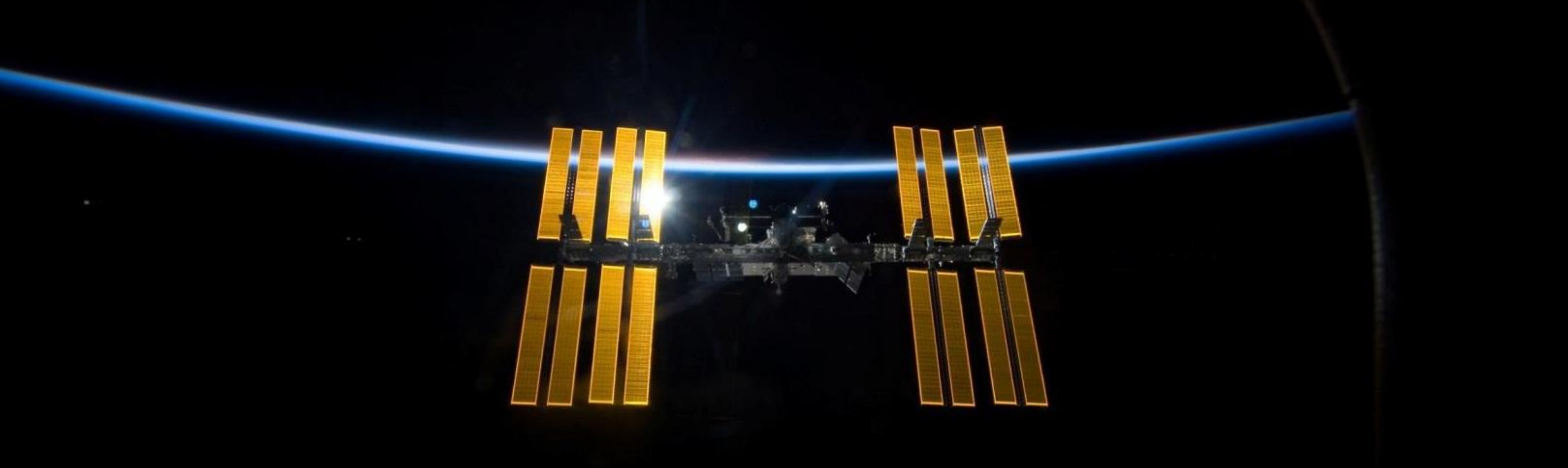
Microbiology and the International Space Station



