Spaceflight Microbiology

From Small Things, Big Things One Day Come

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University of Notre Dame 2020


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


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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
  – 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?

Astronaut Health

- Preventative measures
  - Preflight medical exams and medical consult throughout a mission
  - Preflight crew quarantine
  - Stringent microbiological monitoring of spacecraft and its cargo
- Examples of diseases during spaceflight missions
  - Upper respiratory infections
  - Urinary tract infections
  - Ear infections
  - Herpes Zoster
  - Rashes & skin disorders
  - Gastroenteritis

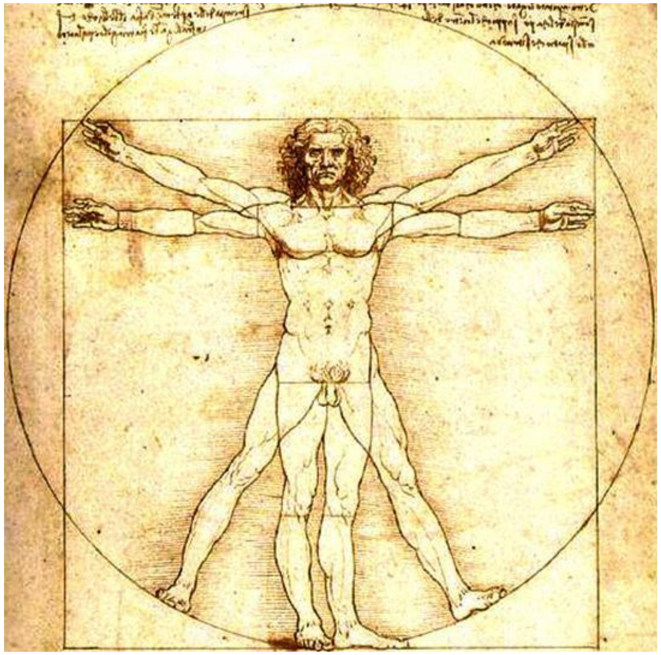

S114E5979

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) (Risin 2009)
  - Infectious disease and allergic symptom rate on the International Space Station (ISS) has been estimated at 3.4 events/flight year (Crucian *et al.* 2016)
- 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)
  - Reactivation of latent viruses
  - Altered cytokine production patterns

Infectious Disease during Spaceflight

- Diagnosis is often based on symptomology
  - Headache
  - Rash
  - Dry hacking cough
  - Diarrhea
- Attributed to infectious disease
  - Upper respiratory infections
  - Ear infections
  - Various fungal infections
  - Herpes Zoster
  - Gastroenteritis
  - Stye
  - Allergic reactions
  - Rashes & skin disorders



