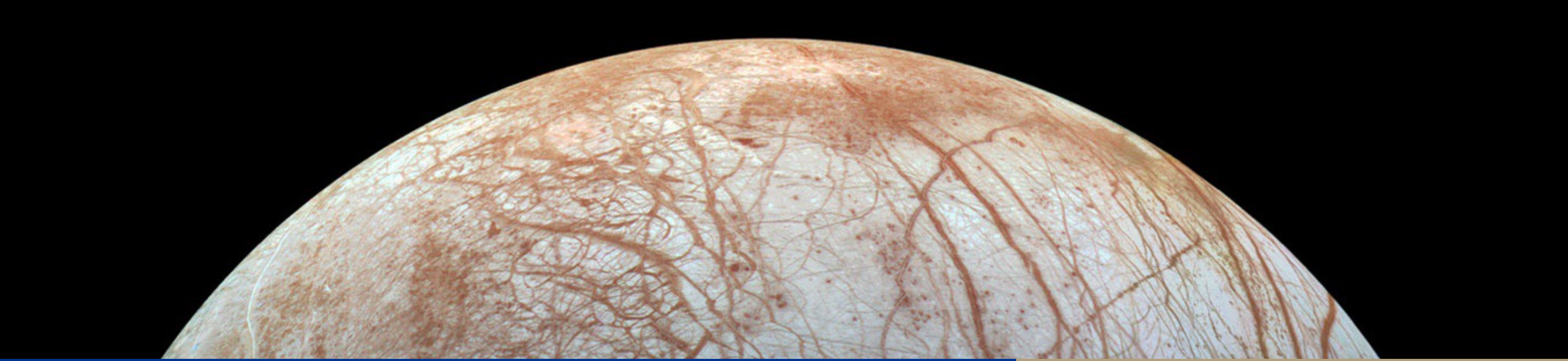
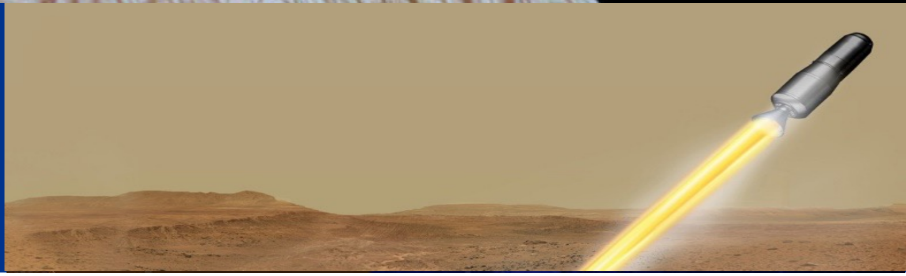


Analysis of Bioburden Associated with Nonmetallic Spacecraft Materials



Chelsi D. Cassilly, Ph.D.

Planetary Protection Microbiologist

Jacobs Space Exploration Group/ESSCA

Space Environmental Effects Team

Nonmetallic Materials and Space Environmental Effects Branch

NASA/MSFC/EM41

Chelsi.D.Cassilly@nasa.gov

Precious Mitchell, Ph.D.

Europa De-Orbit Stage Lead Systems Engineer

Orion Launch Abort System Abort Motor Lead

Solid Propulsion and Pyrotechnic Devices Branch

NASA/MSFC/ER62

Precious.A.Mitchell@nasa.gov

www.jacobs.com | worldwide

CCMPP 2023 Workshop – Goddard Space Flight Center – 14 September 2023


Is there life on EUROPA?

Jovian Moon: Europa

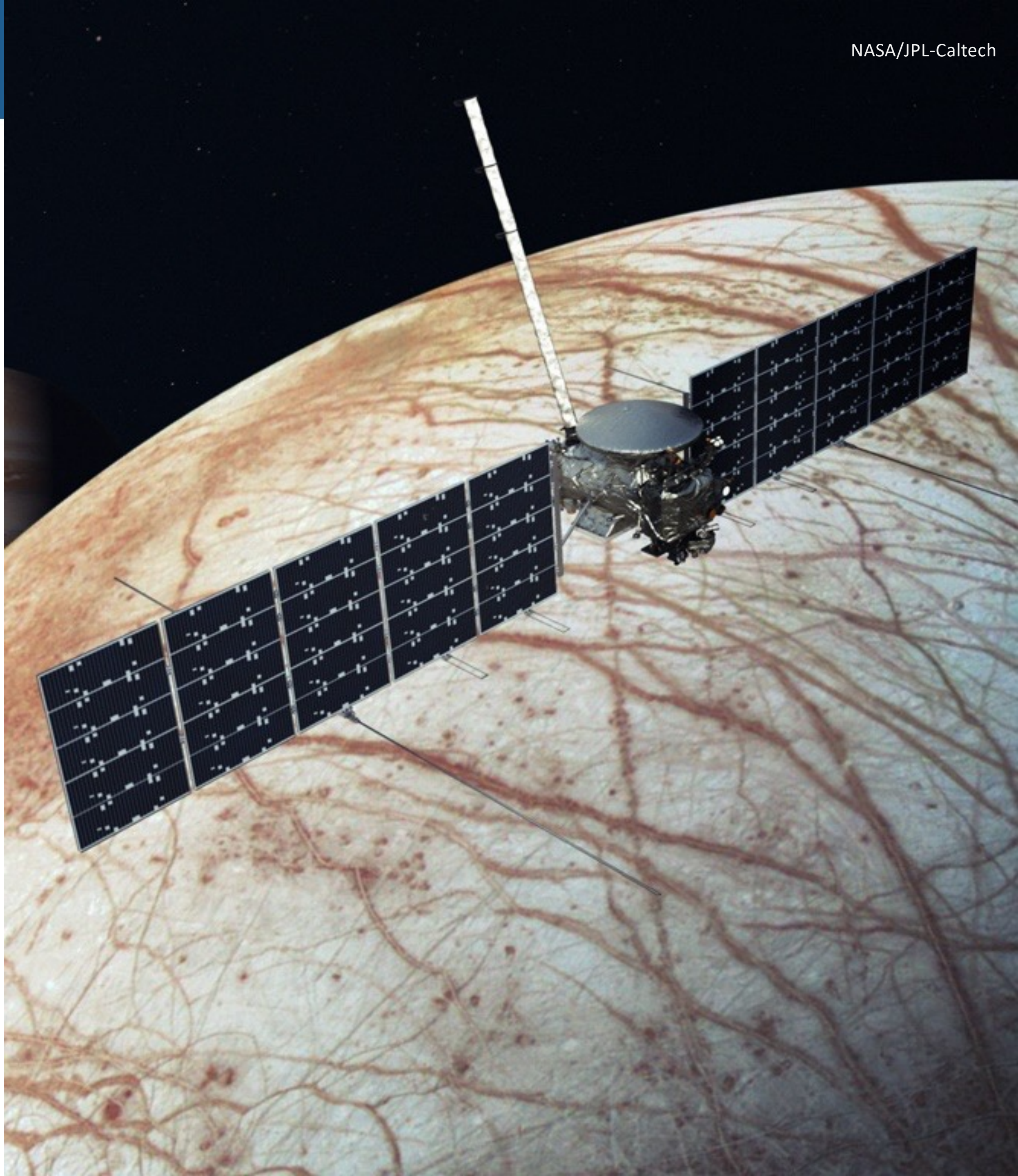
- Possible induced magnetic field
- Thin oxygen atmosphere
- Possible plumes
- Ice shell
- Surface fractures
- Liquid/slushy saltwater ocean
- Possible deep surface hydrothermal vents
- Rocky mantel
- Iron-nickel core

