Advancing a Collection of Microorganisms Associated with Robotic Spacecraft Assembly

Wayne W. Schubert† and James N. Benardini

Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA

†Contact: wayne.w.schubert@jpl.nasa.gov

ABSTRACT

Microorganisms isolated from robotic spacecraft have been preserved as a biological resource for studies relevant to planetary exploration and life detection. An increased understanding of the composition of this collection has emerged from new data for 16S rRNA gene identifications, biochemical identification profiles, salt tolerance and electron acceptor studies. To date, the collection is comprised of approximately 3,500 isolates. New isolates are added incrementally as new missions proceed. Preliminary estimates of a subset show roughly 60% of 1322 characterized isolates belong to the genus *Bacillus* and other spore forming genera and roughly 40% belong to non-spore-forming genera including *Staphylococcus*. More than 30 isolates have been identified as novel species candidates. The collection is comprised of isolates gathered from six Mars missions over a period of 40 years, collected throughout each multi-year assembly process. Most microorganisms were isolated from routine bioassays using cotton swabs or polyester wipes. Some microorganisms originated from special assays including isolation from inside of solid materials, such as paints and thermal insulation, or by studies assessing the frequency of air-borne heat-resistant microbes. Microorganisms isolated from these processes were sub-cultured and preserved as frozen glycerol stocks. The complexity of the assembly process, manipulating the thousands of parts, configuring the hardware for testing, shipment and launch, inadvertently introduces microorganisms despite a multitude of precautions including; cleanroom assembly, dry heat microbial reduction and rigorous cleaning. Improving our understanding of this spacecraft microbial archive collection was advanced through student internships and academic partnering. This is a unique microbial environment with little opportunity for cell division, dominated by the rare microorganism that has survived desiccation and removal by cleaning. These microbes exist in a transport-driven environment that is strongly influenced by human activity and control measures, but not predominately composed of human flora. Despite these factors, the microbial diversity of these cultivable organisms is greater than previously appreciated, with more than 25 genera and 65 species represented.

This work was carried out by the Jet Propulsion Laboratory, California Institute of Technology

BACKGROUND

Example of the surfaces that were sampled on the Mars Science Laboratory. The Mars Science Laboratory flight system samples were collected with cotton swabs and polyester wipes.

***Sources* for the collection;**
- Routine bioassays conducted during the assembly of the spacecraft hardware.
- Special studies to understand microbes encapsulated in organic materials, gasses or fluids.
- Specific environments related to spacecraft hardware assembly in clean-room environments and hardware surfaces.
- Microorganisms embedded in organic materials.

Isolation of the microorganisms:
- The majority of the isolates resulted from routine sample collection methods using swabs or wipes. Samples were taken from spacecraft hardware surfaces during the lengthy assembly process. Microbes from the swabs and wipes were extracted into water and in most instances subjected to a 80ºC heat shock. The suspensions were plated in Tryptic Soy agar and incubated for 3 days at 32ºC. Resulting colonies were sub-cultured and added to the Archive Collections.

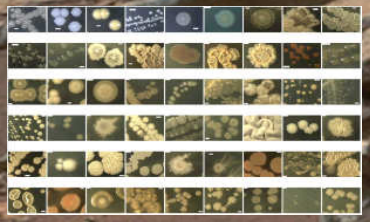
Storage of microbes:
- Frozen Glycerol Stocks and cryobead working stocks.
- Lyophilized cultures for long term preservation.

Microbial Identification:
- Microbial identification was carried out by 16S rRNA gene sequence.
- Strains collected in the 1970's were identified by biochemical utilization patterns.

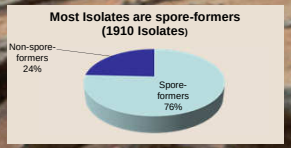
PURPOSE OF THE COLLECTION

These microorganisms were assembled as a reference collection of viable organisms that were present in, on and surrounding spacecraft hardware, that were bound for planetary targets of concern, such as Mars.
- To further understand how to avoid contamination of spacecraft hardware.
- To understand what organisms may be refractory to decontamination procedures such as, routine cleaning, heat microbial reduction (dry, ambient or partial humidity) or vapor phase hydrogen peroxide.
- To aid in understand which assembly processes pose risks for contamination.
- To establish a reference collection of microorganisms that could be used for the evaluation of future life detection instruments and experiments.
- To provide an important resource to the international community and especially the committee on Space Research (COSPAR).