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

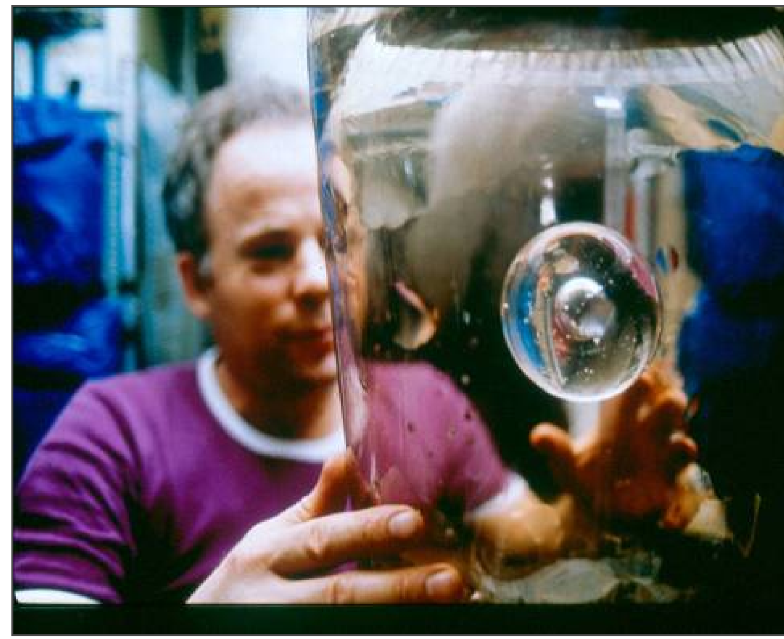
Preflight contamination

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

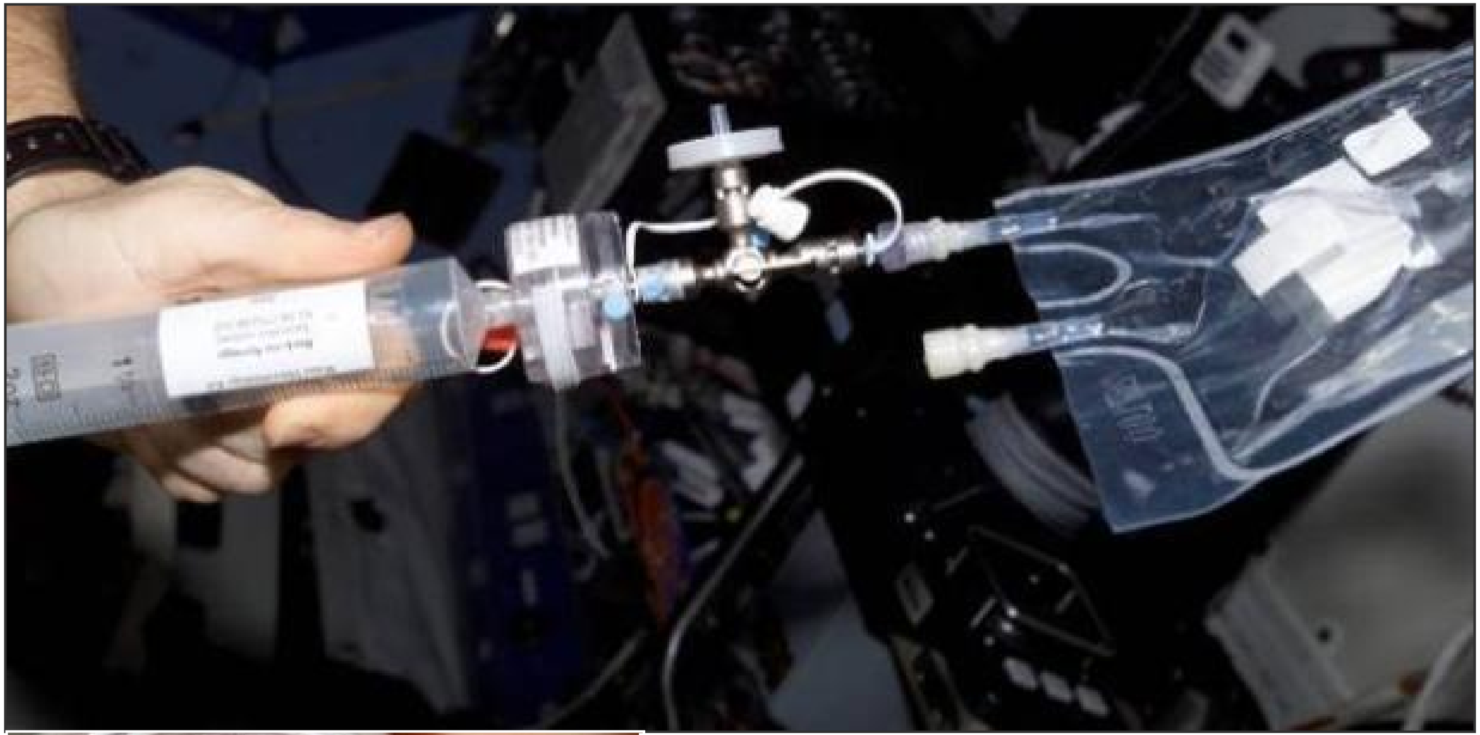
Surfaces

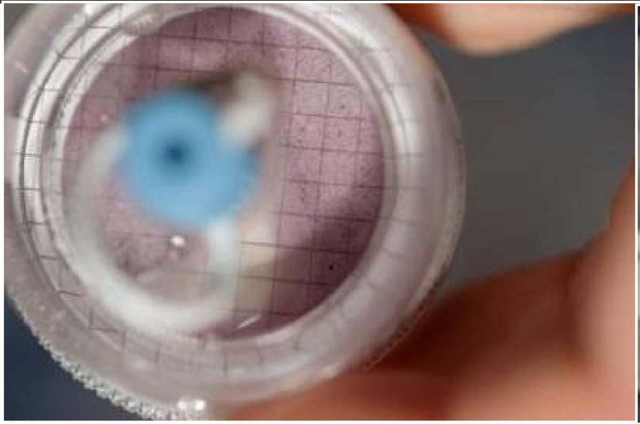
Air

Water

Quantified in-flight and returned to JSC for identification

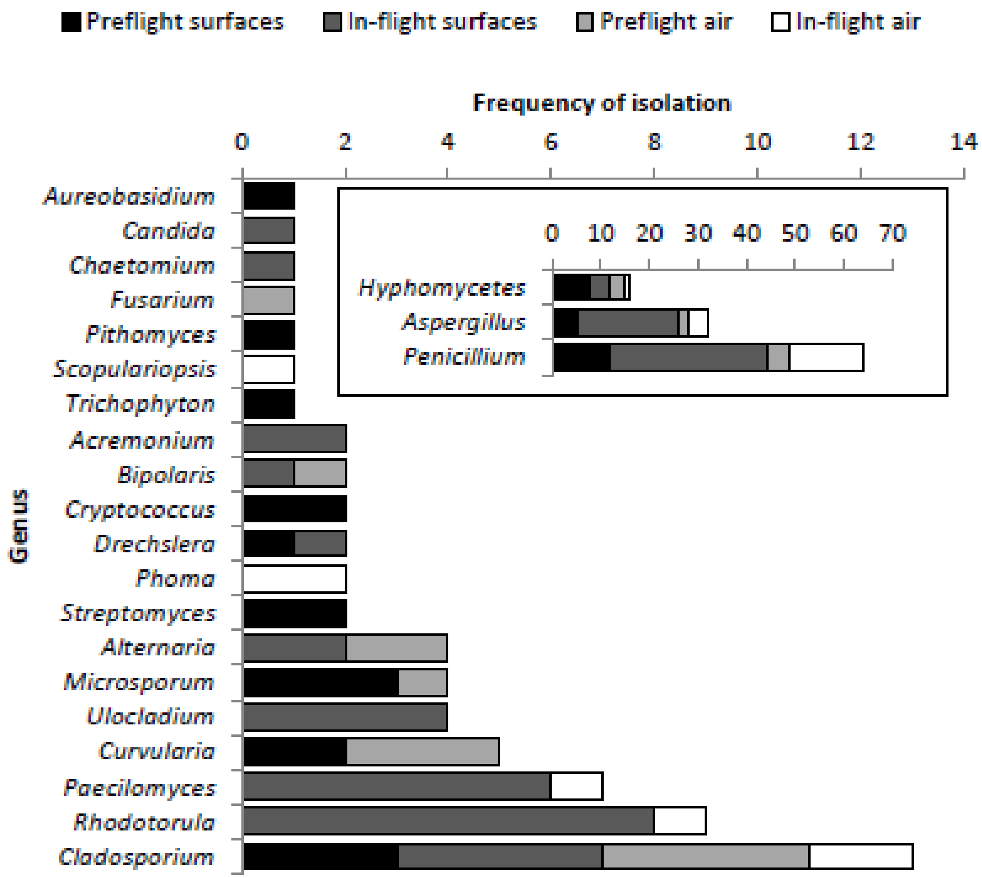
Microbiological Monitoring of Water





ISS Air and Surface Monitoring

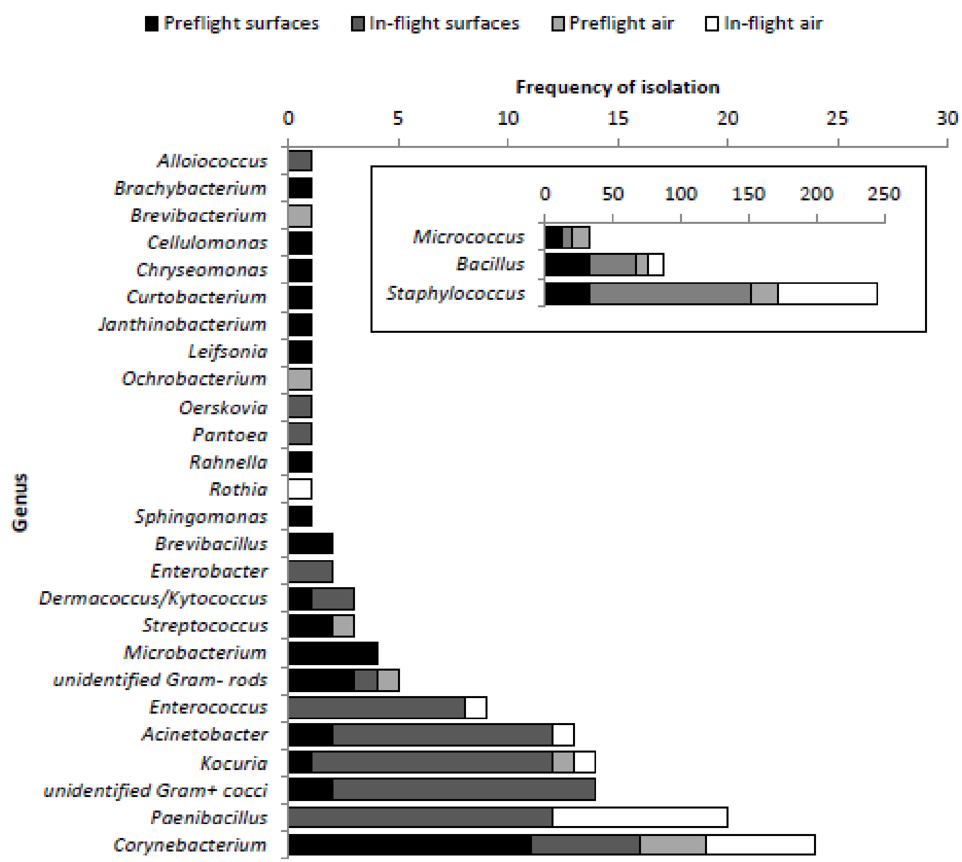
Fungal Isolates