NASA/JPL/University of Arizona

https://solarsystem.nasa.gov/moons/jupiter-moons/europa/in-depth/

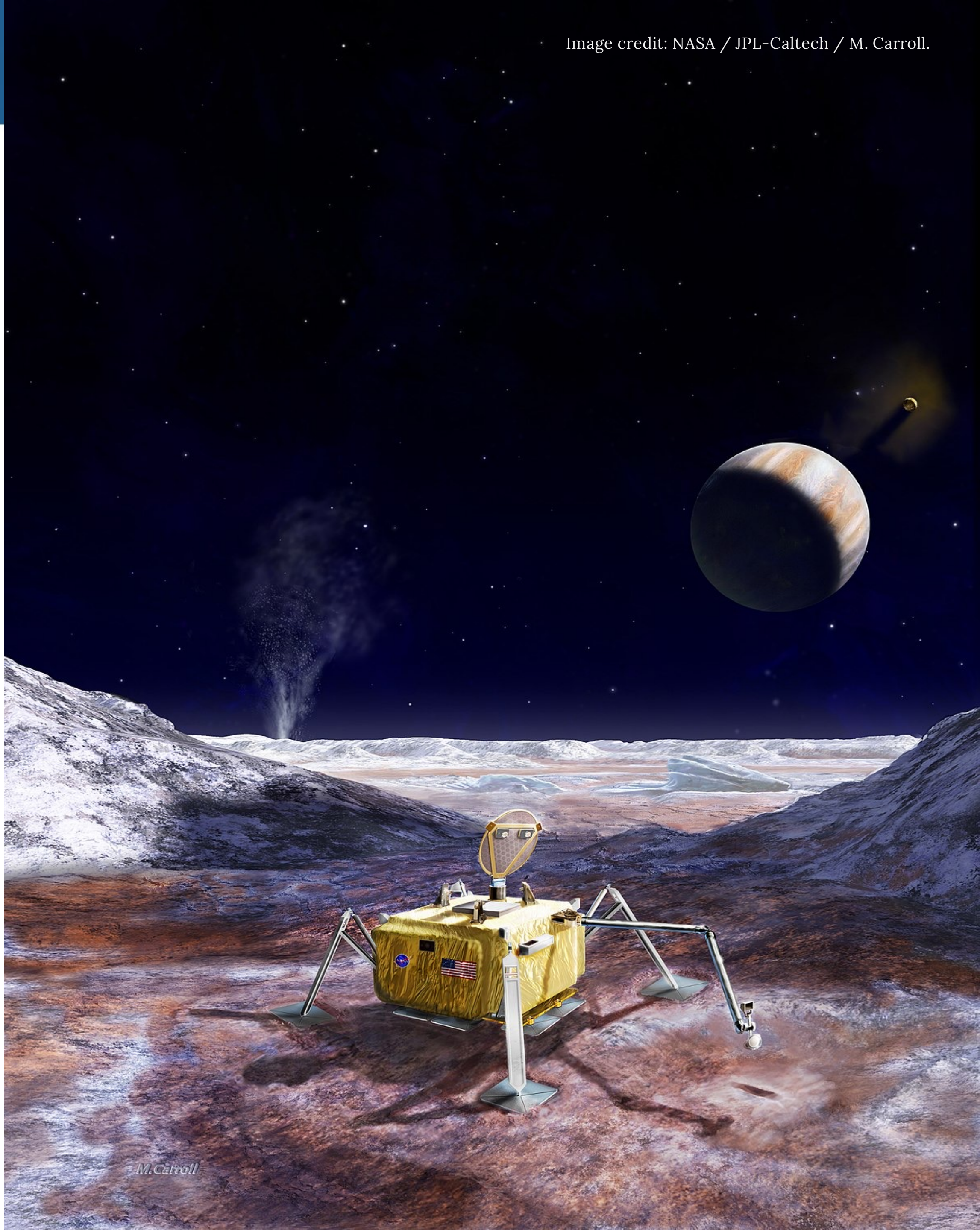
Europa Clipper

- Take measurements to determine if Europa could harbor conditions suitable for life.
- 40 to 50 close passes over Europa
- Expected launch: 2024


NASA/JPL-Caltech

Europa Lander

- Concept for a potential future mission that would look for signs of life in the icy surface material of Europa.
- Collect samples from about 4 inches beneath the surface.
- Analyze in miniature laboratory within the robotic lander.