Collection Sources	Collection & Assembly Locations	Number of Isolates	Comment	Year Collected
Teflon Ribbon Study, Dry-Heat Resistant	Kennedy Space Center (KSC) Florida	234	Characterized Biochemically Preparation for Viking Mission and evaluation of sterilization procedures.	1972
Viking (Mars Landers & Orbiters)	KSC	1296	Characterized Biochemically	1975
Mars Pathfinder	Jet Propulsion Laboratory, California Institute of Technology (JPL) & KSC	47	First US Lander since 1975. A small rover.	1996
Odyssey (Mars orbiter)	Denver, CO	90		2001
Mars Exploration Rovers	JPL	191		2003 2005
Mars Exploration Rovers	KSC	204		2003
Phoenix (Mars)	Denver, CO	48	Verification Bioassays	2007
Phoenix (Mars) Fairing	KSC	21		
Phoenix (Mars) Robotic Arm	JPL	44		
Phoenix (Mars) MECA Instrument	JPL	16	Non spore-formers	
Phoenix (Mars) Fairing & Final Assay	KSC	80	Routine & Verification Bioassays	
Mars Science Laboratory	JPL & KSC	455	Frozen glycerol isolates originating from JPL & KSC	2011
	KSC	816	Temporary slants. Id	
InSight	JPL, Vandenberg AFB	3	Current Mission	2014
Mars 2020	JPL & KSC	0	Future Mission	2014 - 2020
Subtotal of Spacecraft-Related Isolates		3545	Date: May 2014	

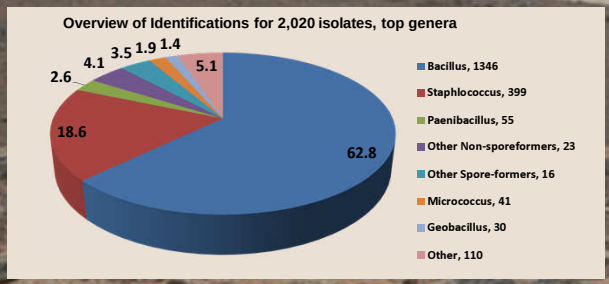


The rogues gallery of microbial colonies isolated from the Mars Science Laboratory. A wide variety of colony morphologies are evident.



An estimate based on the 16S rRNA gene identifications indicates that 76% of the isolates are from spore-forming genera. Two sub-collections had higher frequency of non-spore-formers: the fairing of the Phoenix mission and the Viking collection. Half of the Viking samples were not heat shocked prior to bioassays, permitting the recovery of less heat tolerant organisms.

RESULTS



This graph depicts the abundance of the most prominent genera as identified by 16S rRNA gene sequencing. Data from several sub-collections were compiled. The collection is dominated by the genus Bacillus and other spore-forming genera.

CONCLUSIONS

-The collections arise from unique environments strongly influenced by human intervention and elaborate measures to reduce the number of microorganisms. These measures include: dry heat, alcohol wipes, precision cleaning, HEPA-filtered air and cleanroom attire.
-63% of the 16S sequences are from the genus *Bacillus*
-76% are spore-formers
-Novel species candidates: 97 out of 1495 isolates, about 6%, have homologies <97.5%.
-A sub-collection selected for dry heat resistant microbes, had 30% (55/174) of it's isolates exhibiting homologies <97.5%.

ACKNOWLEDGEMENTS

Further Information:
- For additional information on the characterization of isolates associated with spacecraft assembly, please visit posters:

- Keith Arora-Williams, Garrett Smith, Nicholas Sanchez, Wayne Schubert, Stephanie Smith, Susan E. Childers, Andrzej Paszczynski, and James N. Benardini III, "Phylogenetic and Phenotypic Diversity of Microbial Isolates from the Mars Exploration Rover"
- Alissa K. Tenuto, Stephanie A. Smith, Emmaleen Wear, Kyle Peterson, Connor Chapek, James N. Benardini III, Wayne W. Schubert, Susan E. Childers and Andrzej Paszczynski, "Survival of Microbial Isolates Collected from Viking Spacecraft Surfaces Subjected to Extreme Environmental Conditions Similar to Those of the Martian Surface"
- Aleksandra Checinska, Wayne Schubert, Andrzej Paszczynski, Stephanie A. Smith, Susan E. Childers, James N. Benardini III, "Archiving, Preservation and Characterization of Teflon Ribbon Isolates from the Viking Lander and Associated Cleanroom Facilities"
- Stephanie A. Smith, James N. Benardini III, David Anderl, Matt Ford, Emmaleen Wear, Michael Schrader, Wayne Schubert, Linda DeVeaux, Susan E. Childers, Andrzej Paszczynski, "Identification and Survival of Isolates Collected from the Mars Rover, Curiosity"
- Harold Rohde, Stephanie A. Smith, James N. Benardini III, David Anderl, Matt Ford, Emmaleen Wear, Michael Schrader, Wayne Schubert, Linda DeVeaux, Susan E. Childers, Andrzej Paszczynski. Characterization of Isolates Collected from the MSL at Kennedy Space Center

Collaborations:
- Collaborations that further the science and microbiology of this collection are welcome!

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Photo Credit :NASA The background image includes an area of Mars called "Yellowknife Bay." This area is thought to be a Martian environment favorable for past microbial life. The area has relatively low salt, neutral pH and iron and sulfur containing minerals. Mission public website, image PIA17603.