Pierson, *et al.* 2012

ISS Air and Surface Monitoring

Bacterial Isolates



Pierson, *et al.* 2012

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.* 2012


SPACE STATION
CONSTRUCTION
SPEED
LIMIT
17500
28000
km/h
EXIT
To A/L

Environmental Health

- Microbial monitoring by JSC 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.

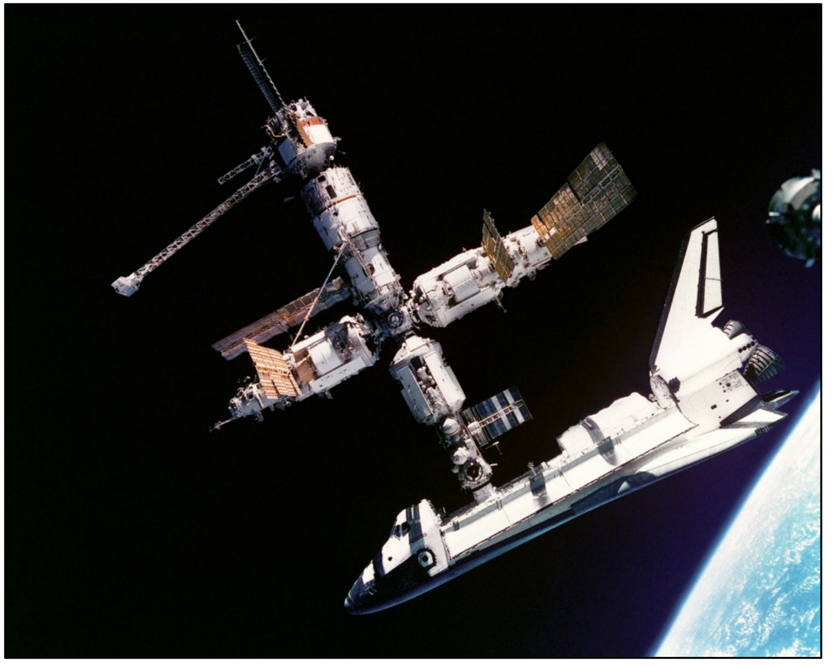
Pierson, *et al.* 2012; Lang, *et al.* 2017; Blaustein, *et al.* 2019

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.