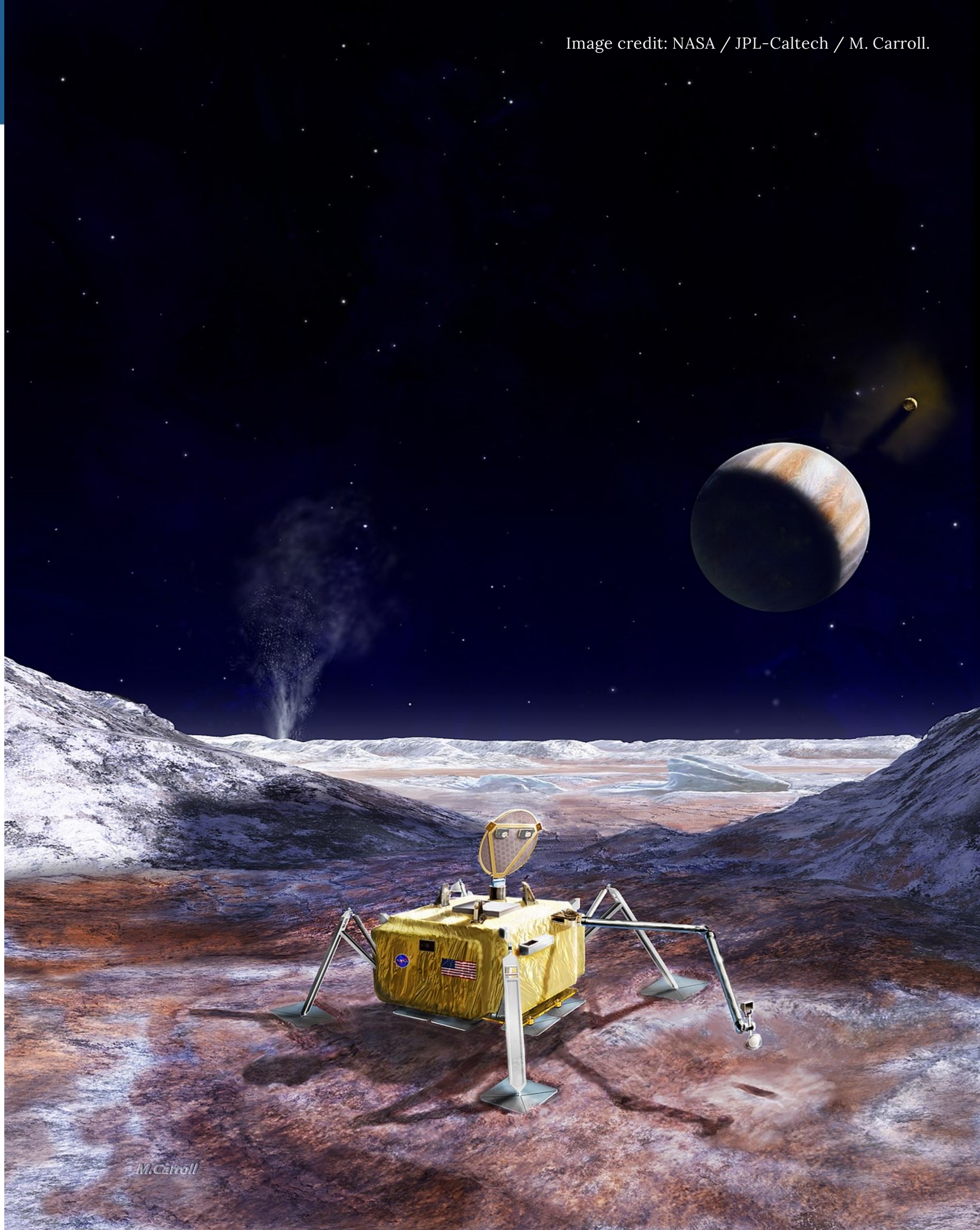


Europa Lander

1. Search for evidence of biosignatures on Europa.

2. Assess the habitability of Europa via *in situ* techniques.

3. Characterize the surface and subsurface of Europa.



JACOBS

MSFC Support of Europa Lander Risk Reduction

- Lander Missions commonly use solid rocket motors (SRM)
  - Risk: SRM case overpressure resulting unburned SRM materials/components encountering a planet’s surface
- NASA MSFC Solid Propulsion and Pyrotechnic Devices and Non-Metallics and Space Environmental Effects Branches collaborated to investigate the bioburden of SRM materials.
- Goal was to identify and document microbial content of SRM materials to:
  - Enhance material selection process
  - Reduce possibility of exceeding allowed bioburden limit
  - Understand what microbes can survive space environments
- MSFC Tech Excellence award and Jacobs Innovation Grant funding allowed us to address this question

Where do microbes hide in SRM materials?