C. Mark Ott, PhD

Microbiology Laboratory

NASA Johnson Space Center, Houston, TX

Microbiological Monitoring on the ISS

Surfaces

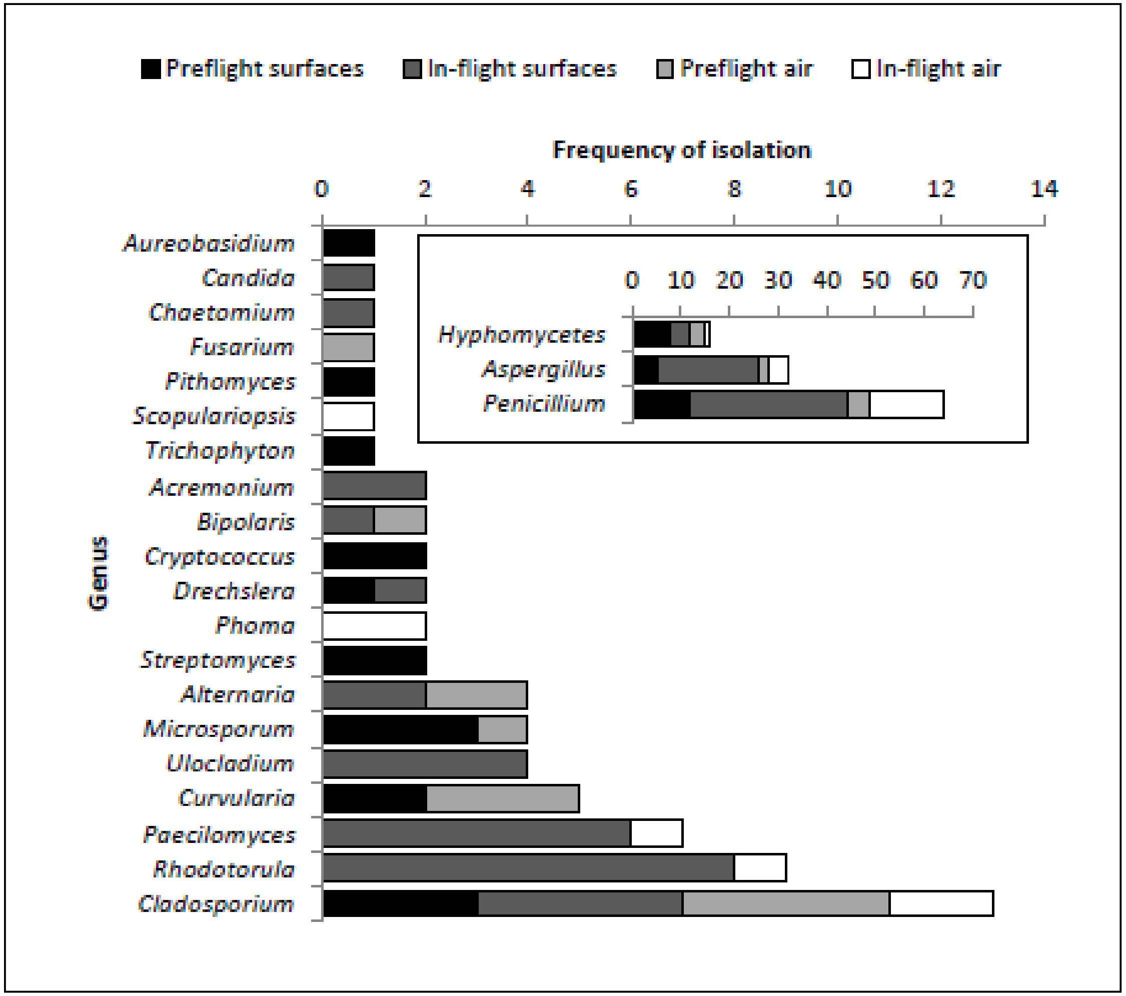
Air

Water

Quantified in-flight and returned to JSC for identification

ISS Air and Surface Monitoring

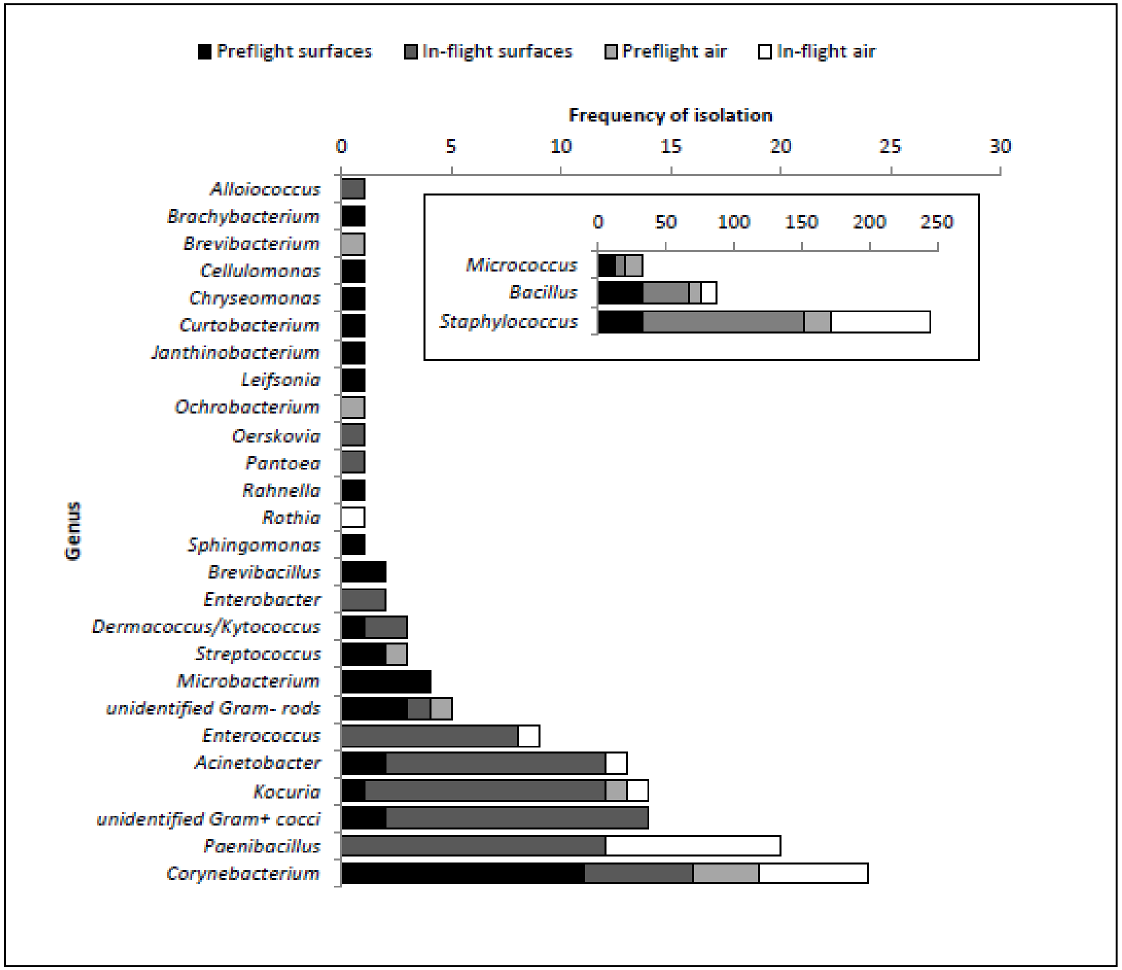
Fungal Isolates



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

ISS Air and Surface Monitoring

Bacterial Isolates



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

Adverse Effects of Microorganisms

- Biodegradation
- Systems failure
- Food spoilage
- Release of volatiles

"…(fungi) feeding behind control panels, slowly digesting the ship's air conditioner, communications unit, and myriad other surfaces."

Gareth Cook, Boston Globe Staff (10-1-00)

Microbial Mutation and Evolution

- Craig Everroad, NASA Ames Research Center
  - Experimental Evolution of *Bacillus subtilis* Populations in Space; Mutation, Selection and Population Dynamics
- Wayne Nicholson, University of Florida
  - Global Transcriptome Profiling to Identify Cellular Stress Mechanisms Responsible for Spaceflight-Induced Antibiotic Resistance
- Cheryl Nickerson, Arizona State University
  - High Dimensional Biology to Understand the Functional Response of *Salmonella* to Long-Term Multigenerational Growth in the Chronic Stress of Microgravity

Biofilm Studies

- Robert McLean, Texas State
  - Polymicrobial Biofilm Growth and Control during Spaceflight
- Luis Zea, University Colorado, Boulder
  - Characterization of Biofilm Formation, Growth, and Gene Expression on Different Materials and Environmental Conditions in Microgravity

Human Health

- Cheryl Nickerson, Arizona State University
  - Investigation of Host-Pathogen Interactions, Conserved Cellular Responses, and Countermeasure Efficacy During Spaceflight using the Human Surrogate Model *Caenorhabditis elegans*
- Clay Wang, University of Southern California
  - Influence of Microgravity on the Production of *Aspergillus* Secondary Metabolites (IMPAS) - a Novel Drug Discovery Approach with Potential Benefits to Astronauts' Health
- Sheila Nielsen, Montana State University
  - Genotypic and phenotypic responses of *Candida albicans* to spaceflight
- Grace Douglas, NASA Johnson Space Center
  - The Integrated Impact of Diet on Human Immune Response, the Gut Microbiota, and Nutritional Status During Adaptation to Spaceflight

Human and Environmental Microbiomes

- Hernan Lorenzi, J. Craig Venter Institute
  - Study of the Impact of Long-Term Space Travel on the Astronauts' Microbiome
- Fred Turek, Northwestern
  - Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms and Impact on Multi-System Physiology
- Crystal Jiang, Lawrence Livermore National Laboratory
  - International Space Station, Microbial Observatory of Pathogenic Virus, Bacteria, and Fungi (ISS-MOP) Project
- Kasturi Venkateswaran, NASA Jet Propulsion Laboratory
  - ISS Microbial Observatory - a Genetic Approach
  - Bacterial, Archaeal, & Fungal Diversity of the ISS--HEPA Filter System

Microbiology Laboratory
NASA Johnson Space Center

- Debbie Aldape
- Audry Almengor, Ph.D.
- Bekki Bruce
- Victoria Castro
- Christian Castro
- Brandon Dunbar
- Todd Elliott
- Tanner Hamilton
- Jane McCourt
- Cherie Oubre, Ph.D.
- Duane Pierson, Ph.D.
- Joan Robertson
- Melanie Smith
- Sarah Stahl
- Sarah Wallace, Ph.D.

Prevention

Vehicle Design Controls

- HEPA air filters
- In-line water filters
- Contamination resistant surfaces
- Water biocides
- Water pasteurization systems
- Minimize condensation
- Contain trash and human waste