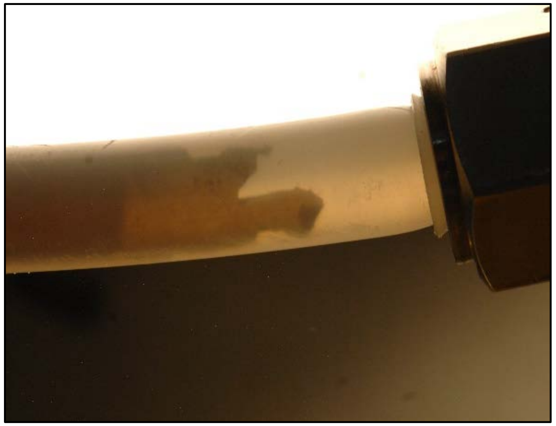
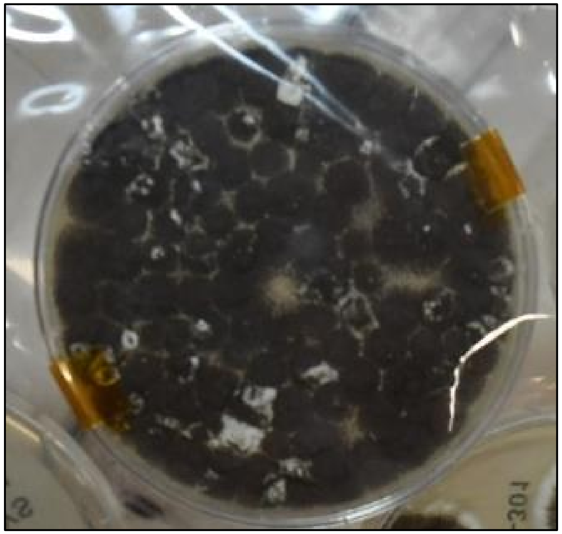
ISS016E034855

Environmental Anomalies



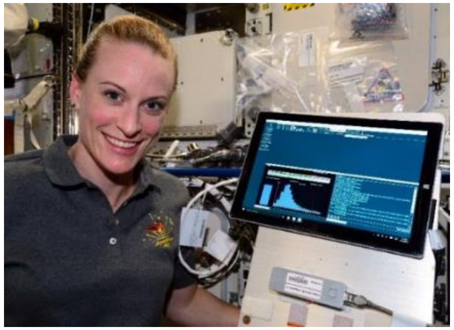
- Mir Space Station
  - Power failures and problems with temperature control eventually caused large amounts of free-floating condensate
  - Samples of condensate revealed high microbial diversity, including ***Escherichia coli, Serratia marcescens, Legionella*** species, and **protozoa** (Ott *et al*., 2005)
- ISS anomalies
  - Surface contamination is often associated with uncontrolled water
  - Air contamination and unpleasant odors do occur; however, the sources are often unclear. These events have been transient and/or handled by the HEPA filtration system

ISS Environmental Anomalies

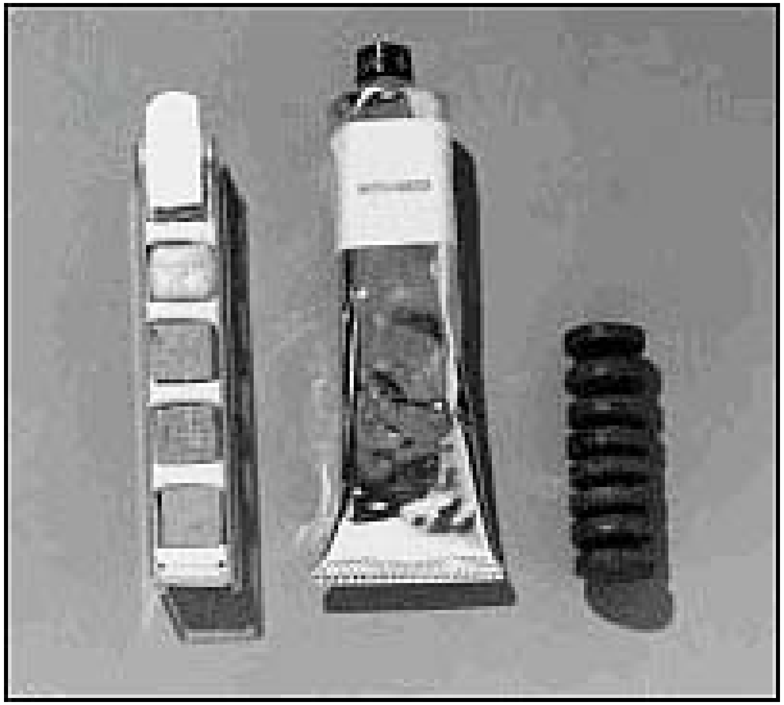
Advances in Environmental Monitoring

- 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

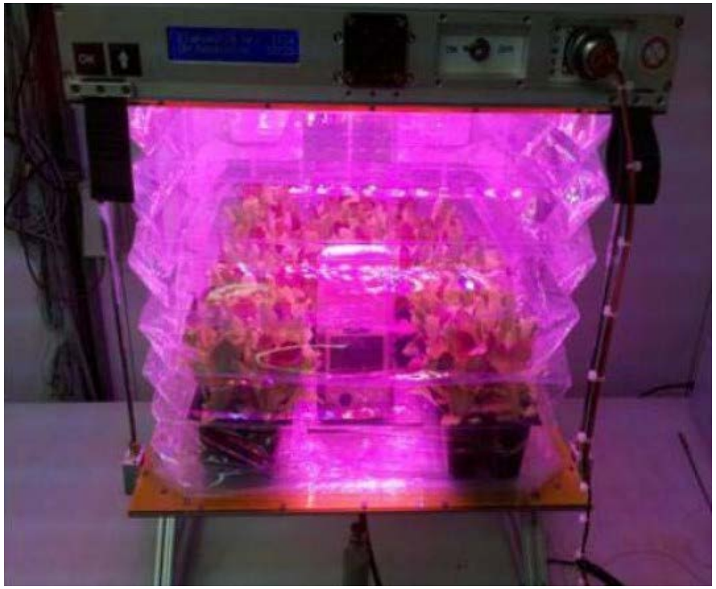
International Space Station (ISS)

