A close-up photograph of three petri dishes containing microbial cultures. The dishes are arranged in a slightly overlapping manner. The leftmost dish shows a yellowish, textured growth. The middle dish shows a more uniform, light-colored growth. The rightmost dish shows a darker, more granular growth. The background is a neutral, light gray.

# The Need for Earth-Based Experiments to Inform Microbial Evolution on Planetary Surfaces

Science and Planetary Protection in Advance of Human Missions

Virtual Seminar

31 October 2024

**Chelsi D. Cassilly**

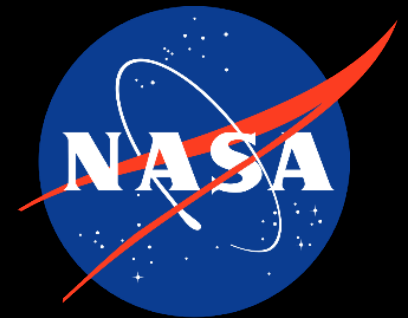
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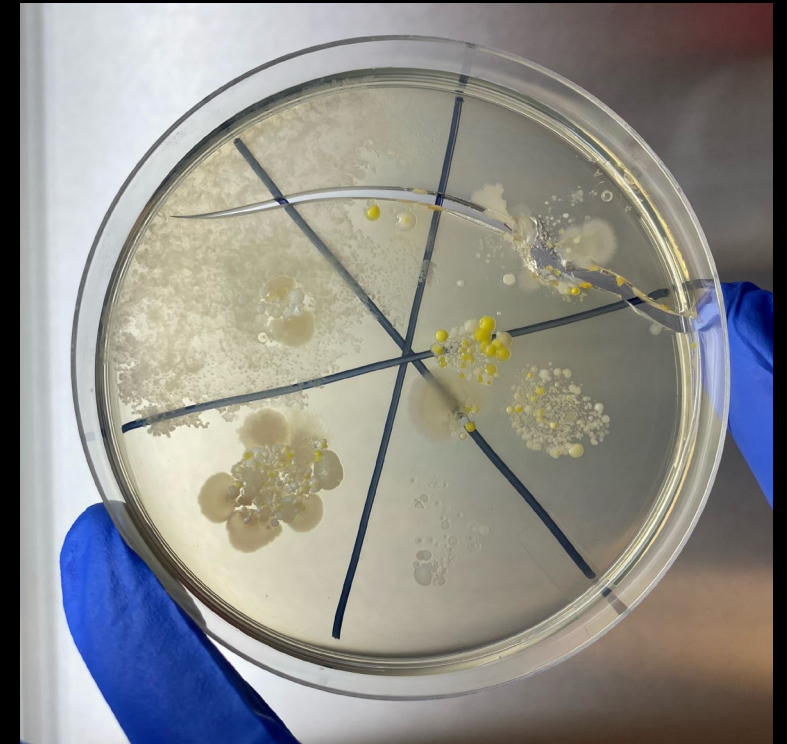
# Introduction

- The goal of forward planetary protection at NASA is to mitigate the risk of contaminating sensitive target bodies with biological life
  - Typically microorganisms



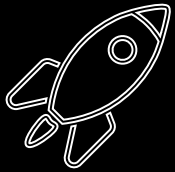
# When Humans Go...

- ...microbes will too.
- Human skin harbors millions of bacteria, fungi, and viruses
  - Halotolerant and desiccation resistant
- Microbes aboard the International Space Station evolve and adapt to life in low earth orbit
- Possible harmful effects to crew and impacts to planetary or astrobiological science



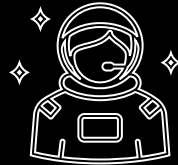
# Risks of Microbial Evolution

1



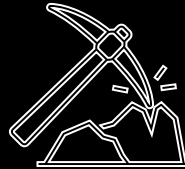
Microbes carried by humans will evolve to new environments even before landing on Mars, during the several month cruise phase.

2



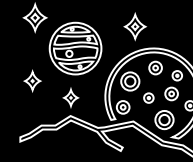
Once landed, microbes will encounter different stressors within the crew habitats on Mars.

3



During extravehicular activities, venting, or other release events, microbes will find their way out onto the Martian surface.

4



The induced environments around crewed systems will create potentially-favorable conditions for microbes to continue evolving on Mars

5



Eventually, microbes may find their way beyond the close confines of the crewed area and continue evolving so as to fill new or distant niches on the Martian surface.