Operational Controls

Health Stabilization Program

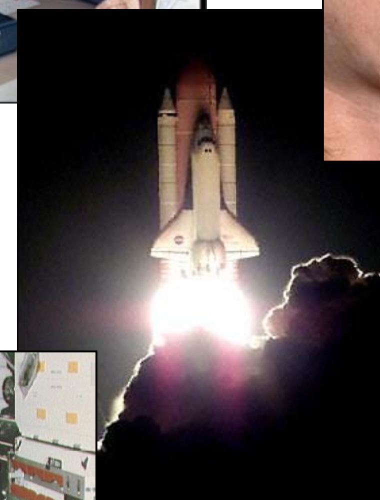
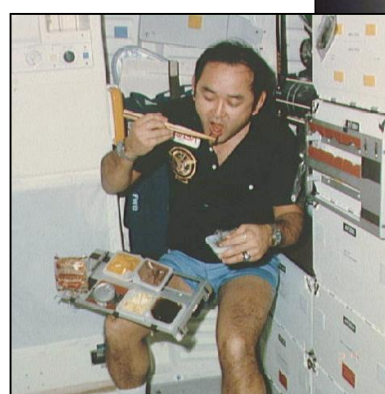
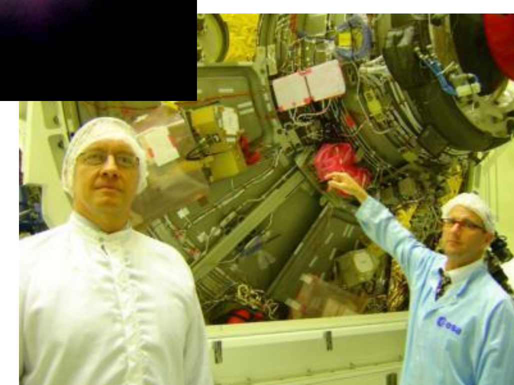
Mission	Illness (Crew)
Apollo 7	Upper respiratory infection (3)
Apollo 8	Viral gastroenteritis (3)
Apollo 9	Upper respiratory infection (3)
Apollo 10	Upper respiratory infection (2)
Apollo 11	
Apollo 12	Skin infection (2)
Apollo 13	Rubella (1)
Apollo 14	
Apollo 15	
Apollo 16	
Apollo 17	Skin infection (1)
Skylab-2	
Skylab-3	Skin infection (2)
Skylab-4	Skin infection (2)

*Billica, Pool, Nicogossian, *Space Physiology and Medicine*, 1994

Preflight Microbiological Monitoring

- Crewmembers
- Food
- Potable water
- Vehicle surfaces
- Vehicle air
- Cargo
- Biosafety review of payloads



Acceptability Limits

Air	
Total bacteria	1,000 CFU/m^3
Total fungi	100 CFU/m^3
Surfaces	
• Total bacteria	10,000 CFU/100 cm^2
• Total fungi	100 CFU/100 cm^2
Water	
• Heterotrophic plate count	50 CFU/ml
• Total coliform bacteria	Not detected in 100 ml

Preflight Monitoring Synopsis

- Few reported clinical infections
  - Dermatitis
  - Urinary tract infection
  - Upper respiratory infection
- Common environmental flora*
- Opportunistic pathogens*
  - *Burkholderia cepacia*
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus*

***Castro, *et al, Microb Ecol* 2004**

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*

Contamination Potential

Preflight contamination

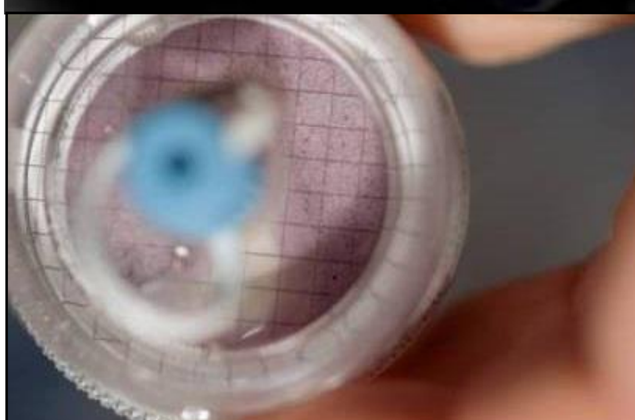
Spacecraft are complex (cluttered)

Astronaut activities, such as eating and hygiene

Microbial Monitoring during Spaceflight

- Safety concerns
- Minimal
  - Power
  - Weight
  - Volume
  - Crew Time
- No phase separation

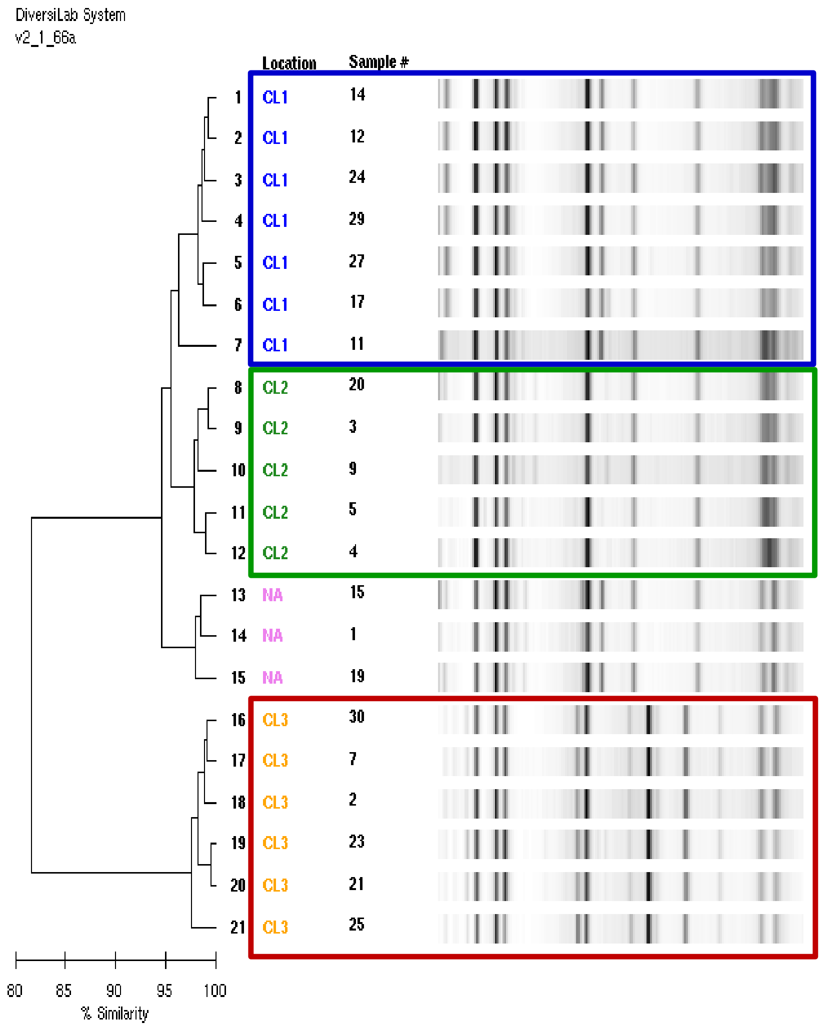
Microbiological Monitoring of Water



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*

Staphylococcus aureus



- No Methicillin Resistant *S. aureus* (MRSA) have been recovered from ISS
- 48% of coagulase negative staphylococci were methicillin resistant
- **Blue** - isolated from the crew of ISS-5, the crew of ISS-4, and in-flight environmental isolates
- **Green** - isolated from the crews of ISS-1, ISS-4, and ISS-5
- **Red** - isolated from the crew of ISS-1 and ISS-4 and from an in-flight environmental surface

Free Floating Condensate

Wolf spent several hours working with Vinagradov to mop up a basketball-size drop of water…"I didn't realize I bought myself anywhere from two to six hours per day doing this for the rest of the mission."