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*

Spaceflight Food

The Future of Food Requirements

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

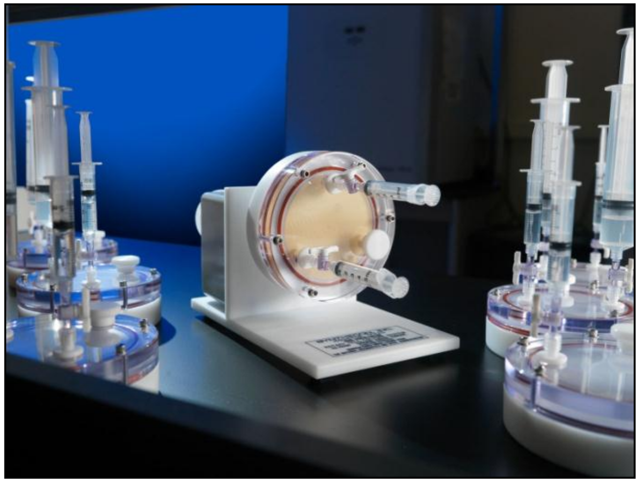


Microbial Research in Space

- 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

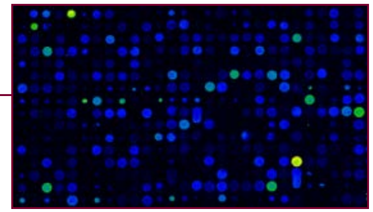
Concordia Station (Antarctica)

MICROBE

Shuttle Atlantis, STS-115, launch September 2006

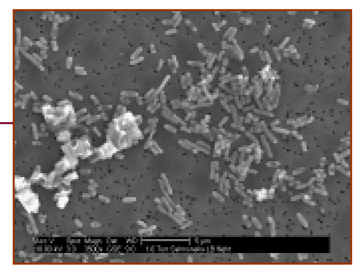
Salmonella Typhimurium experiment design

In-flight hardware



Gene expression

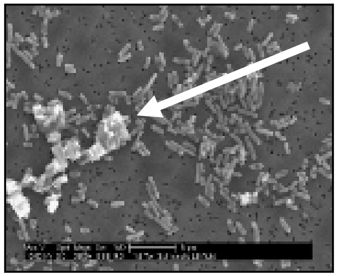
Disease potential



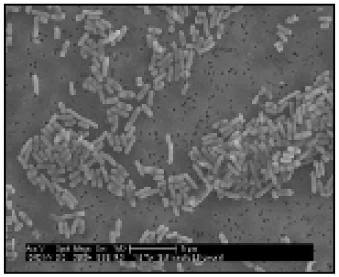
Cellular morphology

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

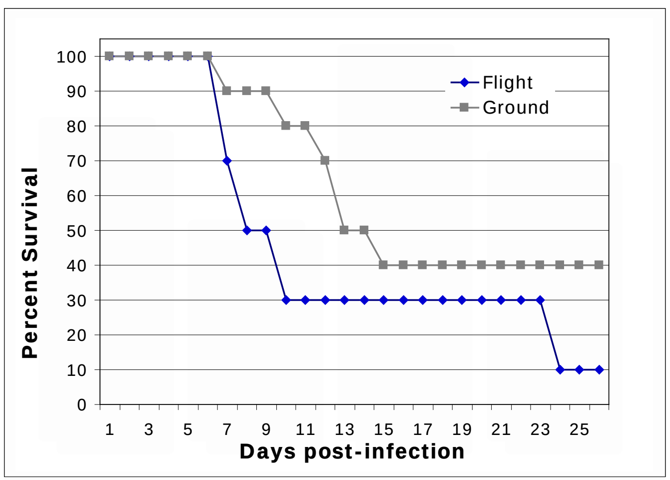
Salmonella Typhimurium Response to Spaceflight Culture



