.
- Potable water reflects common environmental 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

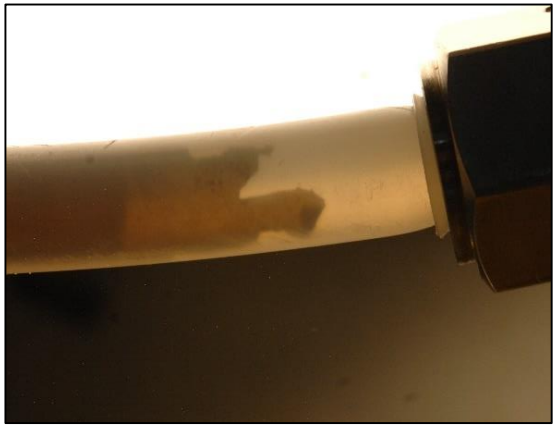


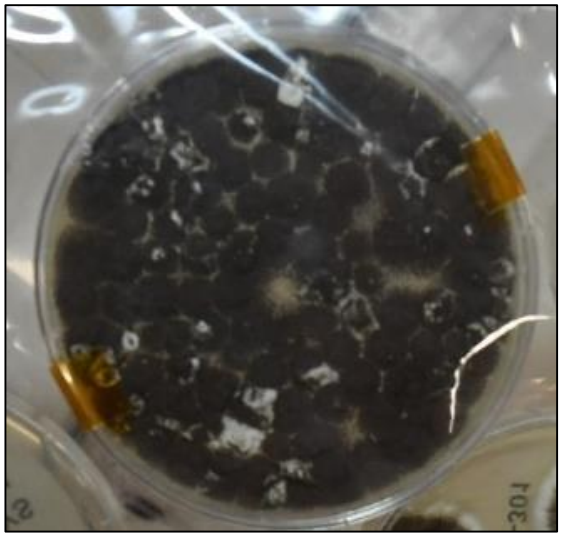
- Mir Space Station
  - Power failures and problems with temperature control eventually caused large amounts of free-floating condensate
  - Samples of condensate revealed high microbial diversity, including ***Escherichia coli, Serratia marcescens, Legionella*** species, and **protozoa**
- ISS anomalies
  - Surface contamination is often associated with uncontrolled water
  - Air contamination and unpleasant odors do occur; however, the sources are often unclear. These events have been transient and/or handled by the HEPA filtration system