From DRAGONFLY by Bryan Burrough

MIR Condensate Samples

NASA 6 "Slimy"

<u>Fungi</u>

- *Acremonium* species
- *Candida guilliermondii*
- *Candida krusei*
- *Cladosporium* species
- *Fusarium* species
- *Penicillium* species
- *Rhodotorula rubra*

<u>Bacteria</u>

- *Alcaligenes eutrophus*
- *Alcaligenes latus*
- *Enterobacter agglomerans*
- *Escherichia coli*
- *Hydrogenophaga flava*
- *Kingella denitrifican*
- *Methylobacterium* species
- *Pseudomonas vesicularis*
- *Serratia liquefaciens*
- *Stentrophomonas maltophilia*

Escherichia coli

NASA 7 "Slimy"

<u>Fungi</u>

- *Acremonium* species
- *Candida guilliermondii*
- *Candida krusei*
- *Cladosporium* species
- *Fusarium* species
- *Penicillium* species
- *Rhodotorula rubra*

<u>Bacteria</u>

- – *Alcaligenes faecalis*
- – *Bacillus* species
- – *Bacillus circulan*
- *Bacillus coagulans*
- – *Bacillus licheniformis*
- – *Bacillus pumulis*
- – *Citrobacter brackii*
- – *Citrobacter freundii*
- *Comamonas acidovorans*
- *Corynebacterium* species
- *Flavobacterium meningosepticum*
- *Serratia liquefaciens*
- *Serratia marcesens*
- *Yersinia frederiksenii*
- *Yersinia intermedia*

Serratia marcescens

NASA 7 "Fresh"

<u>Fungi</u>

- *Acremonium* species
- *Candida guilliermondii*
- *Candida krusei*
- *Cladosporium* species
- *Fusarium* species
- *Penicillium* species
- *Rhodotorula rubra*

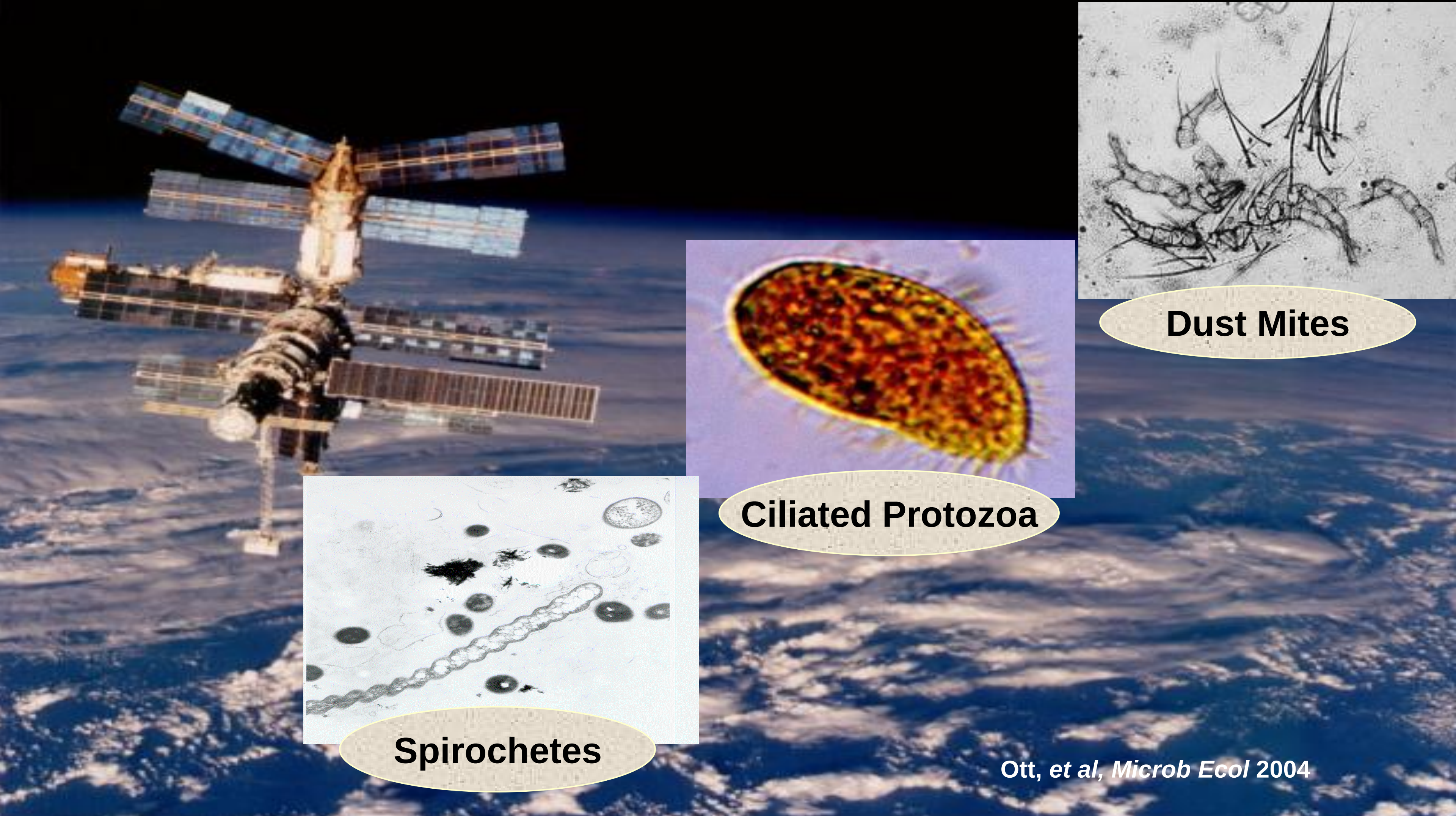
<u>Bacteria</u>

- *Bacillus coagulans*
- – *Bacillus licheniformis*
- – *Bacillus pumilus*
- – *Enterobacter cloacae*
- – *Legionella* species
- *Pseudomonas* species
- *Rhodococcus* species
- *Serratia liquefaciens*
- *Serratia marcerans*
- *Sphingobacterium thalpophilum*
- *Yersinia frederiksenii*
- *Yersinia intermedia*

***Legionella* species**

Ott, *et al, Microb Ecol* 2004

MIR Condensate Residents



Ott, *et al, Microb Ecol* 2004

“Establish a “microbial observatory” program on the ISS”

– National Research Council

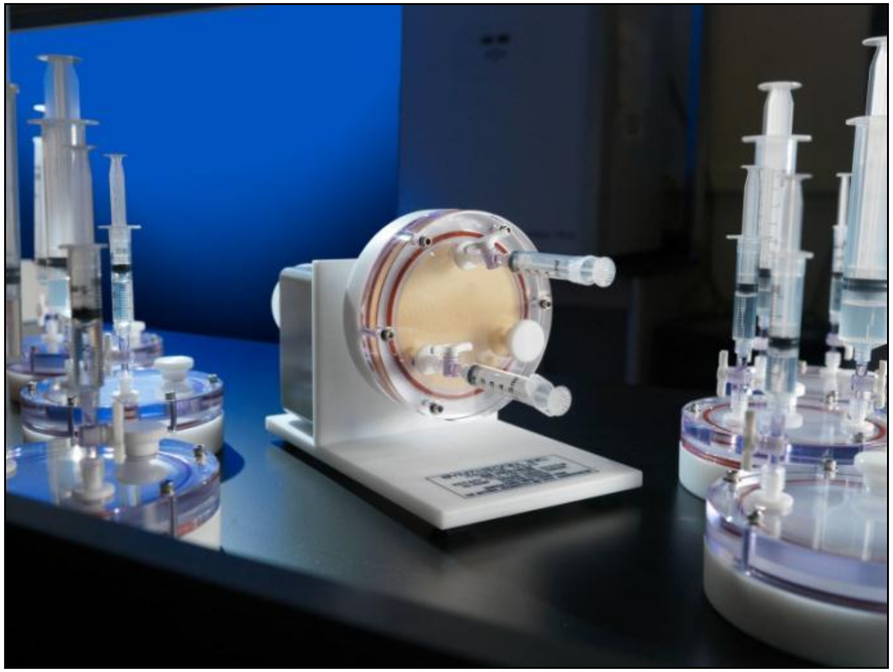
Microbiological Spaceflight Research

- 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
- The vast majority of microbial ecology data is based on media-based analysis
- The impact of radiation on microbial responses/mutational rates is not known