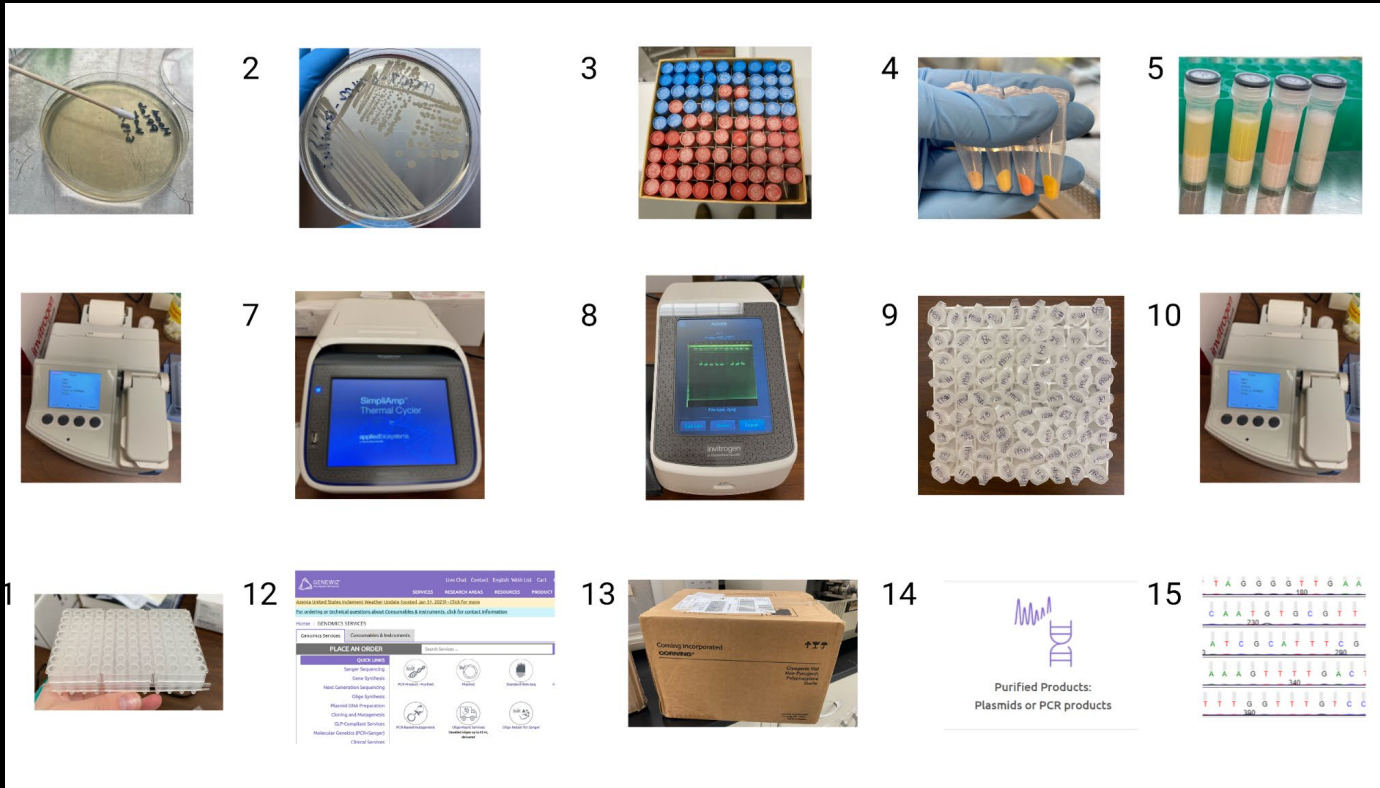
# Examples of Evolution on Earth

- Serial passaging by Richard Lenski shows dramatic changes *E. coli* undergoes within a laboratory setting
- Michael Baym demonstrated the power of single mutations in microbial development of antibiotic resistance
- Could we do similar experiments for space-like stressors? Space environments?
- What experiments can we have the crew do during cruise phase to Mars?