Ott, *et al.* Microb Ecol, 2005

ISS Environmental Anomalies





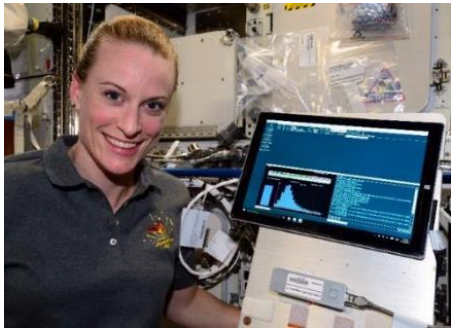


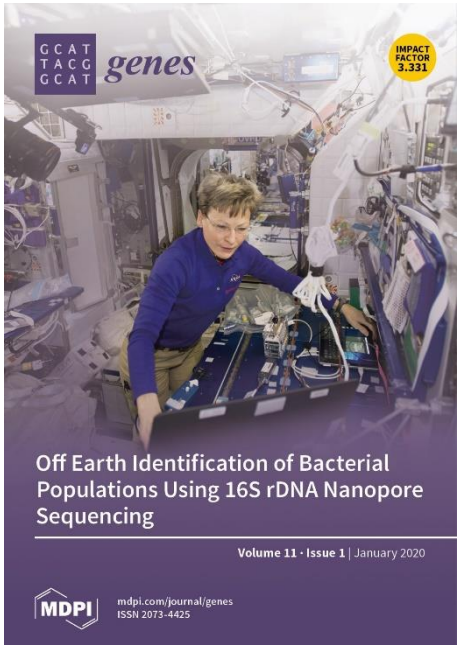




Advances in Environmental Monitoring

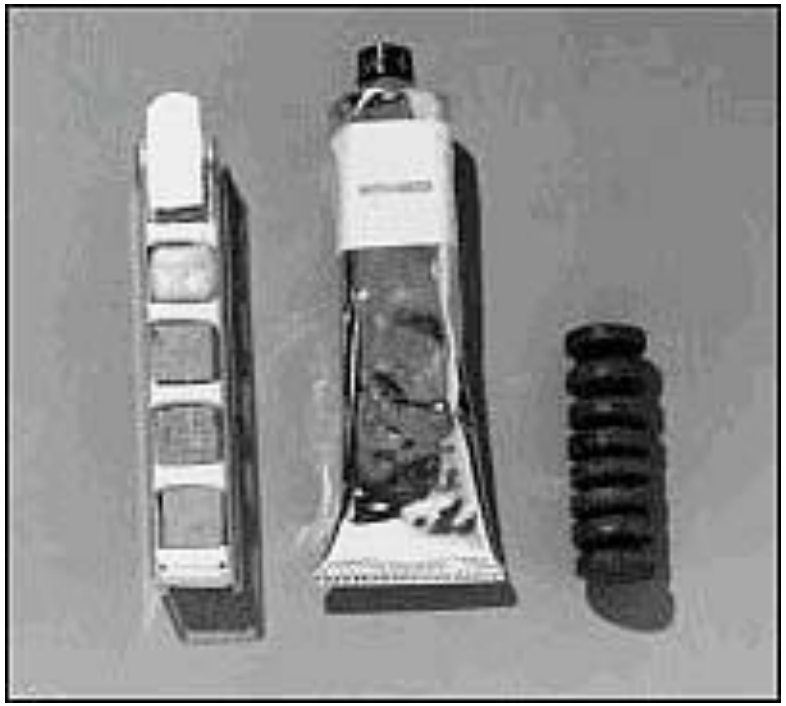
- DNA sequencing in space
  - First performed in 2016 using Oxford Nanopore's MinION using lambda bacteriophage, *E. coli*, and mouse DNA
- In-flight identification of environmental samples
  - Three microbial colonies from a media plate used for ISS surface sampling were processed and sequenced on ISS.
  - The MinION accurately identified the three isolates that were selected.
- Major push to convert our current monitoring hardware to this technology, especially for deep space missions.





Castro-Wallace *et al.* 2017; Burton *et al.* 2020

Spaceflight Food





Early Disqualified Food Samples

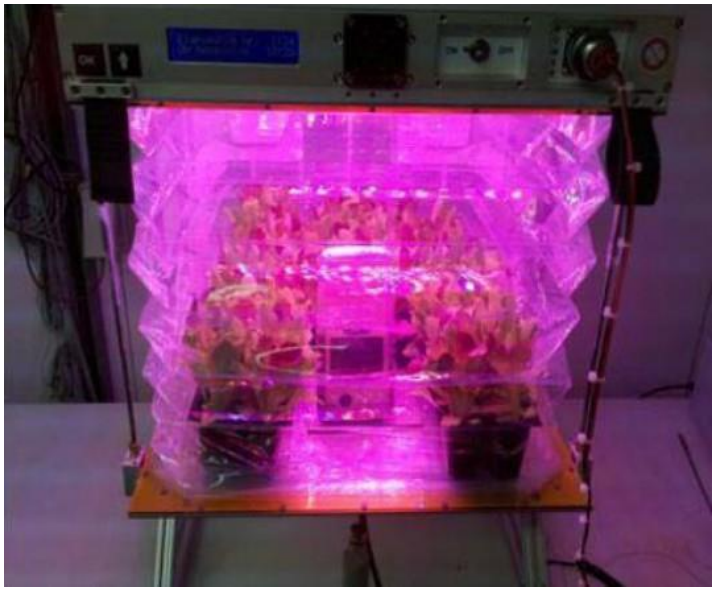
International Space Station (ISS)

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*

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The Future of Spaceflight Food

- The VEGGIE spaceflight experiment grew lettuce on ISS
  - Can the crew eat it?
  - What about other crops besides lettuce?
- Probiotics are being evaluated as an immune booster
  - What organisms do we monitor for safety?
- We are planning to prepare and cook more food on spaceflight
  - How do we control contamination during processing?