Flight Sample

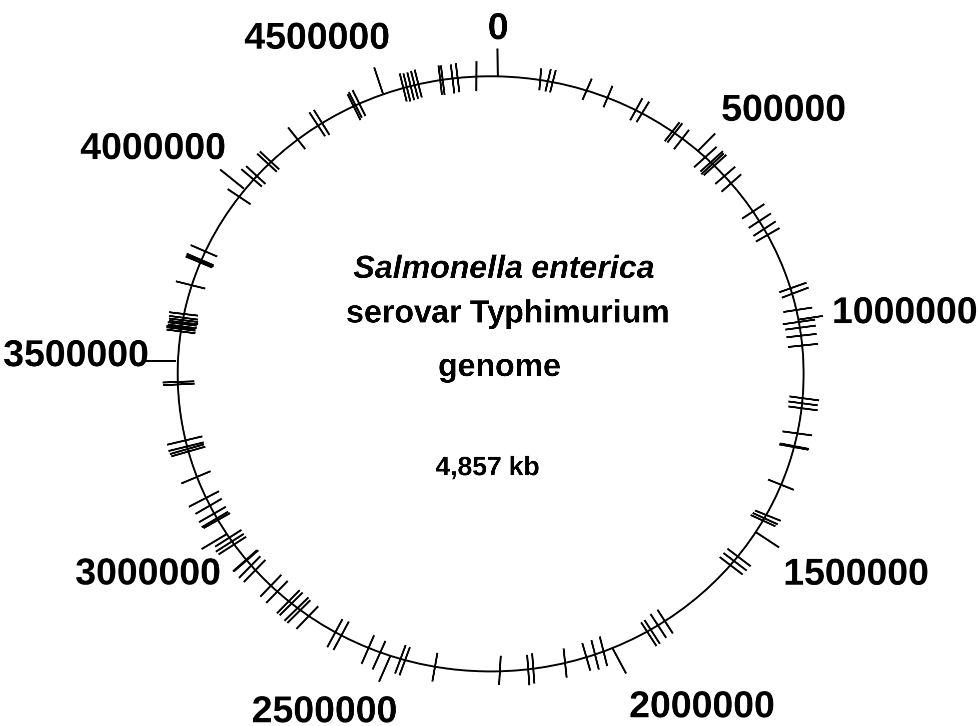


Ground Control



- 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

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. Proc Natl Acad Sci USA* 2007

MDRV

Shuttle Endeavour, STS-123, launch March 2008

Salmonella Typhimurium experiment design

In-flight hardware

Disease potential

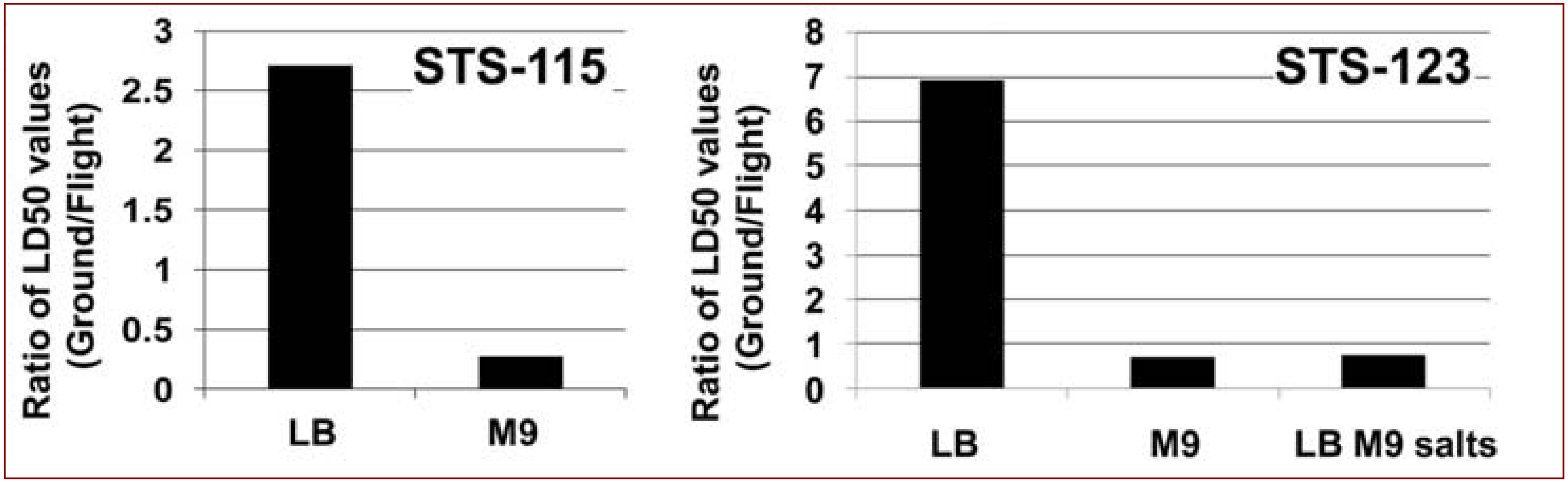
Independent validation of the STS-115 results

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



- Using Lennox Broth, the LD_{50} in the second spaceflight experiment again decreased (6.9 fold)
- This trend did not occur when M9 media was used or when the Lennox Broth media was supplemented with the inorganic ions used in M9 media

Wilson *et al. PLOS One* 2008

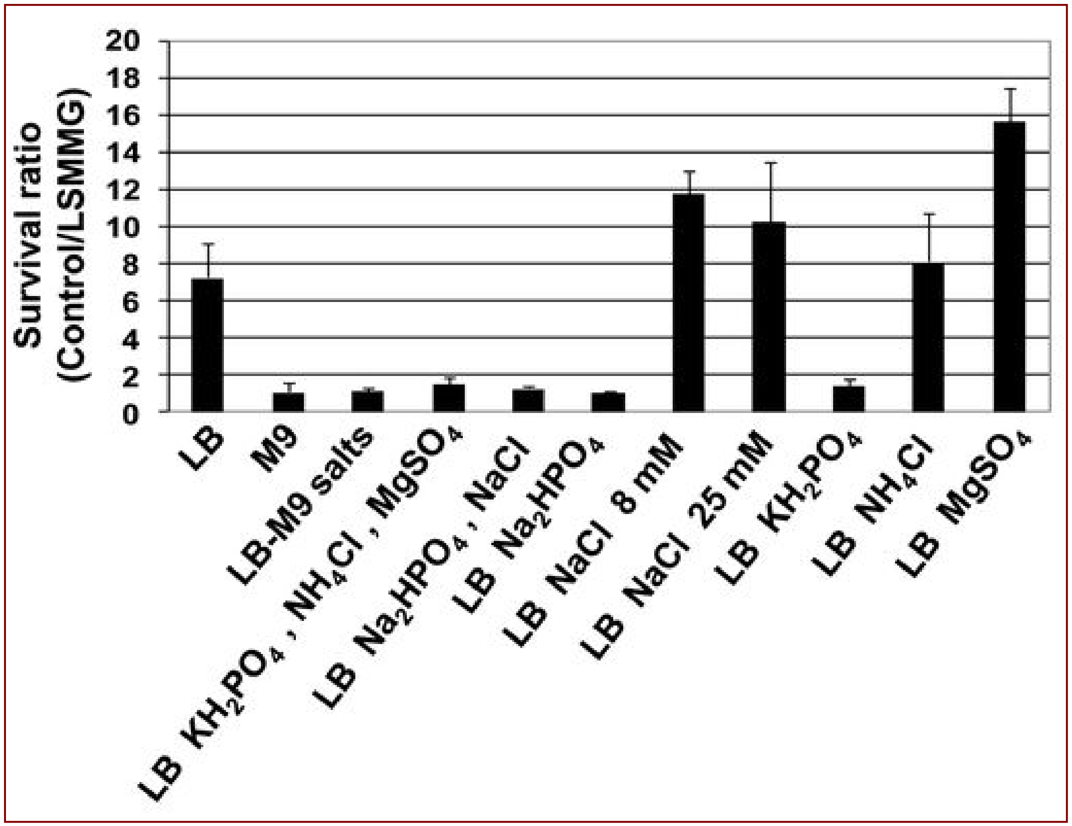
Is there a Spaceflight Contribution?