- Surfaces
- Encapsulated within material
- Lodged between mated areas

Embedded spores are ~10x more heat resistant than surface

JACOBS

NASA-Approved Bioburden Reduction

- Vapor Hydrogen Peroxide (VHP) – surface
- Heat Microbial Reduction (HMR) – total

ECSS-Q-ST-70-57C - DFR 1
23 January 2013

Table D-1: D-values for 2 to 3 orders of magnitude reduction

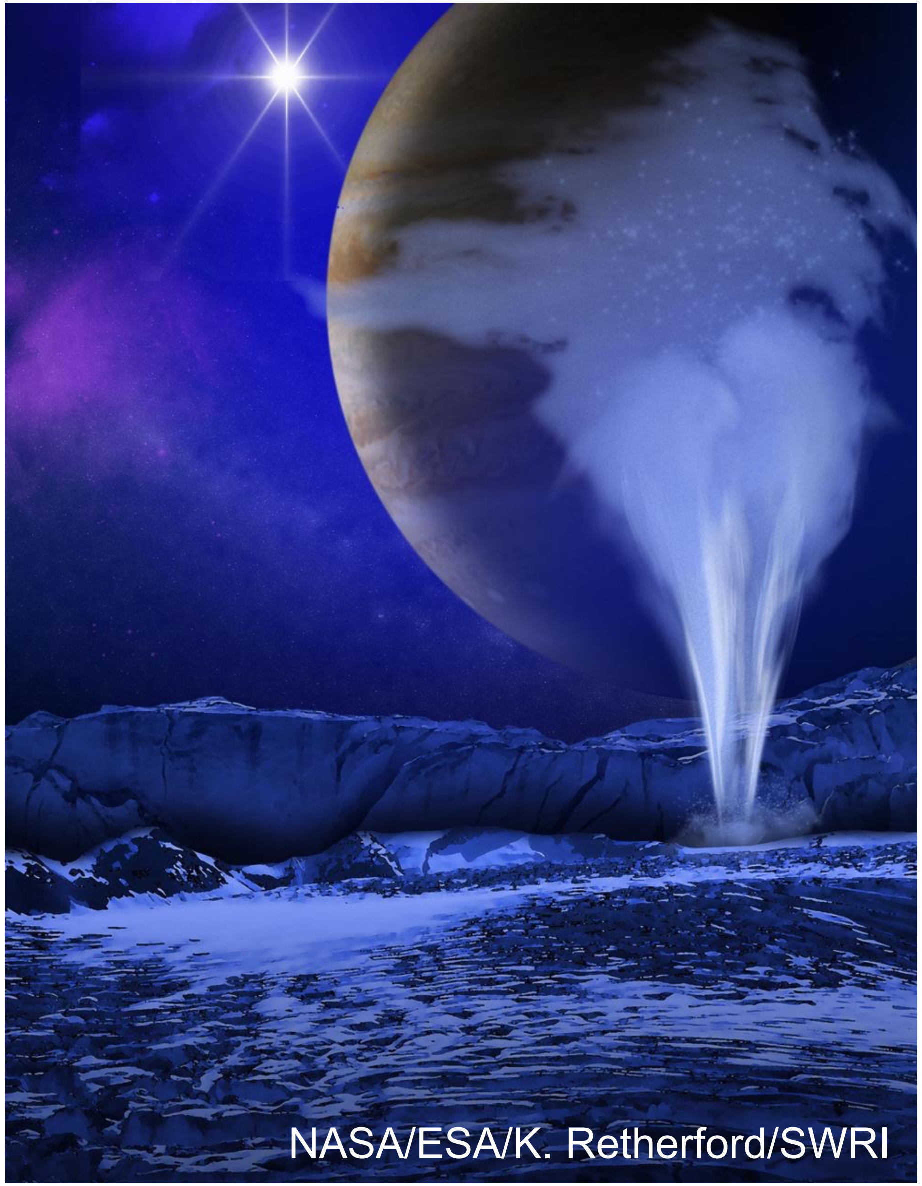
Temperature T (°C)	dry surface D_{value} (min)	ambient surface D_{value} (min)	dry mated D_{value} (min)	ambient mated D_{value} (min)	uncontrolled humidity (surface, mated) and encapsulated D_{value} (min)
110	155,4	268,7	310,8	537,5	1533,8
111	139,2	236,5	278,5	473,0	1392,5
112	124,8	208,1	249,6	416,2	1247,9
113	111,8	183,1	223,7	366,2	1118,3
114	100,2	161,1	200,4	322,2	1002,1
115	89,8	141,8	179,6	283,5	898,1
116	80,5	124,7	161,0	249,5	804,8
117	72,1	109,8	144,2	219,5	721,2

D-value - time required to achieve inactivation of 90% of a population of the test microorganisms under stated conditions

Must be sustained temp/humidity

Bioburden Reduction Limitations

- Some materials and missions have bioburden reduction limitations
  - Material incompatibility
  - Material integrity/aging
  - Unknown mission-related risks (deep space)
- NASA applies "specification values" to materials that cannot be bioburden reduced



NASA/ESA/K. Retherford/SWRI

JACOBS

NASA Specification Values

- NASA describes encapsulated bioburden specification values:
  - 30 spores/cm^3 of nonelectronic materials
  - 130 spores/cm^3 of mixed nonmetallic assemblies
  - 150 spores/cm^3 of electronic piece parts
- Specification values are **conservative**
  - May represent an order of magnitude greater abundance than true numbers
  - May be overestimated because many materials contain toxic substances