Ground-based Analogue

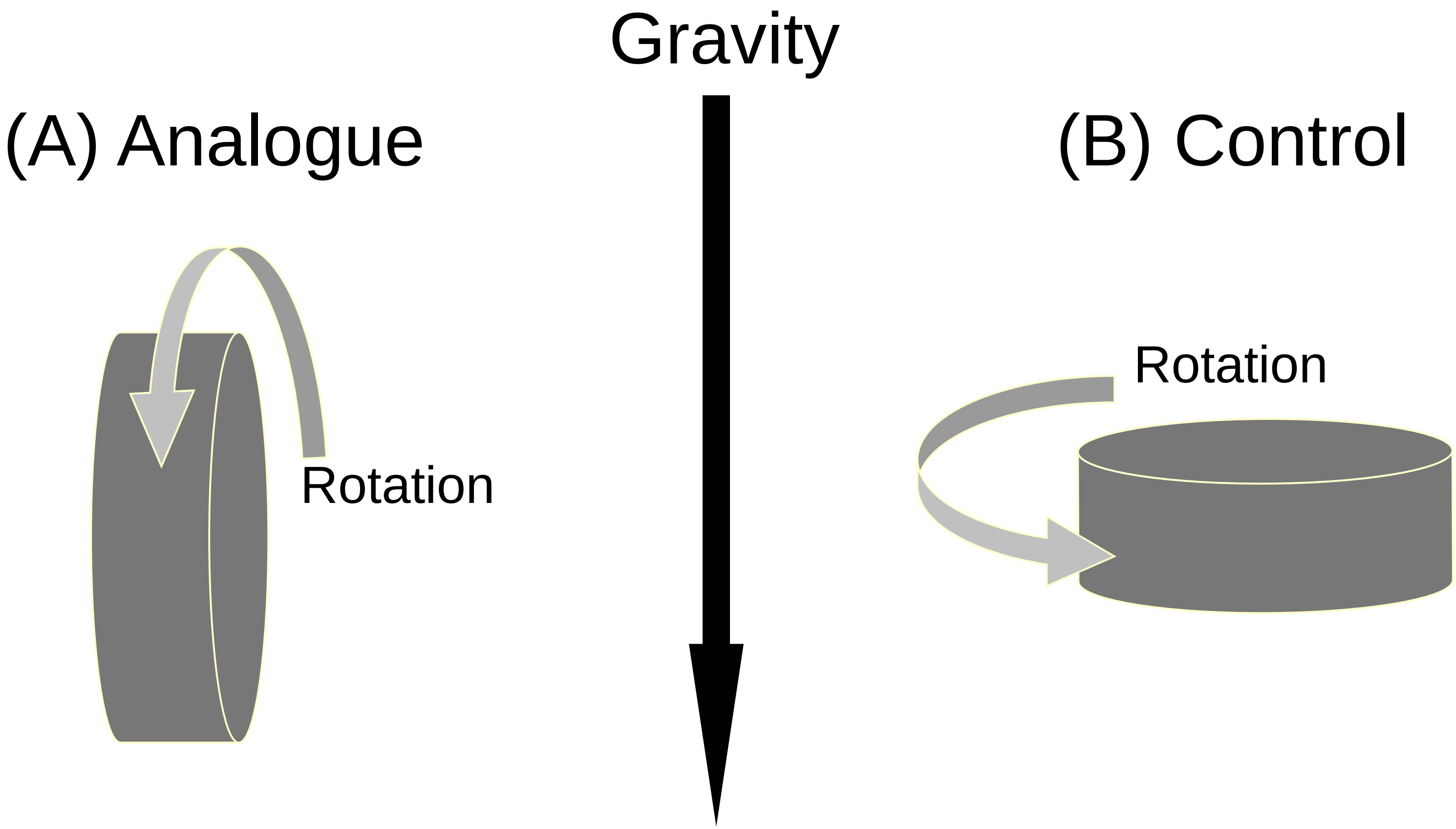
The Rotating Wall Vessel (RWV)

- Solid body rotation in the reactor simulates several aspects of culture in microgravity
- Enables relatively high throughput
- Provides good indicators for spaceflight experiments
- Capability to follow up spaceflight findings without the delays associated with true spaceflight experiments

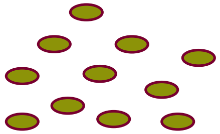


The low shear culture conditions has initiated the term Low Shear Modeled Microgravity (LSMMG) environment

Microgravity Analogue Model Control

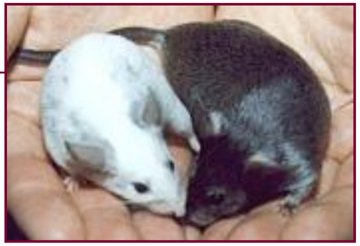


Microgravity Analogue Model Results

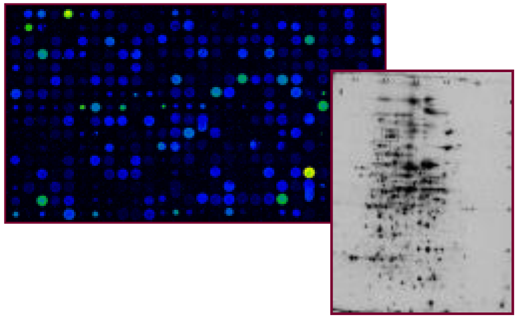


Salmonella typhimurium

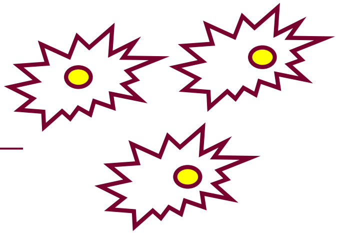
Rotating Wall Vessel bioreactor



Virulence ↑



Gene expression ↑↓



Stress Resistance ↑↓

(Macrophage, acid, thermal, osmotic, oxidative)

Nickerson *et. al. Infect Immun* 2000; Wilson *et al.*, *Proc Natl Acad Sci USA* 2002; Wilson et al. *Appl Environ Microbiol* 2002; Nickerson, *et al*. *Microbiol Mol Biol Rev* 2004.