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

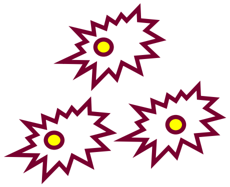
Which component of the media?



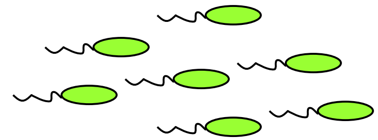
Increased phosphate ion concentration prevents altered *S. typhimurium* acid tolerance in analogue culture

Spaceflight data supplemented with ground-based model

Summary *Pseudomonas aeruginosa*



Stress Resistance



Pseudomonas aeruginosa

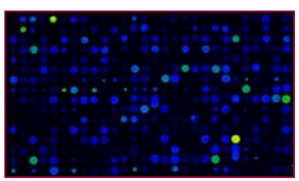
Production virulence factors

Biofilm production

RWV Bioreactor

Spaceflight

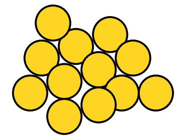
Gene Expression



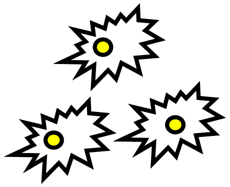
Hfq - evolutionarily conserved low fluid shear response regulator in other pathogens

Crabbé *et. al.* 2008; Crabbé *et al.* 2010; Crabbé *et al.* 2011

Summary *Staphylococcus aureus*

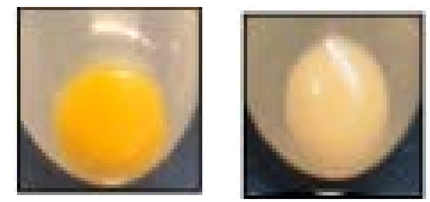


Staphylococcus aureus



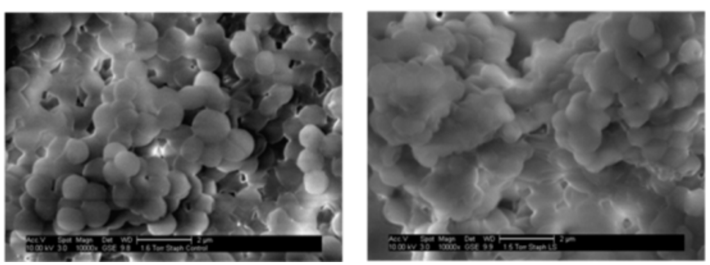
Stress Resistance

Carotenoid Production (Virulence Factor)



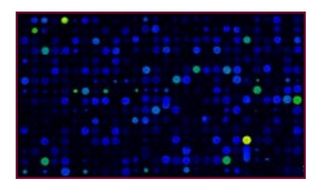
RWV Bioreactor

EPS production



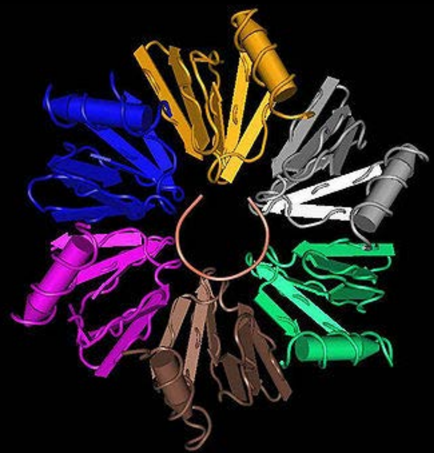
Control LSMMG

Gene Expression



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

Castro *et al.* 2011



Hfq link identified for other microbes in response to short duration spaceflight/spaceflight-analogue culture

S. Typhimurium

Wilson et al. 2007 PNAS
Wilson et al. 2008 PLoS ONE

P. aeruginosa

Crabbe et al. 2010
Envion Microbiol
Crabbe et al, 2011
Appl Environ Microbiol

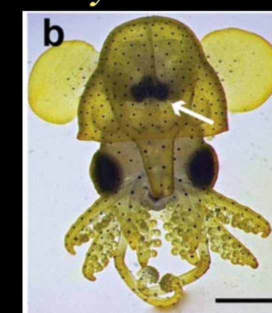
S. aureus

Castro et al. 2010
Appl. Environ. Microbiol.