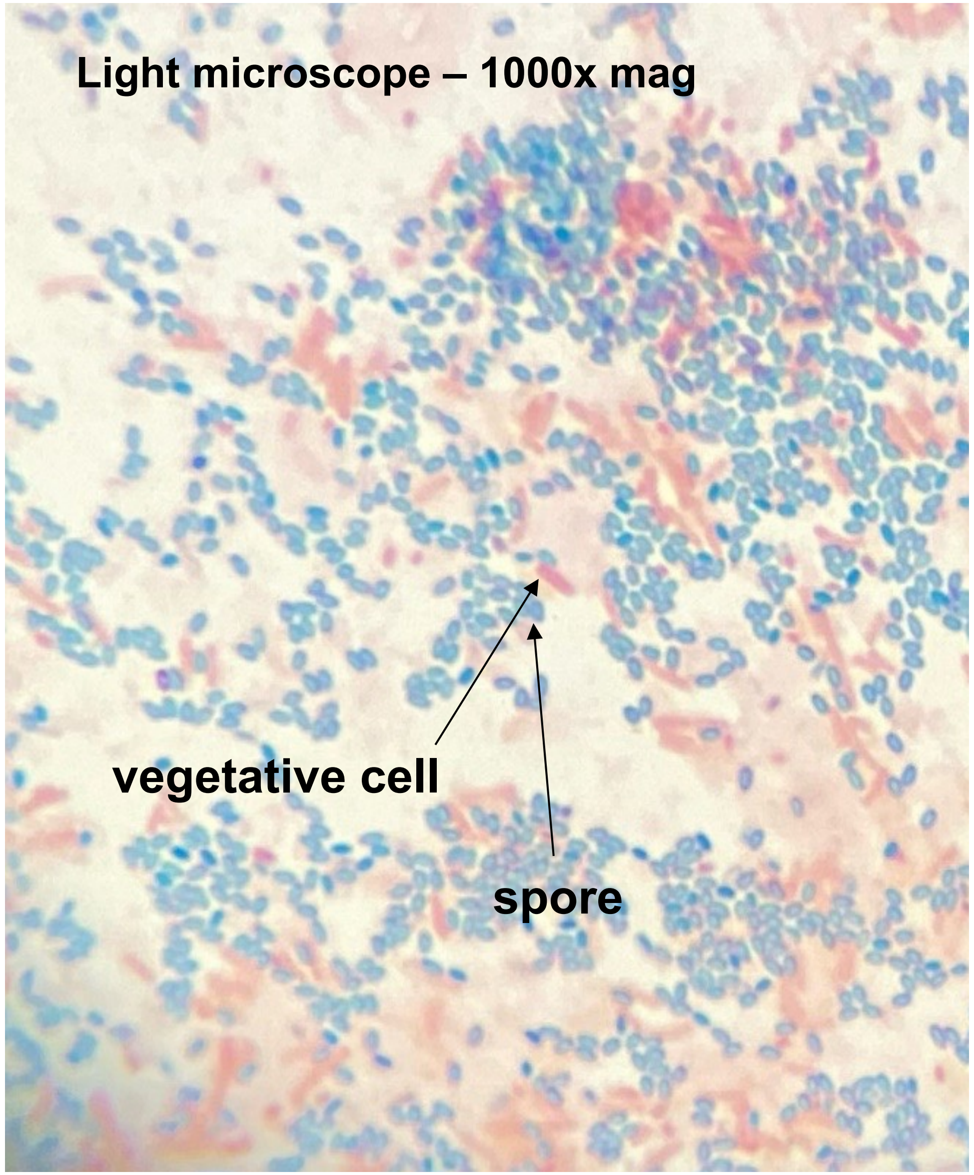
Previous Research at JPL

- Use of destructive assays to determine true microbial load of certain spacecraft materials limited by bioburden reduction methods
  - Epoxy Adhesive (Scotch-Weld-3M 2216 B/A Gray)
  - Shielded twisted-wire pair
  - Spacecraft lubricant
  - Electrolyte components of a lithium-ion battery

Wayne W. Schubert, Laura Newlin, Shirley Y. Chung, Raymond Ellyin, Assessment of bioburden encapsulated in bulk materials, Advances in Space Research, Volume 57, Issue 9, 2016, Pages 2027-2036, ISSN 0273-1177, https://doi.org/10.1016/j.asr.2016.02.012.

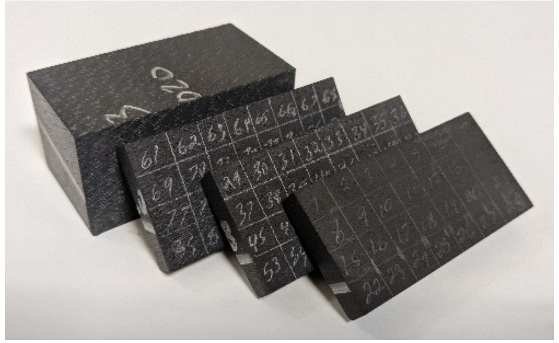
Previous Research at JPL

- They studied **total** viable microorganisms (not just spores)
- **Key result: ALL materials were below NASA specification values**
- Future work: assess a more diverse suite of materials, as well as greater quantities



Bioburden Assessment using Destructive Assays

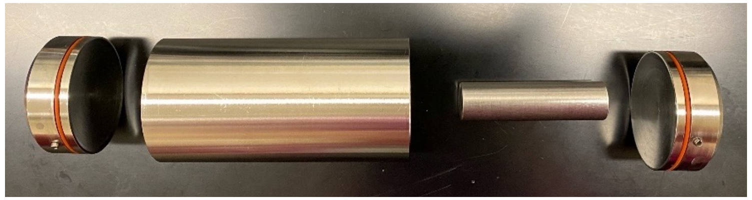
- We collected 14 different nonmetallic materials, consisting of six categories:
  1. Adhesives
  2. Composites
  3. Insulations
  4. Liners
  5. Inert propellants
  6. Thermal protection systems