Unique Microbial Responses

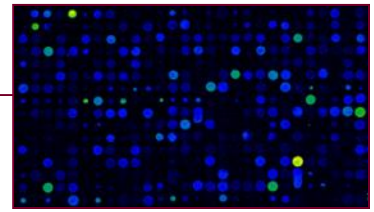
- Investigations by Dr. Cheryl Nickerson at Arizona State University evaluating microbial gene expression, morphology, and virulence
- Experiments aboard space shuttle missions STS-115 (2006) and STS-123 (2008)

MICROBE

Shuttle Atlantis, STS-115, launch September 2006

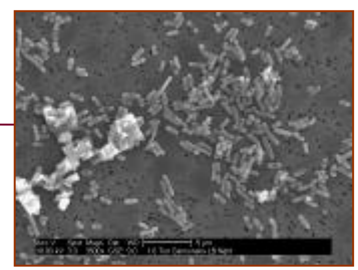
***Salmonella* Typhimurium experiment design**

In-flight hardware



Gene expression

Disease potential

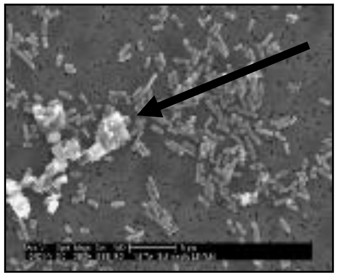


Cellular morphology

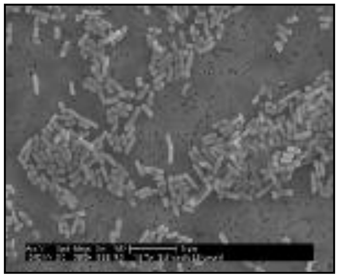
**** Synchronous ground controls maintained under identical conditions as those on-board Shuttle*** *- ground and in-flight hardware loaded with same sample.*

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

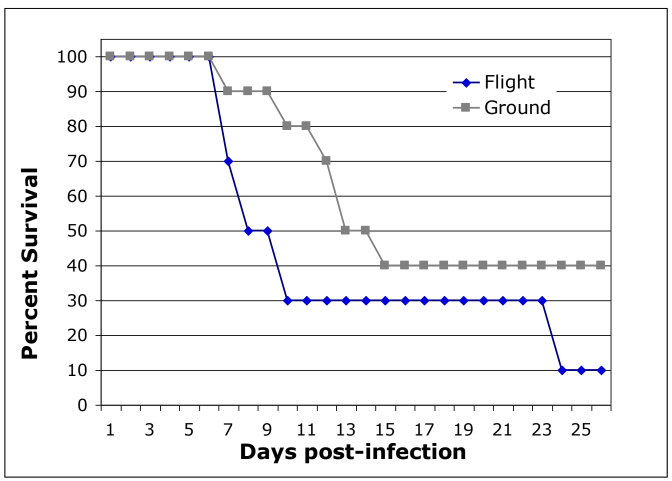
Salmonella Typhimurium Response to Spaceflight Culture



Flight Sample



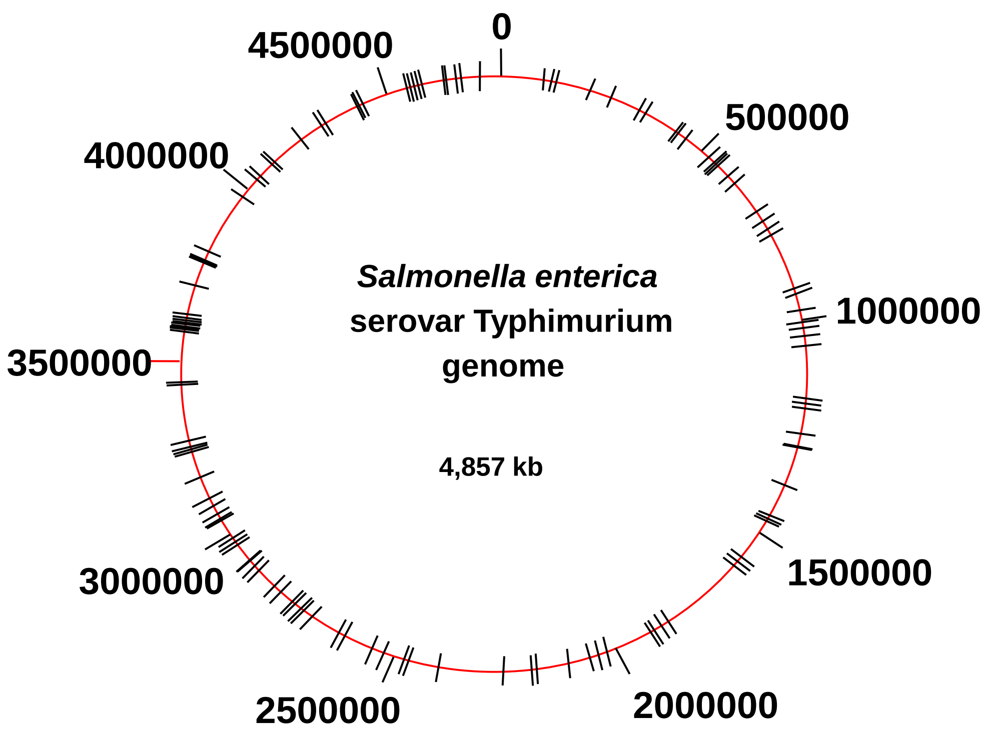
Ground Control



- 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

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

MDRV

Shuttle Endeavour, STS-123, launch March 2008

Salmonella Typhimurium experiment design

In-flight hardware

Disease potential

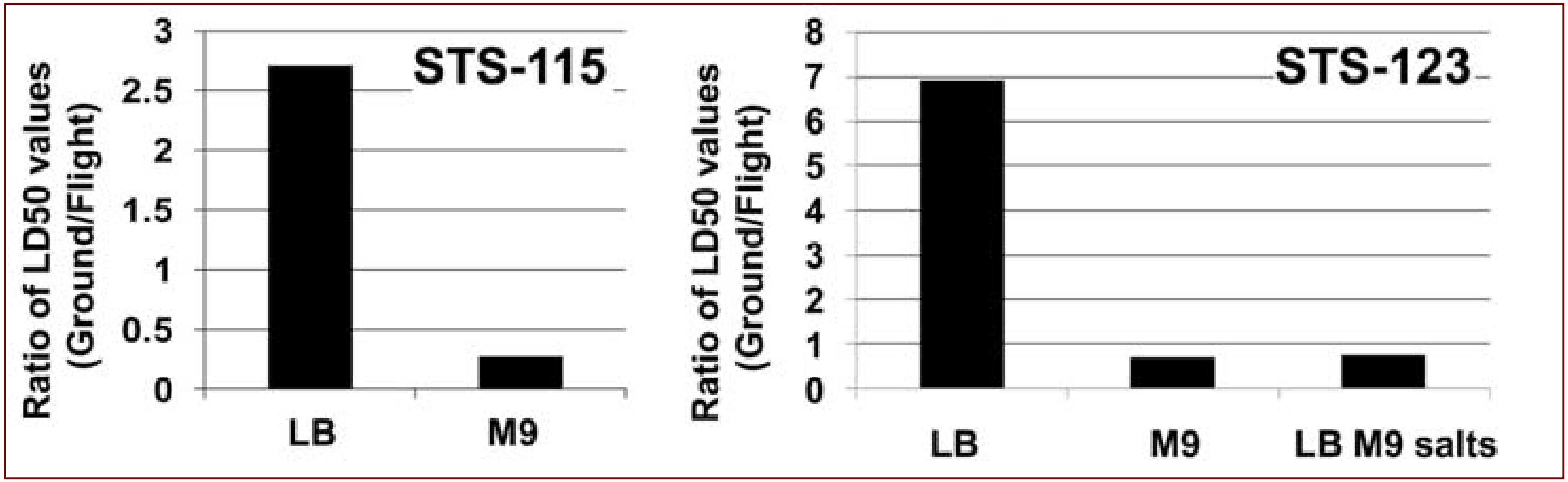
Independent validation of the STS-115 results

