E. Coli biofilm adhesion to porous and nonporous surfaces in spaceflight conditions

Debbie Adam[1], Michelle Fang[1], Niki Gooya[1], Pali Keppetipola[1], Apurva Raghu[1]
[1]University of Maryland, Terps in Space
**All authors contributed equally

Abstract

Biofilms are communities of microorganisms that have the capacity to facilitate the development of diseases. Previous literature has found that biofilm growth is affected by surface properties: for example, in some cases there is reduced biofilm formation on porous surfaces compared to non-porous surfaces. As humans continue to explore space, understanding the behavior of biofilms in spaceflight conditions will become critical. Research has indicated that bacterial colonies within microgravity environments exhibit atypical behaviors of increased growth and virulence. To help shed some light on these aspects of biofilm growth, our study analyzed the formation and adhesion of E. coli on porous and nonporous 99.99% aluminum on Earth and in space. The experiment was conducted both on Earth and at the International Space Station to determine if the presence of gravity impacts biofilm physiology on these surfaces. E. coli growth on nonporous and porous aluminum were analyzed using scanning electron microscopy (SEM). Qualitative analysis reveals a possible size difference between the Earth and space bacteria. However, no significant qualitative differences were observed between gravity and microgravity samples on porous and nonporous aluminum surfaces. We are currently analyzing our samples to corroborate or invalidate the presence of structural differences on biofilms in porous vs. nonporous surfaces and Earth vs. space settings. Further research is required to assess the morphology of individual bacteria on these aforementioned materials and growth settings.

Research Question

How does biofilm formation on porous and smooth aluminum surfaces differ between an Earth and microgravity environment?

Introduction

- Biofilms form when cell colonies reproduce and adhere to a surface
- Bacterial in biofilms behave differently from non-aggregate bacteria:
  1. Increased growth and virulence[2]
  2. Differing levels of gene expression[4]
- Bacteria in space exhibit different traits than on earth, including:
  1. Morphology
  2. Higher antibiotic resistance
- Surface porosity decreases biofilm formation[3]
- Aluminum one of many metals used on ISS

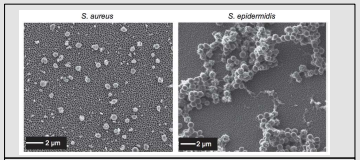

Image reprinted from Feng et. al.[3]
Fig 1: Scanning Electron Microscopy (SEM) images of S. aureus and S. epidermidis cells at 2µm on porous aluminum surfaces (100nm diameter).

Methodology

Tube Preparation
- 2 silicone tubes partitioned into 3 chambers (see Fig. 2)
- Clamps used to separate each chamber
- Aluminum chips glued to chamber 2 with silicone adhesive

Experimental Procedure
- Earth and Space tube
- Samples at 4°C for 3 wks
- Space tube sent to ISS
- Day 0: Clamp A released, *E. coli* exposed to growth medium and aluminum
- Bacteria grew for 3 days (21-24°C)
- Day 3: Clamp B released, fixative to fill chamber
- Refrigerated for 4 wks, space sample returned to Earth

Tube Harvesting
- Tubes cut open
- Aluminum chips extracted for SEM
- Chips submerged in 1.0-2.0 ± 0.3 mL PBS
- Wells stored at 40°C
- Chips imaged using SEM
- Broth OD determined using spectrometer

Data Analysis
- SEM images analyzed using ImageJ line tool to measure bacterial length
- Statistical significance determined using R

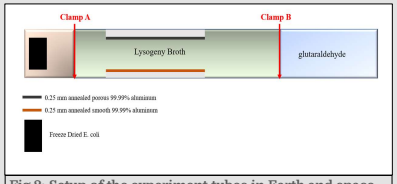

Fig 2: Setup of the experiment tubes in Earth and space. Section 1 contained 2.4 mL with 0.3 g *E. coli*. Section 2 contained 4.5 mL with 2 aluminum surfaces (5 chips each) and LB broth (growth medium). Section 3 contained 1.5 mL 10% glutaraldehyde fixative.

Results

	Earth	Space	P-Value
n-value	46	49	
Average	1.647µm	1.9µm	**0.0066**
SD	.007µm	0.6µm	**0.0072**
Cell Length Range	1.04-2.49µm	1.15-4.43µm	

Table 1: Cell length of E. Coli K-12 across Earth and Space Conditions
Average Space cell length values are larger than Earth cell length values (p-value< .01). Red p-values indicate categories of statistical significance.

Results

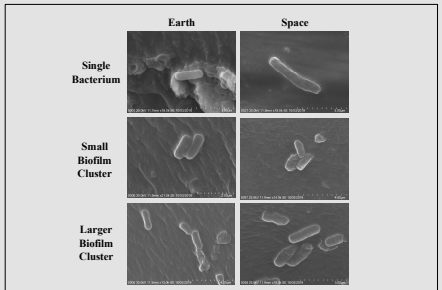

Fig. 3 SEM images of nonporous Earth and space aluminum samples. Varied sizes of bacterial clusters were found on both samples, however bacterial size may be larger for the space sample. Both samples presented cases of bacterial clustering together in the same regions, likely the start of a biofilm forming.

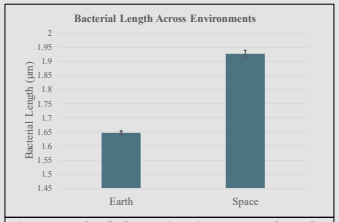

Fig 4. Length of a bacterium in space and Earth. E. coli from the space samples were observed to possibly have a greater length than the samples on Earth, along with a greater variation in length. Typical Dimensions of K12 E. coli length are 2.68 - 4.00 µm

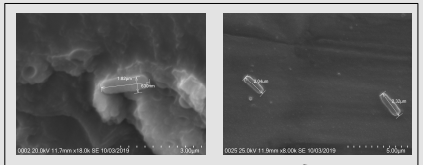

Fig 5. Measuring lengths of non-porous (NP) E. coli cells using SEM in Earth and Space Conditions

Discussion

- Slightly higher OD (.154) for space LB broth than earth LB (.196)
  - Due to random variation or increased bacterial growth and metabolism in space
- Strengths:
  - ISS experimental setting
  - Well-characterized bacteria
- To improve upon:
  - No standardized way to pick E. coli to image
  - Imprecise timing of chamber mixing
  - Possible cross-linking of glutaraldehyde

Conclusion

- Significant difference in the bacterial cell lengths between Earth and space samples.
- Variance across conditions was also statistically different, indicating that space samples had greater variability in length than the Earth samples.
- Further research must be conducted in comparing biofilms developed on porous materials.

Future Directions

- Determining the impact of various metal alloys on biofilm growth.
- Exploring the effect of various pore sizes on biofilm development.
- Analyzing the genetic variation and mutations that may be suspect in differing bacterial cell lengths in the space samples.
- Increase experimental trials to decrease uncertainty in sample values.
- Repeat experiment, avoiding glue as adhesive for aluminum chips.

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