

A Collection of Microbes Associated with Spacecraft Assembly



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ABSTRACT

A special culture collection was established for the preservation of microbes associated with the assembly of robotic spacecraft. The collection contains nearly 3,500 independent isolates collected from missions to Mars where there was a concern regarding forward biological contamination. The collection arose in support of the planetary protection effort to control and limit the number of microorganisms on spacecraft hardware on the missions: Viking, Mars Pathfinder, Mars Exploration Rovers, Phoenix, Mars Reconnaissance Orbiter and Mars Science Laboratory. Isolates were collected and saved as each craft was being assembled. The majority of isolates came from spacecraft assembly in cleanrooms, but some originated from uncontrolled environments. Collection sites include NASA cleanrooms at JPL and KSC as well as infrequent samples of instruments from universities and companies. The main focus of this collection was to preserve the resulting microbes that were found in routine bioassays, for reference and further evaluation. The sampling techniques utilized swab and wipe samples taken from spacecraft hardware surfaces, heat shocked at 80°C and grown on a nutrient-rich medium, TSA. Thus the collection is biased towards aerobic, desiccation tolerant, mesophiles with a substantial fraction of spore-formers. Strains were preserved as frozen glycerol or as lyophilized stocks. Efforts to characterize the isolates by 16S rRNA gene and biochemical methods have begun. Initial characterization of 415 isolates from three different spacecraft indicated that the most prevalent genera were *Bacillus* (72%), *Staphylococcus* (7%), *Paenibacillus* (5%) with several other genera in smaller percentages, in contrast to historical biochemical-based identifications of 1296 isolates that were primarily human-related strains (75%), *Bacillus* spp. (23%) as the predominant groups. Historical biochemical-based IDs classified 234 dry-heat resistant isolates as *B. lentus* (42%), atypical *Bacillus* (40%), *Actinomyces* (6%), *B. brevis* (5%) and other *Bacillus* sp. Of 171 MER isolates identified by 16S rRNA gene sequence homology, 2.9% were identified as novel species candidates (< 97% sequence homology). This collection is potentially a rich resource for unique organisms resistant to adverse environmental conditions.

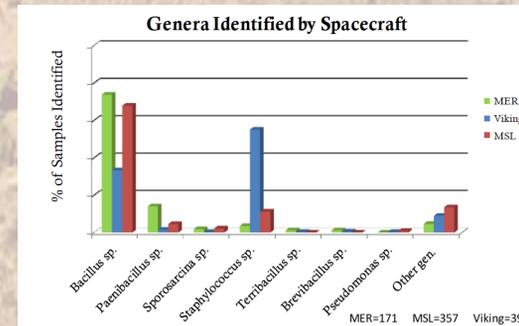
BIOLOGICAL HOLDINGS OF THE COLLECTION

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 understanding 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).

RESULTS



This graph depicts the abundance of the most prominent genera as identified by 16S rRNA gene sequencing, for a sub-collection of the archive. Smith et al. ASM 2013.

Dry Heat Resistant microbes isolated from particulate fallout in spacecraft assembly buildings. Identifications were based on seven biochemical reactions. Molecular identification in progress.

Organism*	Isolates	%
<i>Bacillus lentus</i>	313	42.8
Atypical <i>Bacillus</i>	295	40.3
<i>Actinomyces</i>	41	5.6
<i>B. brevis</i>	35	4.8
<i>B. circulans</i>	16	2.2
<i>B. subtilis</i>	10	1.4
<i>B. coagulans</i>	6	0.8
<i>B. sphaericus</i>	5	0.7
<i>B. firmus</i>	4	0.5
<i>B. pumilus</i>	3	0.4
<i>B. macerans</i>	2	0.3
<i>B. licheniformis</i>	1	0.1
<i>B. polymyxa</i>	1	0.1
Pool of original isolates, Total	732**	100%

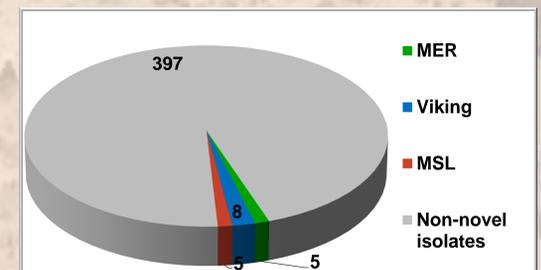
*Identification by seven biochemical responses (1973-1978)

** only ~234 isolates saved for a permanent collection

Ref: Puleo et al. (1978), Thermal Resistance of Naturally occurring Airborne Bacterial Spores. AEM 36:473-479



The rogues' gallery of microbial colonies isolated from the Mars Science Laboratory.



To date 18 candidates (4.3%) of 415 isolates analyzed by for 16S rRNA sequence were identified as candidates as novel species (<97% sequence homology)

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 and Mars Reconnaissance Orbiter	JPL	191	16S rRNA & Biochemical info on 180 isolates	2003 2005
Mars Exploration Rovers	KSC	204		2003
Phoenix (Mars)	Denver, CO	48		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		
Mars Science Laboratory	JPL & KSC	455	Frozen glycerol isolates originating from JPL & KSC	2011
	KSC	816	Temporary slants	
Subtotal of Spacecraft-Related Isolates		3542	Date: March/2013	

CONCLUSIONS

Potential uses and research topics:

- What organisms are most likely to be found on spacecraft hardware during assembly?
- What organisms are potential risks for transfer to other solar system bodies?
- What organisms could potentially be resistant to microbial decontamination procedures?
- What organisms could survive on Mars? Metabolic potential for survival in suspected salt environments?
- What organisms could survive ionizing radiation, UV radiation or desiccation?
- What genetic properties permit organisms to survive harsh environmental conditions?
- Novel species Identification.

Collaborations:

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

Acknowledgements:

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Further Information:

- For additional information on the characterization of isolates associated with spacecraft assembly, please visit posters 1050, 1189, 1627, and 2030.

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 80°C heat shock. The suspensions were plated in Tryptic Soy agar and incubated for 3 days at 32°C. 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.