C. albicans

Crabbe et al. 2013
PLoS ONE

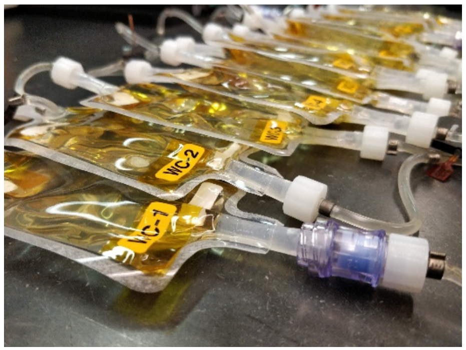
Squid-Vibrio fischeri symbiosis



Grant et al. 2013
Int. J. Astrobiol

Studies by our team and others have implicated Hfq in the response of at least four other microbes to spaceflight and/or spaceflight-analogue culture

Biofilms and Microbial Evolutional



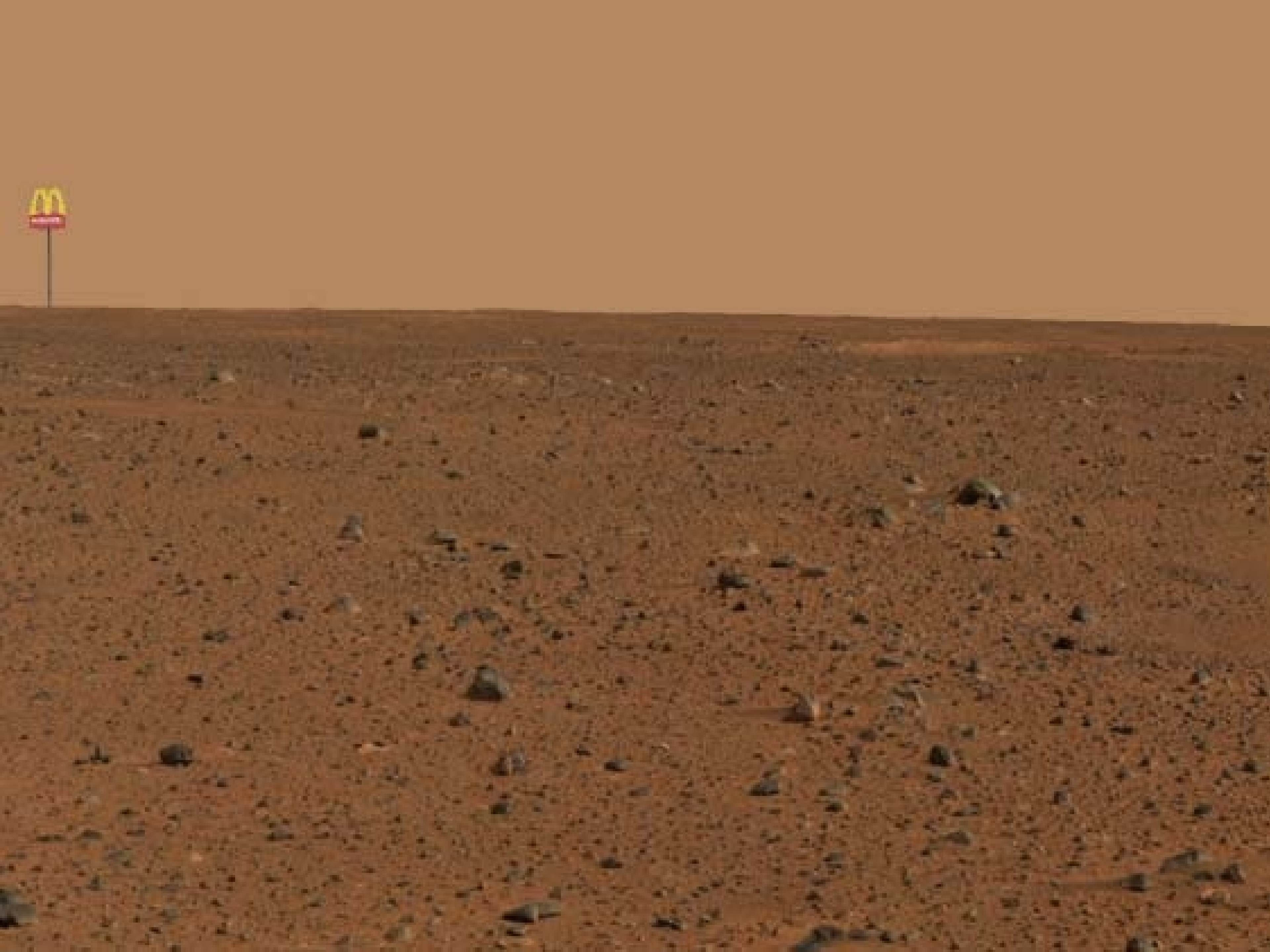
- **PI: Cheryl Nickerson, Arizona State University**
- As most spaceflight studies deal with short duration, batch cultures of microorganisms, no information is available on the long term evolution of microbial pathogens cultured in the spaceflight environment.
- This study will focus on the mutation rate and phenotypic characteristics of *S*. Typhimurium actively cultured over 100 generations aboard ISS.

- **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.

Crew and Vehicle Microbiology

- **PI: Hernan Lorenzi, J. Craig Venter Institute**
- **<u>Baseline</u>** 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.

- **PI: Cherie Oubre, NASA**
- Expansion of our nominal crew health environmental monitoring to include the VEGGIE plant growth system to determine if the bacterial and fungal composition near the plants is different than the general habitat area.
- Information will be used to support future requirements for "pick and eat" foods



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