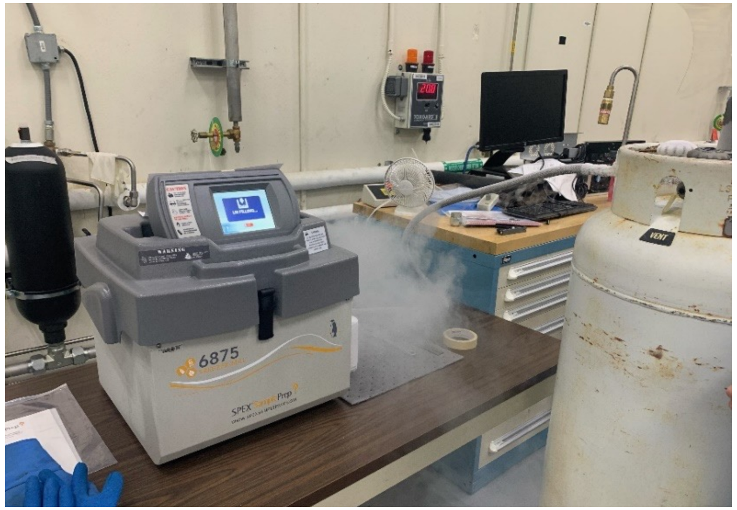




Bioburden Assessment using Destructive Assays

- Developed a protocol based on Schubert *et al*., 2016
- Can be updated over time and used for many materials/projects





Procedure for Cryogrinding Uninoculated Materials

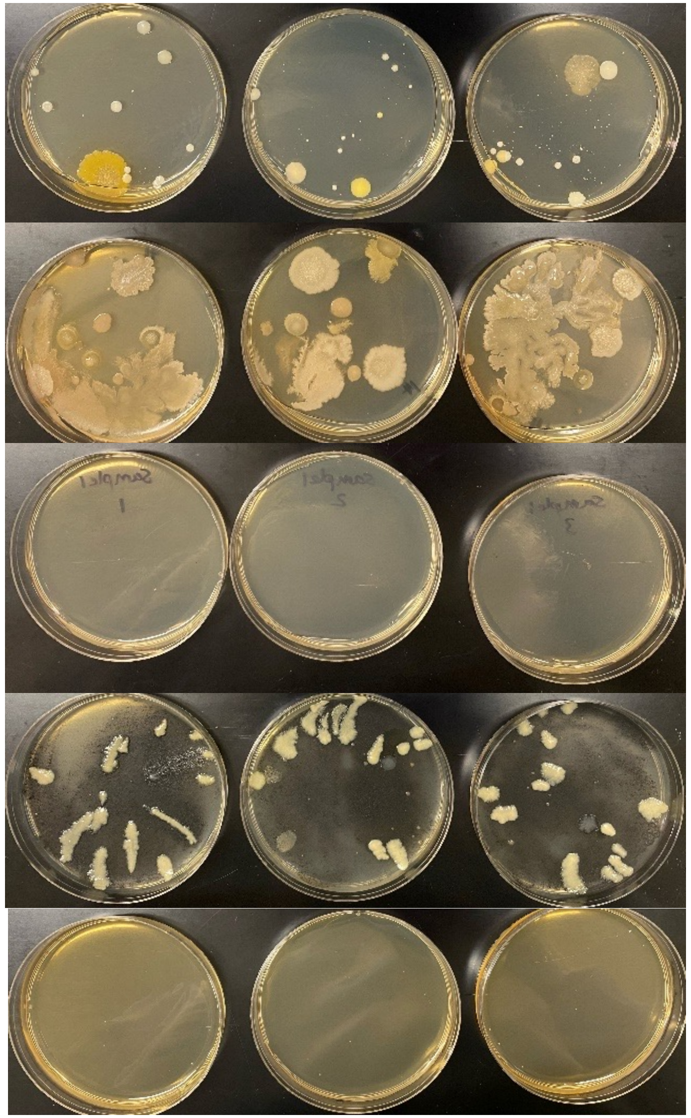
Summary: This document is to guide inexperienced individuals on how to collect and assess microbial content of materials via cryogrinding. The procedure includes preparation of samples and cryovials, the cryogrinding procedure, and the follow-up analysis of the microbial isolates.