# MSFC Cleanroom Microbial Library

- Collected 95 microbial isolates from cleanroom environments at MSFC
- Identified them using 16S or ITS commercial sequencing



# Exposure of Cleanroom Microbes to Simulated Space Environments

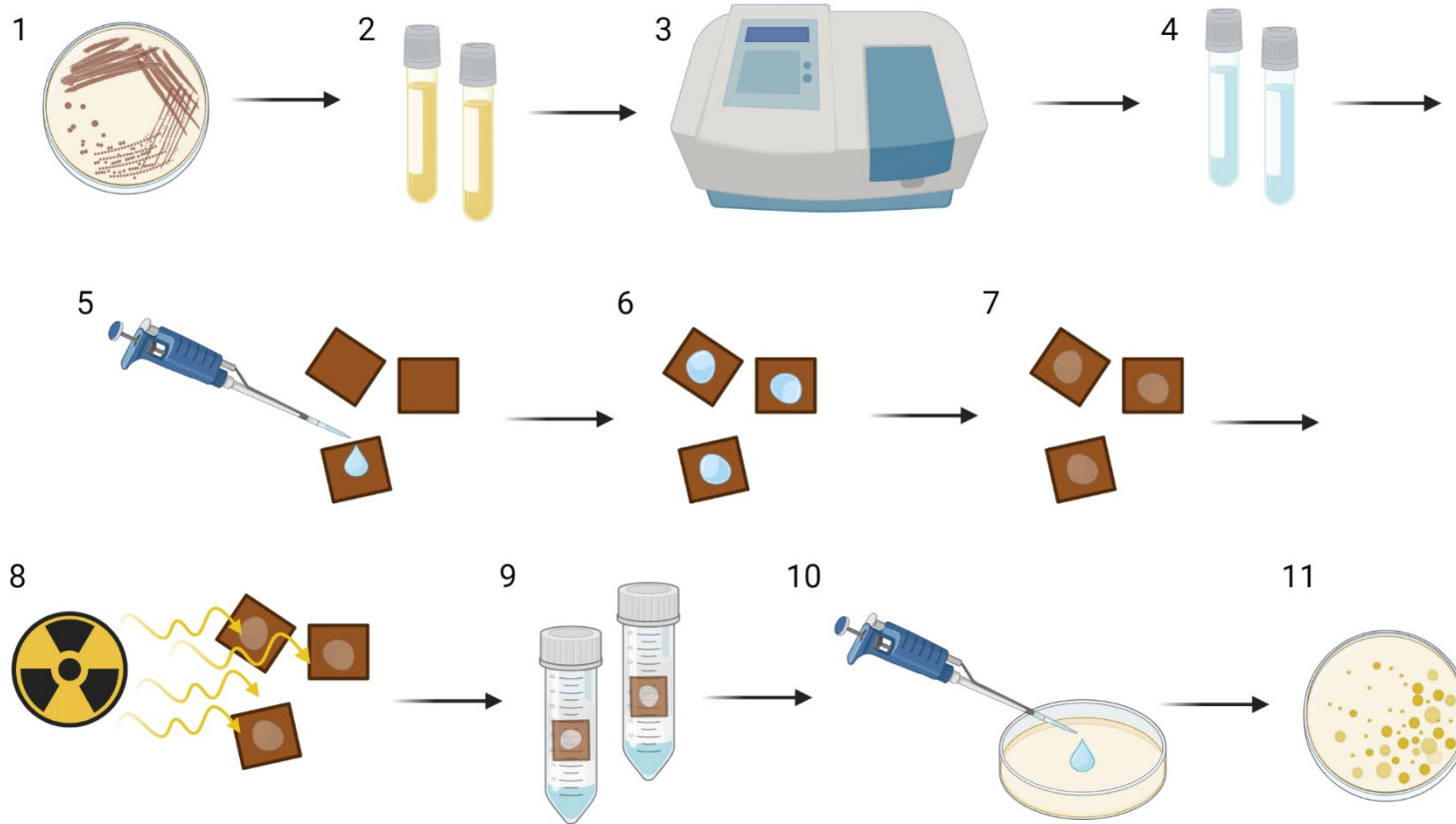
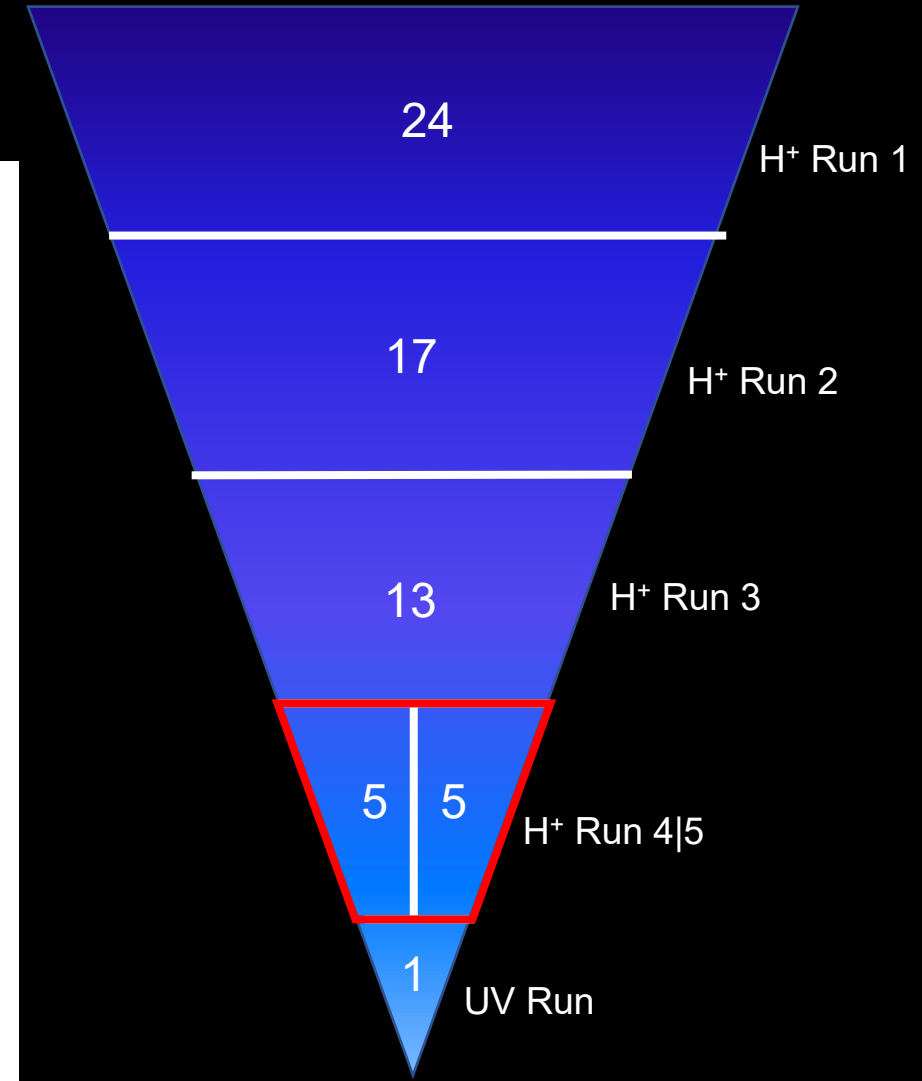