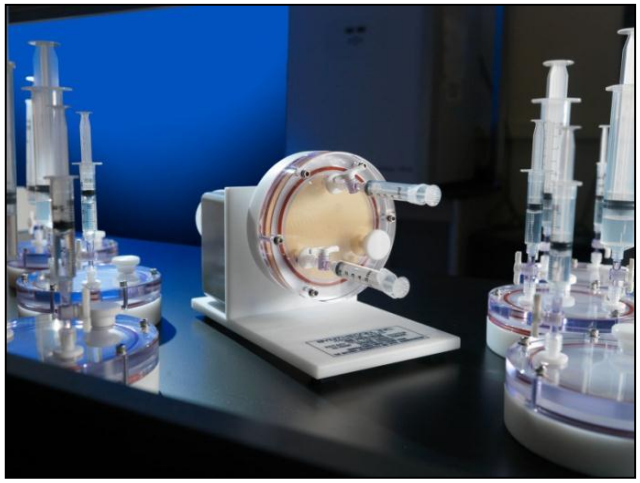
Microbial Research in Space

- Multiple experiments over the past 50 to 60 years have demonstrated **unexpected microbial responses** when microorganisms are cultured during spaceflight, including:
  - Antibiotic resistance
  - Growth kinetics
  - Virulence
  - Gene expression
- Spaceflight research has been burdened by sensationalized or unsubstantiated conclusions.
  - Highly visible field with limited access to rerun experiments for confirmation
  - Misconceptions from these reports can last for years and actually be damaging to many of the real scientific breakthroughs



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)

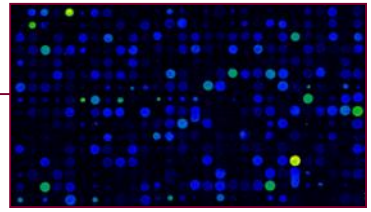
MICROBE

Shuttle Atlantis, STS-115, launch September 2006

***Salmonella* Typhimurium experiment design**



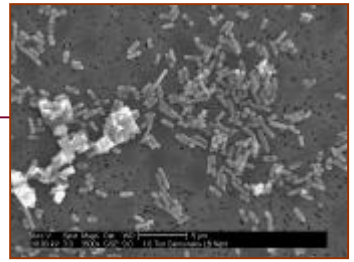
In-flight hardware



Gene expression



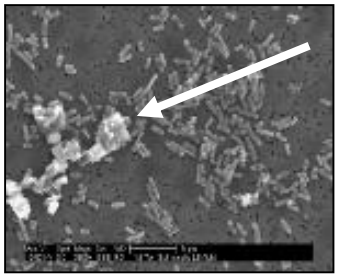
Disease potential



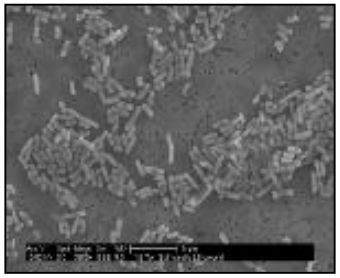
Cellular morphology

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

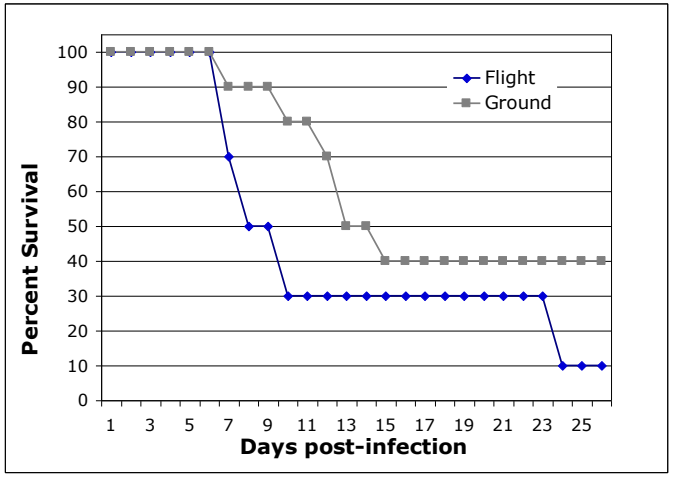
Salmonella Typhimurium Response to Spaceflight Culture



