



Advantages and Context of Omics Approaches for Microbial Risk Assessments of Spacecraft Environments

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Why Monitor Spacecraft Environments



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





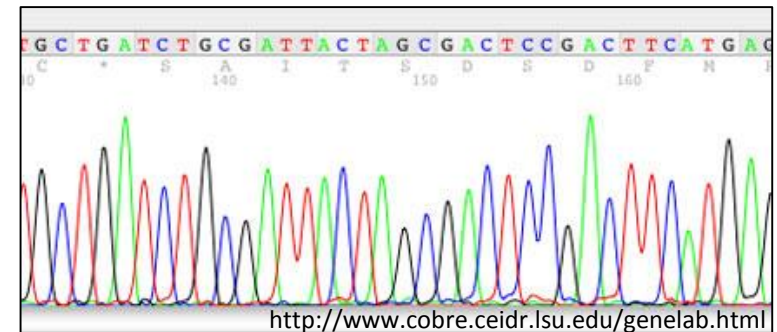
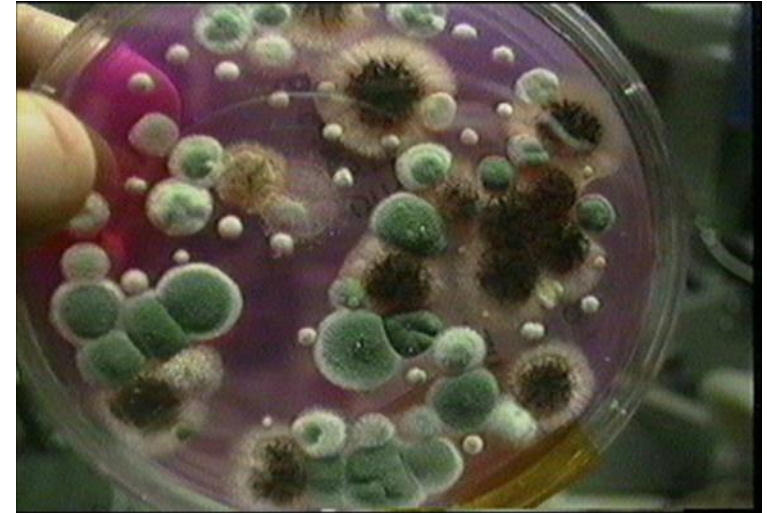
Prevention





Current Microbiological Requirements

- Current spaceflight requirements are based on classical microbiological enumeration using “colony forming units” that reflect the number of microorganisms that grow on a media plate.
 - Example: Flight surface requirement for bacteria = 10,000 CFU/cm²
- Current spaceflight requirements also include identification of medically significant organisms.
 - Identification originally relied on biochemical testing allowing us to describe the genus and species.
 - Technological advances have allowed us to identify microorganisms using DNA based tools.





Preflight Monitoring and Disinfection

- Possible routes of infection during spaceflight missions
 - Crew
 - Spaceflight food and water
 - *Salmonella enterica* serovar Typhimurium
 - *Staphylococcus aureus*
 - Vehicle air, surfaces, and cargo
 - *Pseudomonas aeruginosa*
 - *S. aureus*
 - Experimental Payloads (Biosafety Review)
 - *S. Typhimurium*
 - Methicillin resistant *S. aureus* (MRSA)
- Disinfection criteria
 - High microbial concentrations
 - Medically significant organisms





Contamination Potential



Preflight contamination



Spacecraft are complex



Astronaut activities
(e.g., eating and hygiene)



Microbiological Monitoring on the ISS

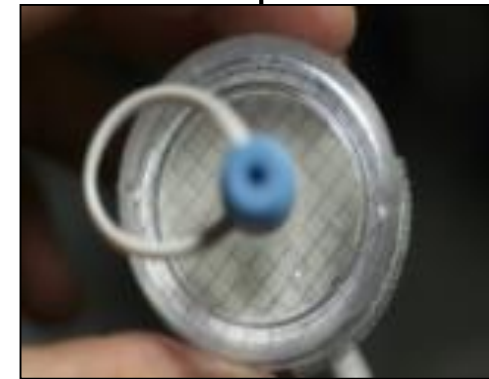
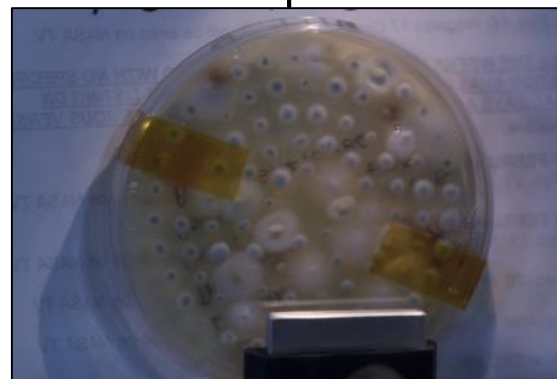
Surfaces



