Advantages and Context of Omic 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

Timbury, et al., 2004
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.

http://www.cobre.ceidr.lsu.edu/genelab.html
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.
The International Space Station: A Research Platform to Understand Environmental and Human Microbiomes

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