Flight Sample



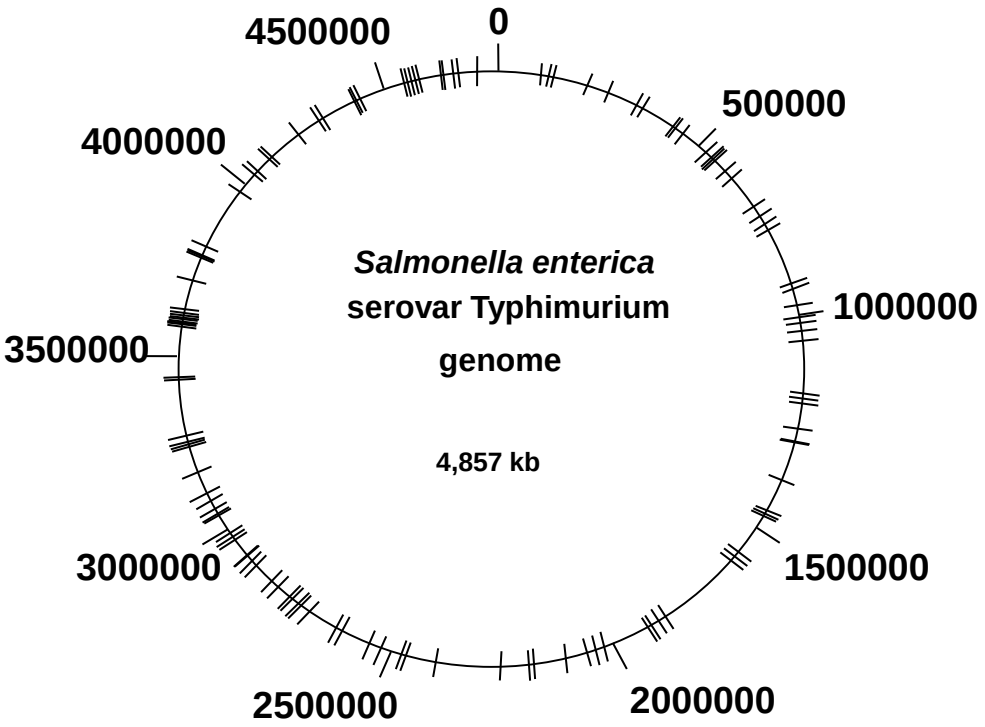
Ground Control



- 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

Wilson *et al.* PNAS, 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

Wilson *et al.* PNAS, 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

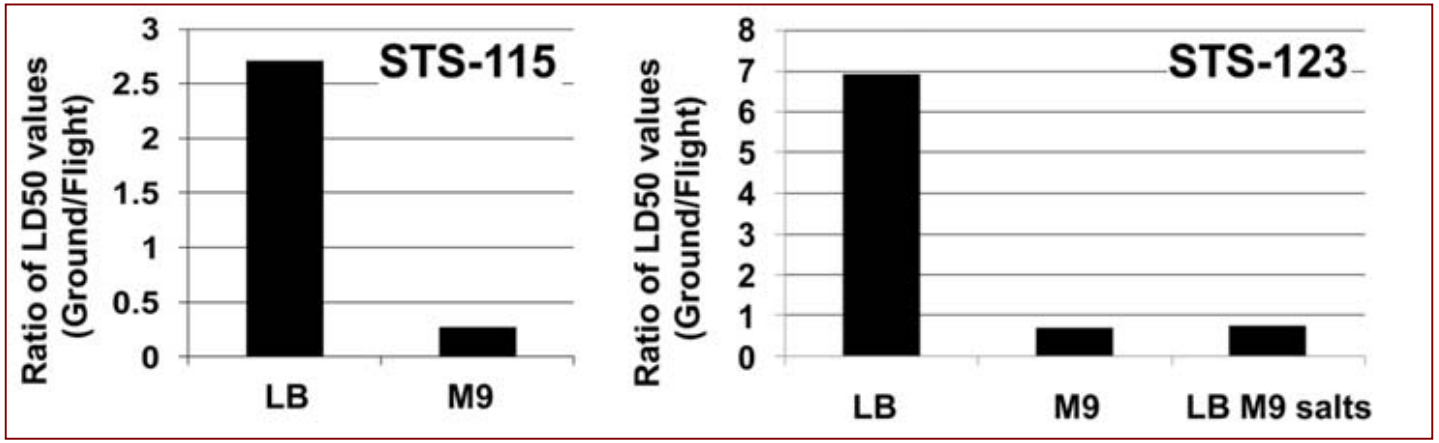


Media composition

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

Wilson *et al.* PLoS One 2008

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

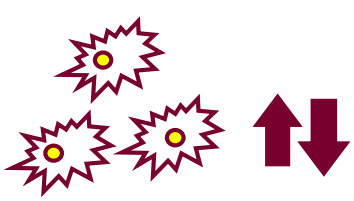


Media	Growth Location	LD_{50} (CFU)	Fold Increase Relative to LB Media - Flight
LB media	Flight	5.81×10^4	1.0
LB-M9 salts media	Flight	7.45×10^5	12.8
M9 media	Flight	3.30×10^6	56.8

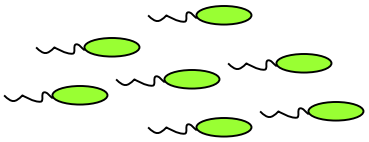
Media	Growth Location	LD_{50} (CFU)	Fold Increase Relative to LB Media - Ground
LB Media	Ground	4.02×10^5	1.0
LB-M9 salts media	Ground	5.73×10^5	1.4
M9 media	Ground	2.30×10^6	5.7

Wilson *et al.* PLoS One 2008

Summary *Pseudomonas aeruginosa*

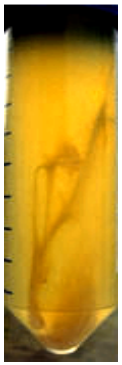


Stress Resistance



Pseudomonas aeruginosa