Image created with Biorender

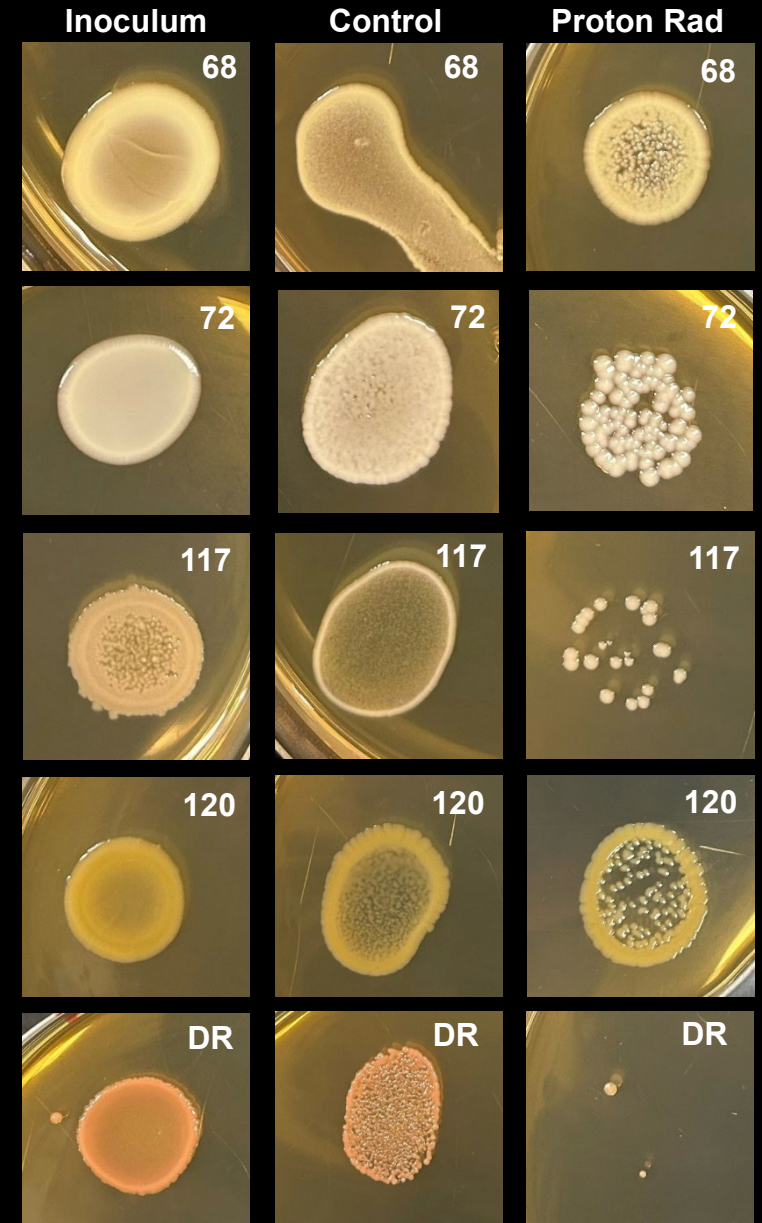




# Four Isolates with Increased Ionizing Radiation Resistance

Run 3

Isolate #	Identity	Inoculum	Control	Proton Rad
PPS55	<i>Alkalihalobacillus gibsonii</i>	+	-	-
PPS67	<i>Brevundimonas vesicularis/nasdae</i>	+	-	-
<b>PPS68</b>	<b><i>Arthrobacter koreensis</i></b>	+	+	+
PPS69	<i>Acinetobacter lwoffii</i> or <i>Prolinoborus fasciculus</i>	+	+	minor
<b>PPS72</b>	<b><i>Paenarthrobacter nitroguajacolicus</i></b>	+	+	+
PPS73	<i>Staphylococcus hominis</i>	+	+	-
PPS74	<i>Brevundimonas</i> sp.	+	minor	-
PPS77	<i>Janibacter hoylei</i>	+	+	-
PPS111	<i>Bacillus licheniformis</i>	+	minor	-
PPS114	<i>Bacillus atrophaeus</i>	+	+	-
<b>PPS117</b>	<b><i>Mycetocola manganooxydans</i></b>	+	+	+
<b>PPS120</b>	<b><i>Erwinia</i> sp.</b>	+	+	+
PPS125	<i>Neocylindroseptoria</i> sp.	+	+	-
ATCC 13939	<i>Deinococcus radiodurans</i>	+	+	minor
Negative	Water	-	-	-



Control: ambient drying

Radiation: 100 keV protons at a fluence of  $2 \times 10^{15}/\text{cm}^2$  at  $\sim 10^{-6}$  Torr