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

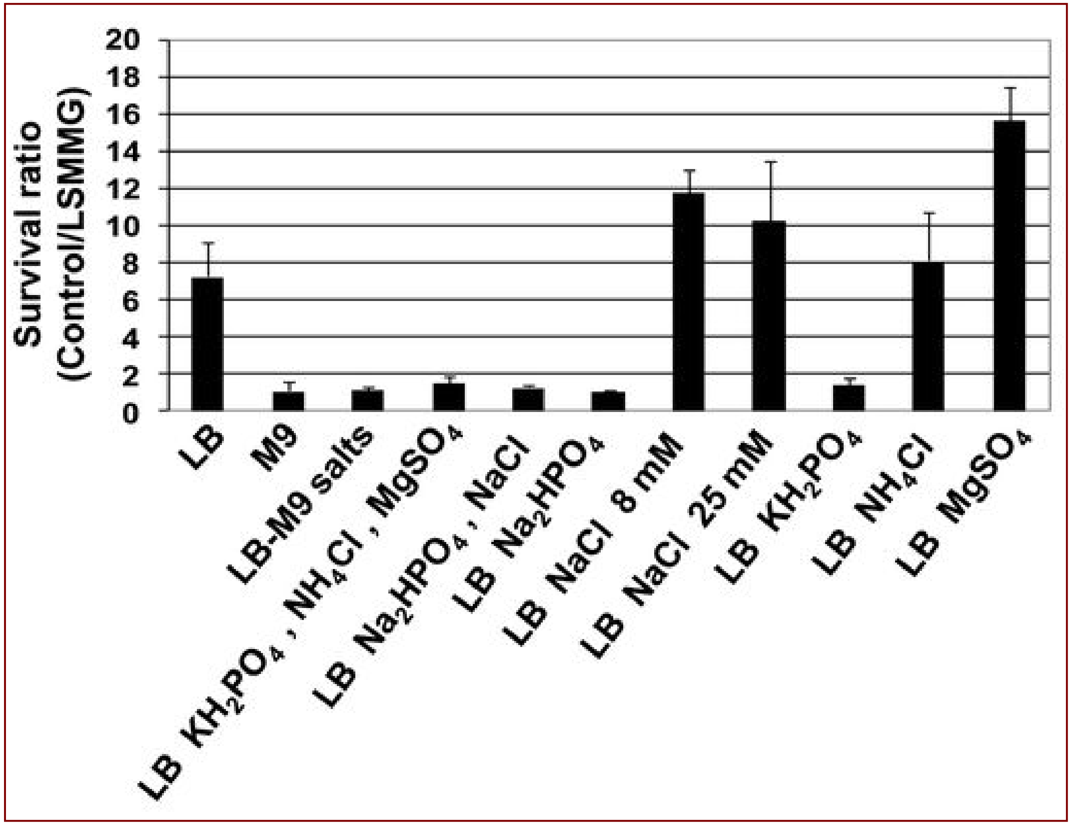
Is there a Spaceflight Contribution?

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?

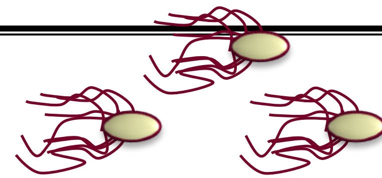


Increased phosphate ion concentration prevents altered *S. typhimurium* acid tolerance in analogue culture

Spaceflight data supplemented with ground-based model

Wilson *et al. PLOS One* 2008

Summary *Salmonella* Typhimurium



***Salmonella* Typhimurium**

Virulence

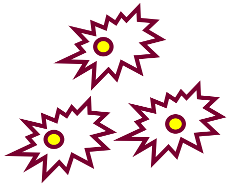
Modulate ion levels

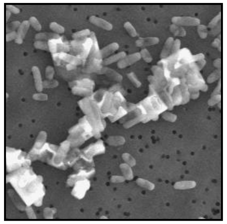
Phosphate controls low fluid shear-associated pathogenesis response