Production virulence factors



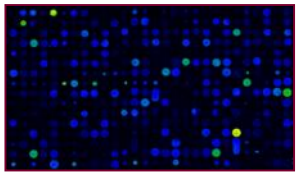
Biofilm production







Gene Expression

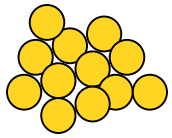




Hfq - evolutionarily conserved low fluid shear response regulator in other pathogens

Crabbé *et. al.* Environ Microbial, 2008;
Crabbé *et al.* Environ Microbial, 2010; Crabbé *et al.* Appl Environ Microbial, 2011

Summary *Staphylococcus aureus*



Staphylococcus aureus

Stress Resistance

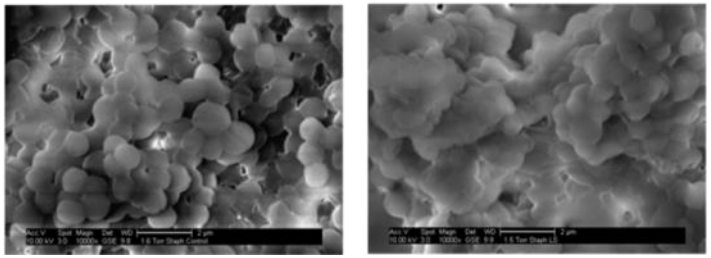
Carotenoid Production (Virulence Factor)





RWV Bioreactor

EPS production

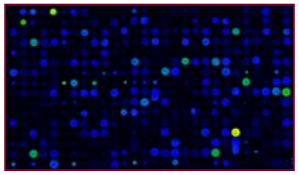


Control LSMMG



Control LSMMG

Gene Expression



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

Castro *et al.* Applied Environ Microbial, 2011

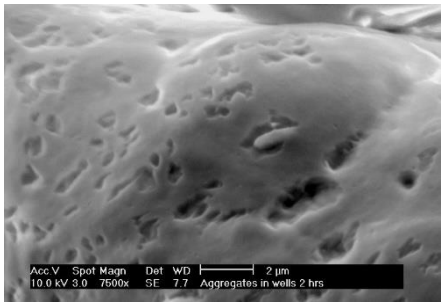
Crew Microbiome



- **PI: Hernan Lorenzi, J. Craig Venter Institute**
- **Baseline** study of skin sites, nostrils, fecal samples from 9 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.
- Set the baseline for astronaut microbiome studies with low sample size and high options for metadata