# Four Isolates with Increased Ionizing Radiation Resistance

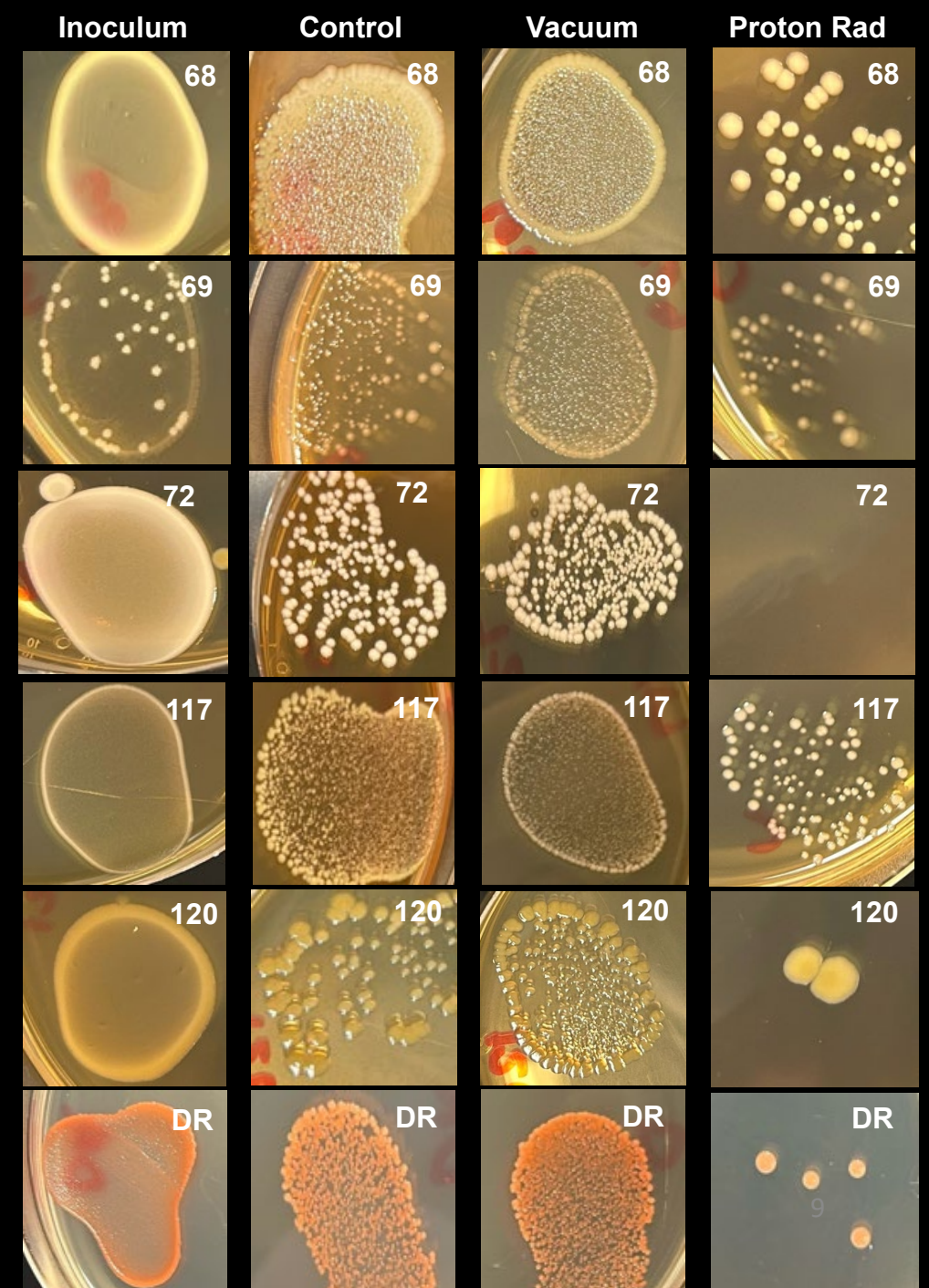
Run 5

Isolate #	Identity	Inoculum	Control	Vacuum	Proton Rad
PPS68	<i>Arthrobacter koreensis</i>	+	+	+	+
PPS69	<i>Acinetobacter lwoffii</i> or <i>Prolinoborus fasciculus</i>	+	+	+	+
PPS72	<i>Paenarthrobacter nitroguajacolicus</i>	+	+	+	-
PPS117	<i>Mycetocola manganooxydans</i>	+	+	+	+
PPS120	<i>Erwinia</i> sp.	+	+	+	minor
ATCC 9372	<i>Bacillus atrophaeus</i> spores	+	+	+	+
ATCC 13939	<i>Deinococcus radiodurans</i>	+	+	+	minor
Negative	Water	-	-	-	-