Chelsi Cassilly

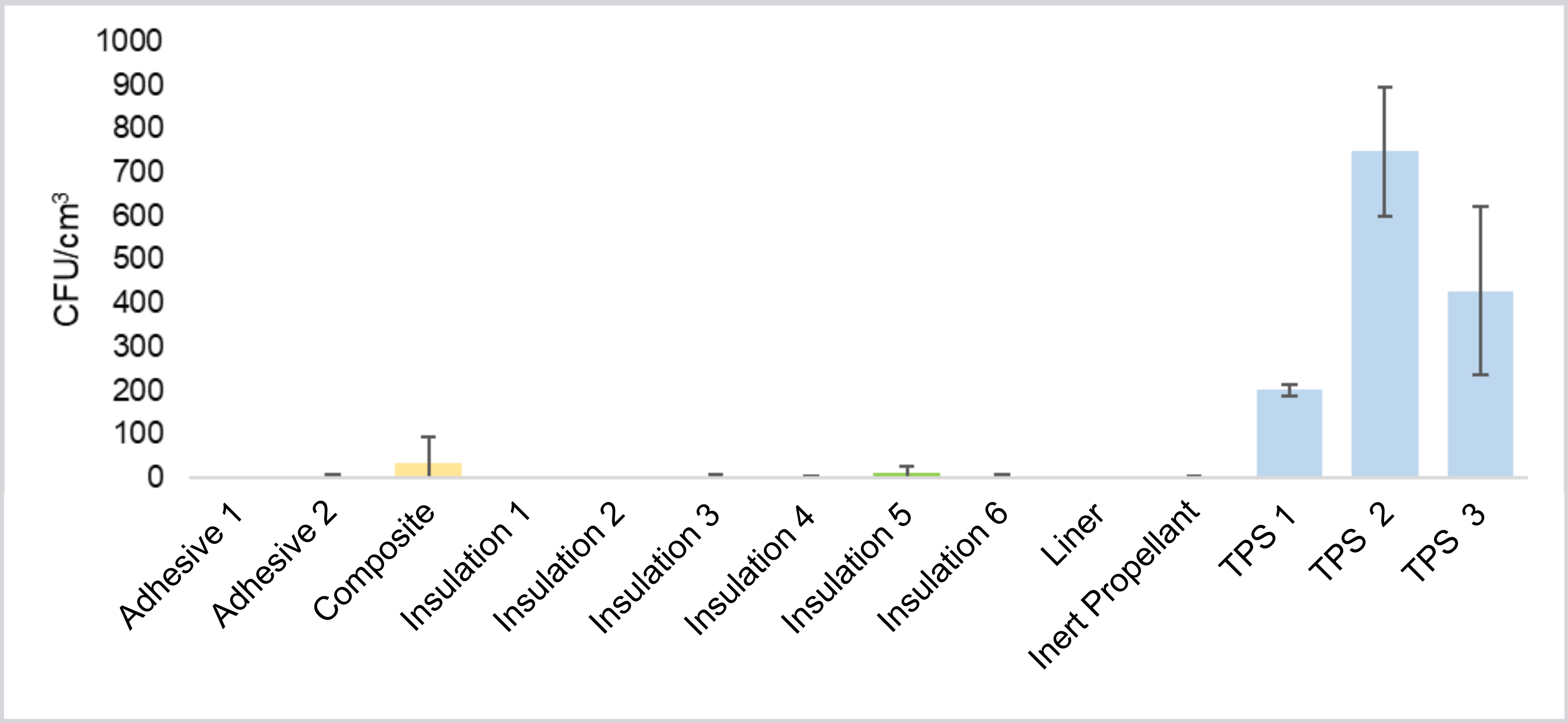
MSFC EM41 PP Lab
September 20, 2022

Contents

Microbial Enumeration of Spacecraft Materials using Destructive Assays



Some Materials Pose Greater Risk than Others



Comparison of Experimental and Applied Bioburden Values

Material	Empirical Total Bioburden Estimate	NASA Specification Total Bioburden Estimate
Adhesive 1	0	360
Adhesive 2	3	360
Composite	33	360
Insulation 1	0	360
Insulation 2	0	360
Insulation 3	4	360
Insulation 4	9	360
Insulation 5	11	360
Insulation 6	3	360
Liner	0	360
Inert Propellant	1	360
TPS 1	200	360
TPS 2	747	360
TPS 3	428	360

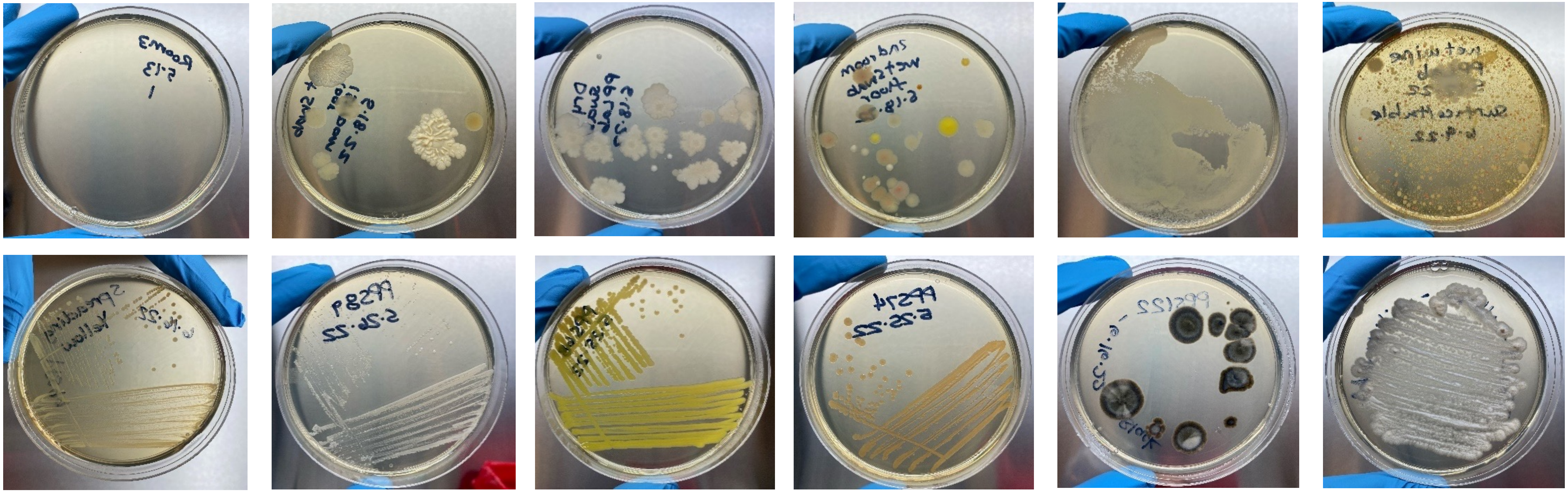
JACOBS

Conclusions

- The majority of materials tested demonstrated smaller total bioburden than the NASA specification values would estimate
- Applying empirically-determined estimates of bioburden can lower total accountable bioburden for certain materials
- Important to gather more data but also evaluate materials on a case-by-case basis

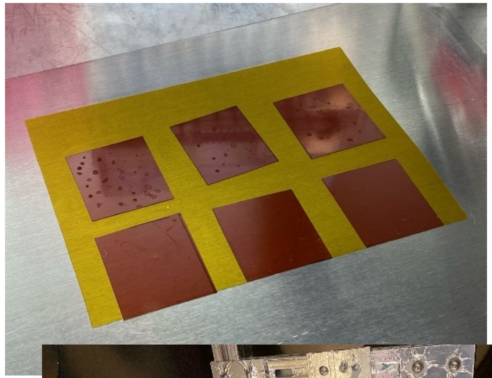
Ongoing Work: Identify Material-Associated Microbes