Air



Water



Quantified in-flight and returned to JSC for identification



Next Generation Spaceflight Monitoring

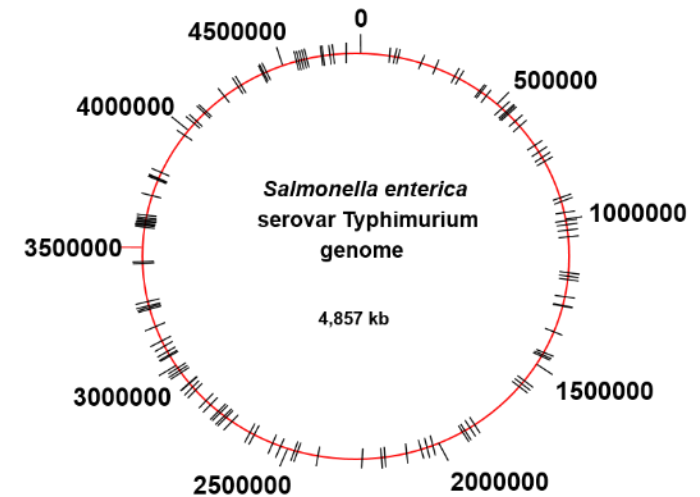
- Spaceflight technology demonstrations
 - Razor EX (Biofire Defense)
 - Targets selected microorganisms or groups of microorganisms
 - Real Time Polymerase Chain Reaction (RT-PCR)
 - Designed for and used by the military
 - Dry chemistry for easier sample prep
 - MinION (Oxford Nanopore)
 - Sequences all organisms in the sample
 - Nanopore technology
 - Both systems performed well in recent ISS technology demonstrations
- As these technologies do not enumerate microorganisms using CFUs, and the goal is autonomous environmental microbiological monitoring, new requirements are needed.





The Impact of the Spaceflight Environment on *Salmonella enterica* serovar Typhimurium

- In 2006, the MICROBE Experiment (PI: Cheryl Nickerson, Arizona State University) identified alterations in microbial virulence in spaceflight grown cultures of *S. Typhimurium*.
 - Proteomic profiling identified 73 differentially regulated proteins, and microarray analysis identified 167 differentially regulated genes compared to ground controls
 - Common molecular regulatory protein (Hfq) associated with 32% of the differentially regulated genes
 - Genes were globally distributed and associated with:
 - 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



Wilson et al. Proc Natl Acad Sci USA 2007



Developing New Requirements

- What information do we really need?
- As a starting point, we begin with information that is equivalent to our current approach.
 - Do we need less information based on lessons learned from the past?
 - Is there information that we did not require in the past that should be required in the future?
 - Toxin production
 - Ability to track a given microbial clone
- What impact will vehicle design have on the required information?



Developing New Requirements

- More devils in the details
 - How important is enumeration? If we do not use CFUs, then what would we use?
 - We want to identify medically significant organisms, but can we provide a list?
 - How will new technologies indicate microbial viability?
 - Antibiotic resistance is often important to know. How will autonomous spacecraft system provide this information?
- Engineering design of the monitoring system
 - What should be the detection sensitivity (*e.g.*, 1 bacterium per liter)?
 - How do we define the required confidence in the data (replicates, accuracy, depth, coverage)?
 - Should it include software to analyze the data (Green light-Red light)?



Moving forward

- The goal is to require monitoring approaches that provide the best available information to maintain both crew health and vehicle integrity.
- We can start out with an equivalent information approach, basically requiring that any new technology on the International Space Station provide information that meet our current standards.
- As we look to future missions, NASA will build requirements through interactions with internal and external experts in fields including computational biology, molecular biology, microbial pathogenesis, microbial risk assessment, infectious disease medicine, and hardware development.



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