Control: ambient drying

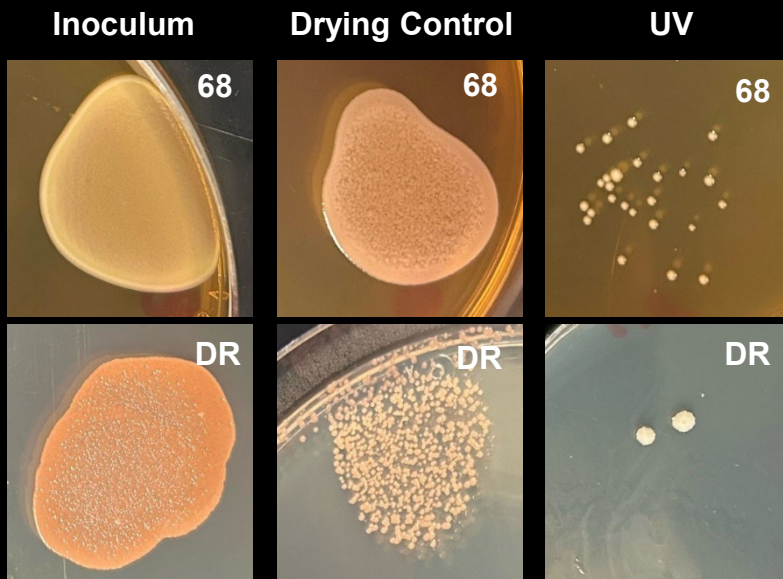
Vacuum:  $\sim 10^{-6}$  Torr

Radiation: 100 keV protons at a fluence of  $4 \times 10^{15}/\text{cm}^2$  at  $\sim 10^{-6}$  Torr



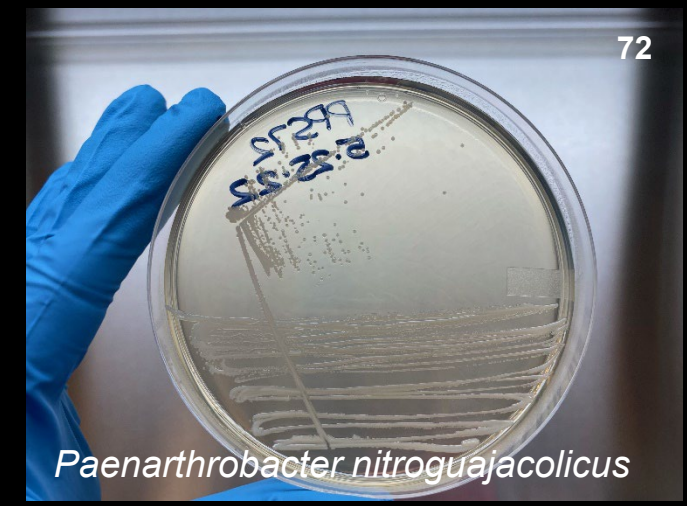
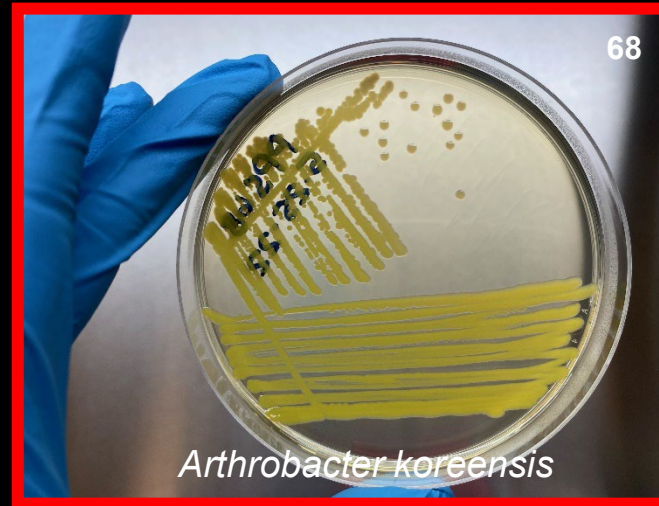


# *A. koreensis* Demonstrates Resistance to UV

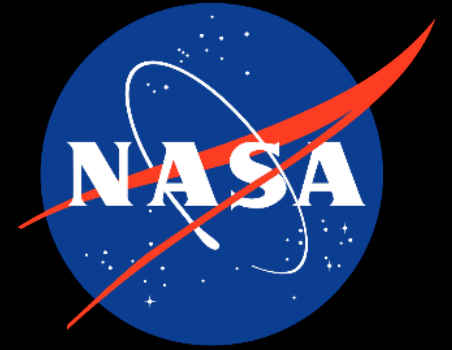


UV: 254 nm wavelength light at an intensity of 80 W/m<sup>2</sup> at ~18 cm for 10 minutes

Four isolates sent for whole genome sequencing



# Acknowledgements



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# Community Discussion

- Short duration exposures are not enough
- Larger and more gradual studies to replicate 1) cruise, 2) to surface habitats, 3) to induced surface environments, and finally true 4) Martian environments
- No Earth-based experiment can perfectly replicate the Martian environment
- We cannot test every possible microbe in simulation experimental regimes
- Examine the evolutionary potential of the “usual suspects” on ISS or in other crewed environments
- What else?