Voorhies *et al.* Sci Rep, 2019

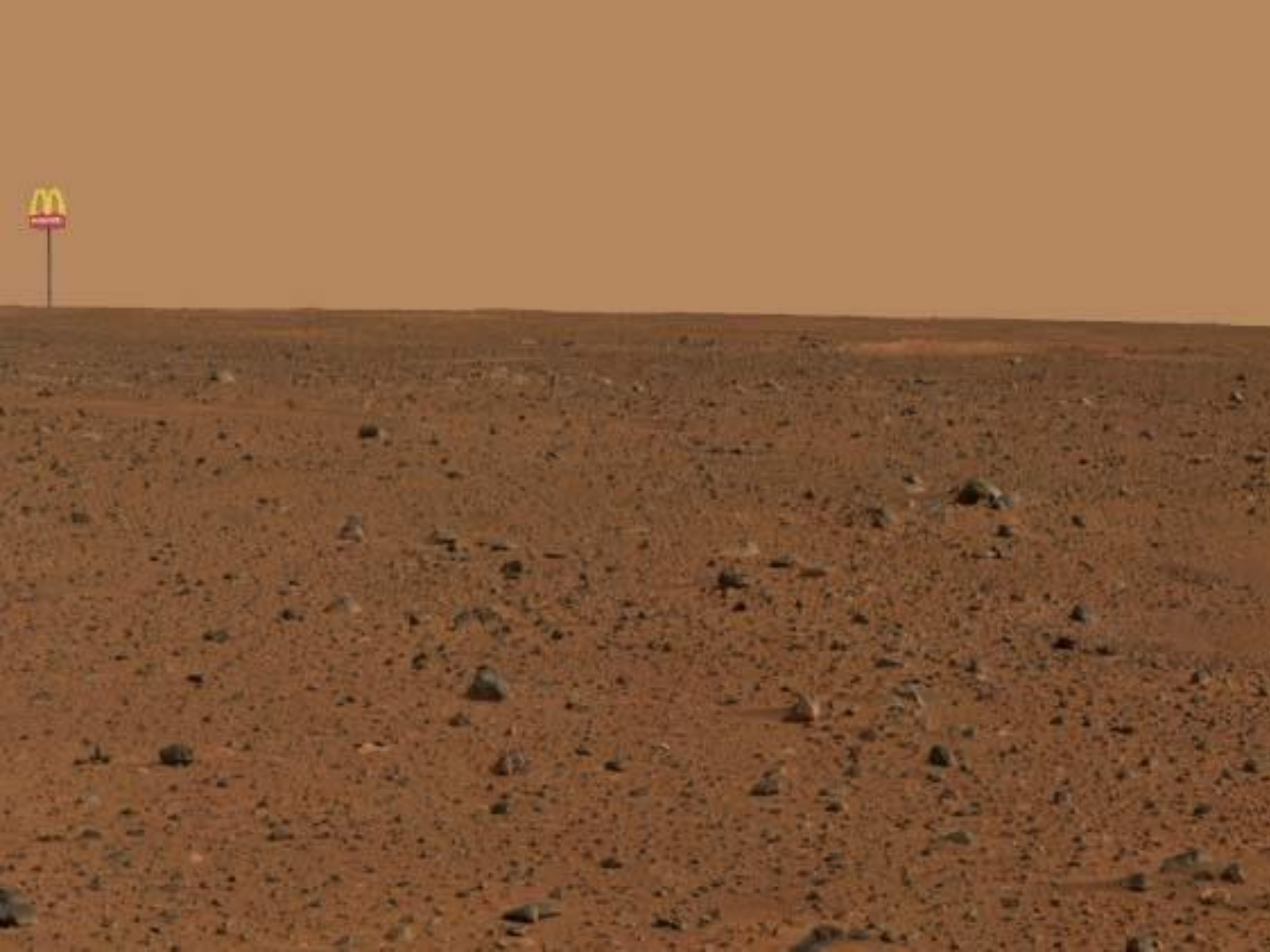
Current Experiments



- **PI: Mark Ott, NASA**
- Collaboration with Chery Nickerson at Arizona State University.
- This ground-based study builds upon the discovery that growth in the spaceflight and spaceflight analogue environments induced changes in *Salmonella* virulence by evaluating the impact of the spaceflight analogue culture on a variety pathogens .



- **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.
- Biofilm studies have been ongoing since the first study was reported by McLean in 2001.





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