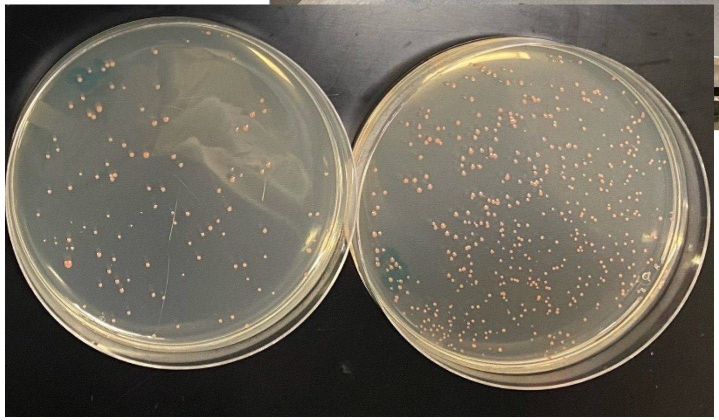
- Collected over 100 microbial isolates which are being identified using MALDI-TOF at University of Chicago, in collaboration with JPL



Ongoing Work: Space Environmental Effects on Microbial Survival

- NASA is interested in how the space environment impacts microbes:
  - Could it reduce bioburden?
  - Could it trigger resistance mechanisms?
- Study microbes to characterize potential risks:
  - Insulation-associated microbe
  - Withstood drying and radiation
  - Submitted grant for additional funding to study:
    - Cryogenic temperatures
    - Ionizing radiation
    - Ultraviolet radiation
    - Vacuum





JACOBS

Future Work

- DNA analysis on materials
  - Isolation of DNA directly from ground materials (MSFC)
  - Amplification/quantification of DNA (JPL)
  - Sequencing (FBI)

JACOBS

Acknowledgements

- Jacobs/ESSCA
  - Ashley Penton
  - Dr. Daneesh Simien
  - Samantha Marcella
  - Daniel Stephens
  - Nate Cox
  - Harold Burtts
- NASA MSFC
  - Mary Nehls
  - Dr. Precious Mitchell
  - Dr. Jarvis Caffrey
  - Todd Schneider
  - Dr. Peter Bertone
  - Dr. Erin Hayward
  - Jason Vaughn
  - Patrick Lynn
- Jet Propulsion Laboratory
  - Lisa Guan
- Federal Bureau of Investigation
  - Michael Brandhagen

Funding:

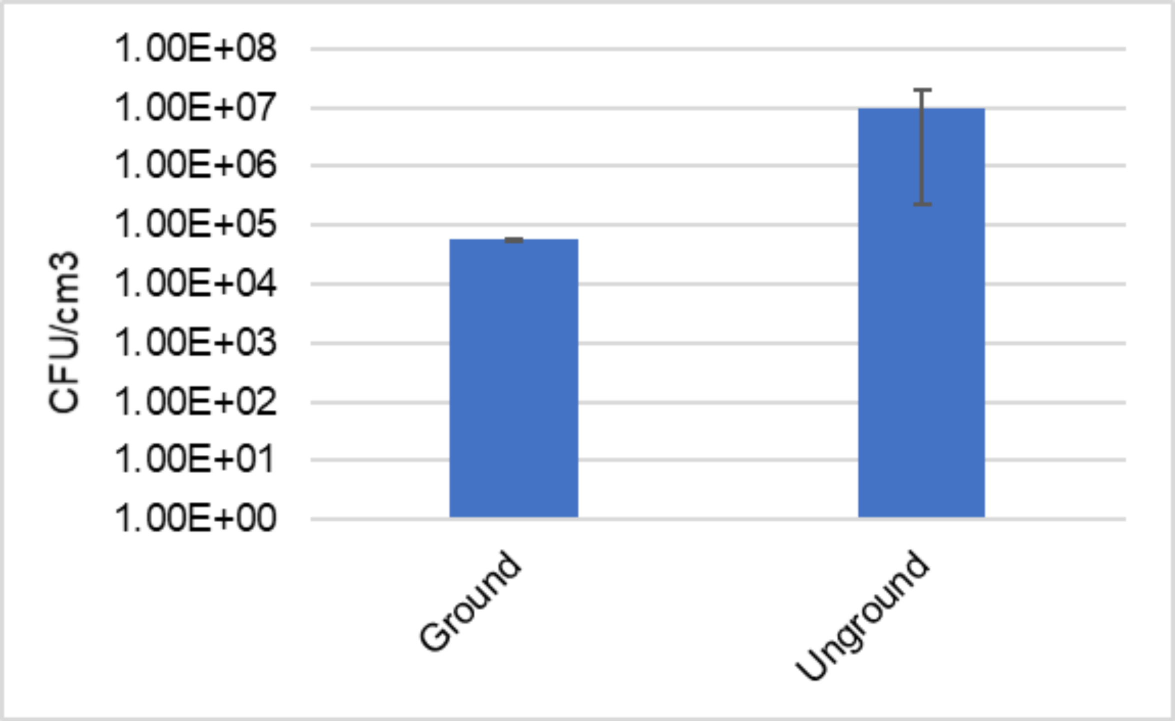
- Europa Lander Risk Reduction
- MSFC Technical Excellence Award
- Jacobs Innovation grant

JACOBS

Questions?

Backup

- The harshness of the procedure could cause a drop in viability of microbes by 2 orders of magnitude



JACOBS

Comparison Between Experimental and Applied Bioburden Values

Material	Empirical Total Bioburden Estimate (CORRECTED)	NASA Specification Total Bioburden Estimate
Adhesive 1	0	360
Adhesive 2	300	360
Composite	3,300 ←	360
Insulation 1	0	360
Insulation 2	0	360
Insulation 3	400 ←	360
Insulation 4	900 ←	360
Insulation 5	1,100 ←	360
Insulation 6	300	360
Liner	0	360
Inert Propellant	100	360
TPS 1	20,000 ←	360
TPS 2	74,700 ←	360
TPS 3	42,800 ←	360