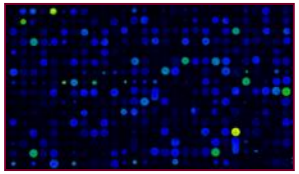
RWV Bioreactor

Spaceflight

Stress Resistance and Biofilm formation





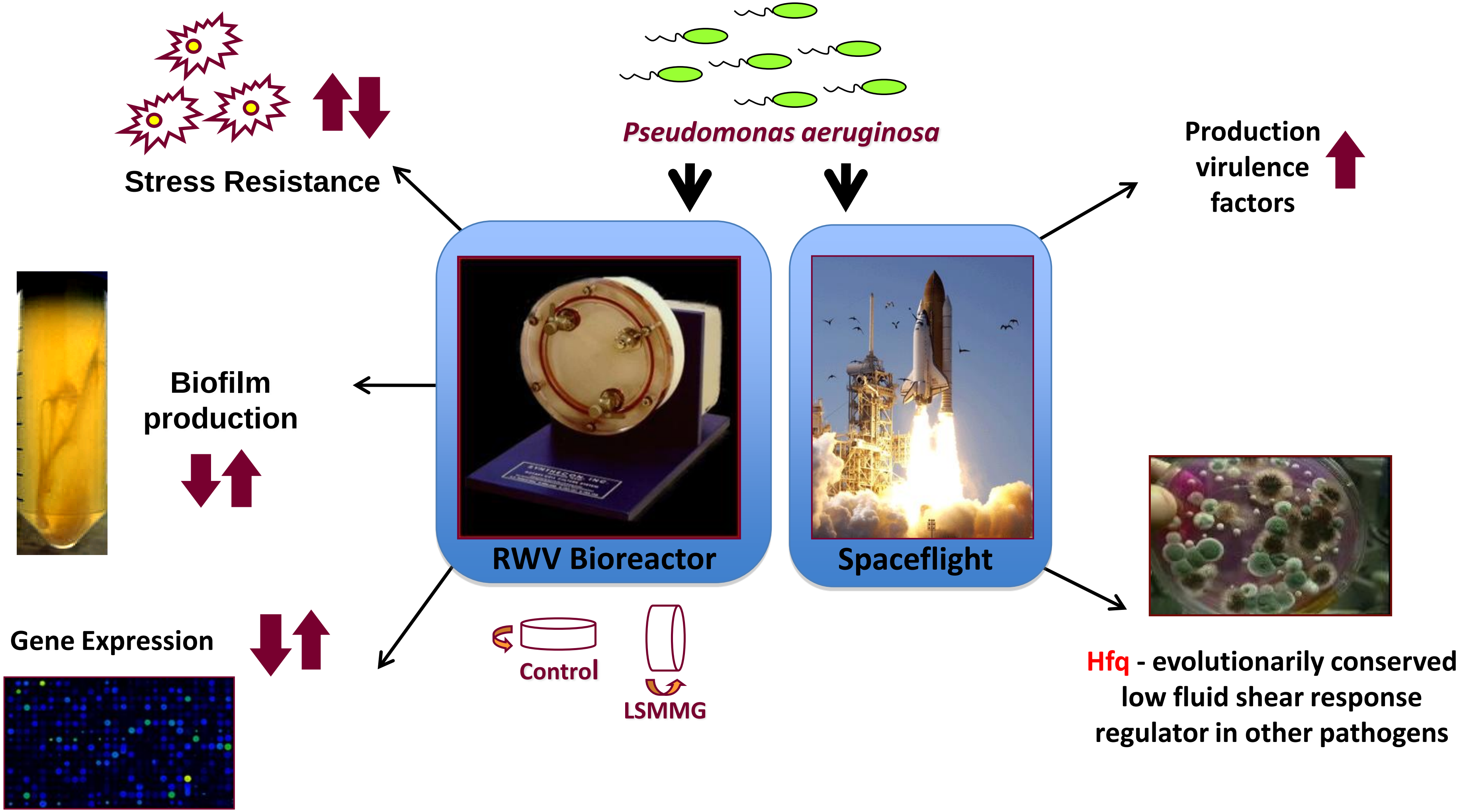


Gene Expression

- 167 genes
- Virulence genes down
- Hfq - evolutionarily conserved low fluid shear response regulator found in other microorganisms

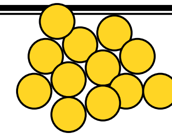
Nickerson *et. al. Infect Immun* 2000; Wilson *et al.*, *Proc Natl Acad Sci USA* 2002; Wilson et al. *Appl Environ Microbiol* 2002; Nickerson, *et al*. *Microbiol Mol Biol Rev* 2004; Wilson *et al. Proc Natl Acad Sci USA* 2007; Wilson *et al. PLOS One* 2008

Summary *Pseudomonas aeruginosa*

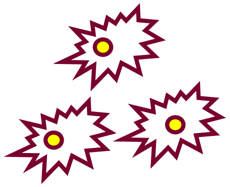


Crabbé *et. al*. *Environ Microbiol* 2008; Crabbé *et al*. *Environ Microbiol* 2010; Crabbé *et al*. *Appl Eviron Microbiol* 2011

Summary *Staphylococcus aureus*



Staphylococcus aureus



Stress Resistance

Carotenoid Production (Virulence Factor)

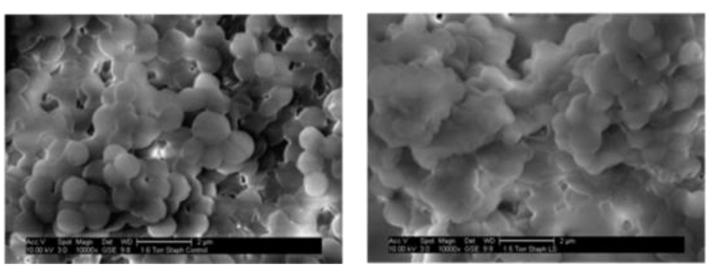
Control LSMMG

RWV Bioreactor

Control

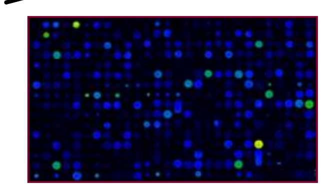
LSMMG

EPS production



Control LSMMG

Gene Expression



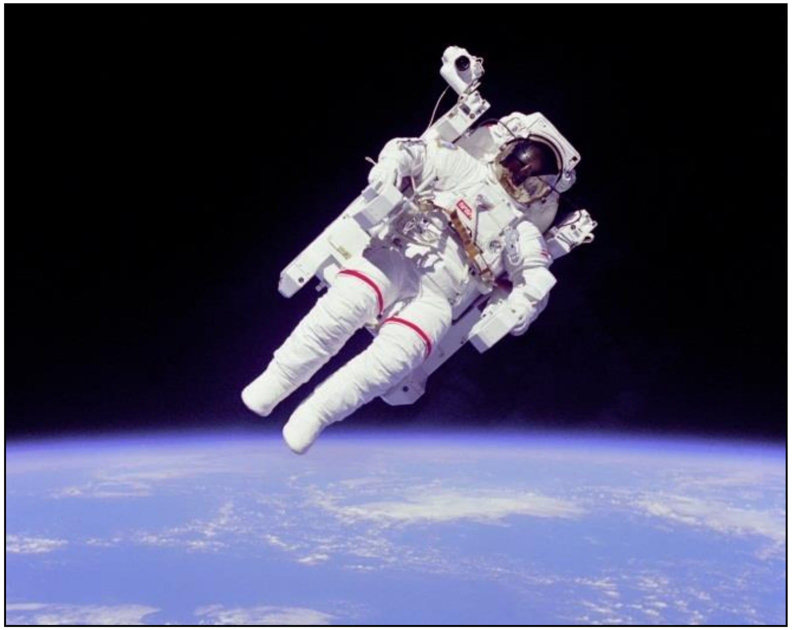
Hfq - evolutionarily conserved low fluid shear response regulator in other pathogens

Castro *et al*. *Appl Eviron Microbiol* 2011

Current Studies in Microbiology

- Astronaut Microbiome
  - *Hernan Lorenzi, J. Craig Venter Institute*
  - Designed to gather information on changes in the crew microbiome during a spaceflight missions
  - Investigation will include preflight, in-flight, and post-flight samples from 9 astronauts
  - Tightly monitored conditions (*e.g.*, temperature, humidity, diet)

Current Studies in Microbiology



- Latent viral reactivation in crewmembers
  - *Dr. Duane Pierson, NASA*
  - 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
  - *VZV can reactivate subclinically in healthy individuals after acute stress.*


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

The ISS as a Microbial Observatory

- The ISS is a semi-closed, well controlled research platform advancing our ability to mitigate microbiological risk to the crew and their vehicle enabling space exploration
- The unique research enabled by access to space provides novel insight into our scientific understanding of life on Earth


United States
Discovery

The Risk of Astronaut Infection

Positives

- Preflight medical exams
- Preflight crew quarantine
- Stringent microbiological monitoring
- Limited exposure to many public health pathogens
- Healthy, well-conditioned crew
- Medical consult throughout a mission

Negatives

- Small enclosed environment
- Recycled air/water
- Stressful conditions
- Dysfunctional aspects of the immune system
- Altered microbial characteristics, including virulence
- Limited diagnostics and treatment on board
- Limited remediation capabilities

Infectious Disease during Spaceflight

- Upper respiratory infections
- Ear infections
- Various fungal infections
- Herpes Zoster
- Gastroenteritis
- Stye
- Allergic reactions
- Rashes & skin disorders