

# Robotic Spacecraft Associated Microorganisms

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## ABSTRACT

Spacecraft assembly areas and clean rooms are unique microbial environments with little opportunity for cell division and dominated by rare microorganisms that have survived desiccation and removal by cleaning. These microbes exist in transport-driven environments that are strongly influenced by human activity, but not predominately composed of human flora. The microbial isolation procedures strongly favor the isolation of spore-formers and surfaces such as aluminum, titanium, carbon fiber laminates, paints, conformal coatings, wires bundles, and electronic boards. Microbial isolates from spacecraft that have been identified by 16SrRNA gene sequence (2318) most frequently belong to the following major genera: *Bacillus* (64%), *Staphylococcus* (18%) and *Paenibacillus* (3%). Preservation of this collection consists of frozen glycerol or lyophilized stocks. Genomic DNA resulting from the identification protocols have been added as an additional resource. A commercial database is used to assist with isolate storage locations and data associated with each isolate. Advancements to the archive of spacecraft isolates and its knowledge base have been made by obtaining MALDI-TOF spectra for isolate identification with a commercial mass spectral microbial database of ~5,800 known organisms. Early results indicate that a substantial fraction of isolates run on the MALDI-TOF from the Planetary Protection Archive are not yet in the database. Therefore, a unique planetary protection spectral library is being constructed to identify isolates from specific clean room environments and to flag organisms that have been isolated on more than one occasion.

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## BACKGROUND

### 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 first identified by biochemical utilization patterns.
- An alternate identification method by mass spectrometry is being evaluated.



Cleanrooms at JPL (left) and Kennedy Space center (right) are used to assemble spacecraft. The center image depicts a swab sampling on a spacecraft part. Gloves and special clean room garments are required.

## 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 Overview

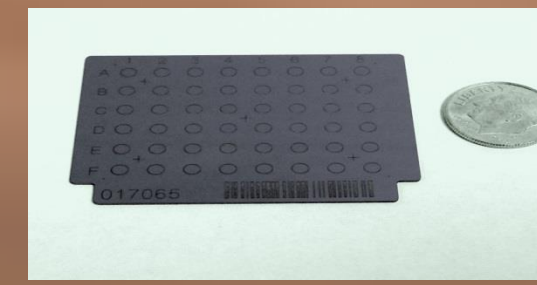
Collection Sources	Collection & Assembly Locations	Number of Isolates	Comment	Year Collected
Teflon Ribbon Study, Dry-Heat Resistant	Kennedy Space Center (KSC) Florida	234	Characterized Biochemically and by 16SrRNA gene sequence. Collected in preparation for Viking Mission and evaluation of sterilization procedures.	1972
Viking (Mars Landers & Orbiters)	KSC	1296	Characterized Biochemically and by 16SrRNA gene sequence.	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	413	Twin rovers. Two assembly locations	2001 - 2003
Phoenix (Mars)	JPL, KSC, Denver, CO	209		2007
Mars Science Laboratory	JPL & KSC	1271	Frozen glycerol isolates originating from JPL & KSC. Includes 816 unprocessed slants.	2011
InSight	JPL, Vandenberg AFB	374	Current Mission	2014-2016
Mars 2020	JPL & KSC	93	Future Mission	2014 - 2020
Subtotal of Spacecraft-Related Isolates		4009	Date: May 2015	

## Identification by MALDI-TOF Mass Spec

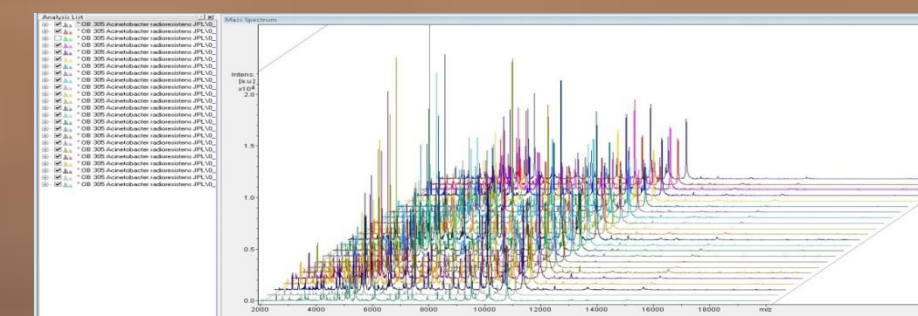
Microbial isolates in this robotic spacecraft collection are being identified by mass spectrometry. Ribosomal proteins in microbial extracts present characteristic spectra that can be used as a fingerprint for identification. We are testing this approach to see if the method will yield comparable results to conventional sequencing approaches. Major steps in the technique are illustrated below.



A MALDI-TOF Mass Spectrometer is used as a rapid identification system for microbial isolate.



Bacterial extracts are distributed to a target plate and overlaid with a matrix compound.



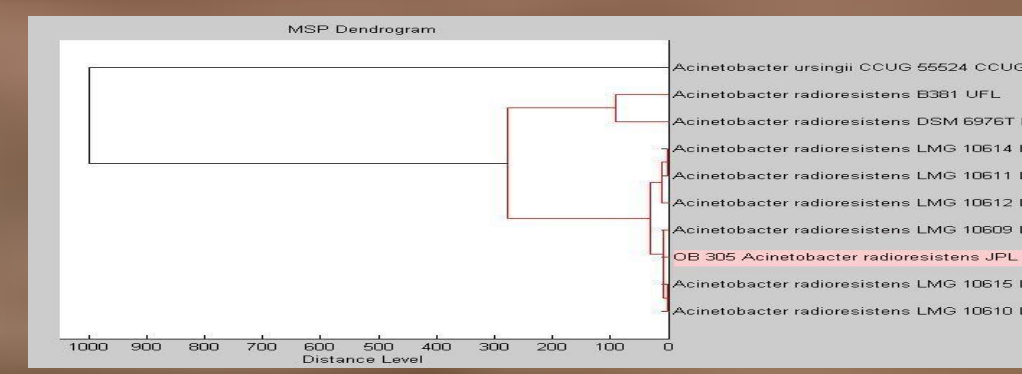
Multiple spectra are obtained for each organism that are processed into a consensus spectrum.

Hit	Detected Species	Log(Score)
1	<i>Acinetobacter radophila</i> DSM 59787 H488	2.2027
2	<i>Acinetobacter radophila</i> L480_10609 L480	2.2026
3	<i>Acinetobacter radophila</i> L480_10610 L480	2.2018
4	<i>Acinetobacter radophila</i> L480_10611 L480	2.2017
5	<i>Acinetobacter radophila</i> L480_10612 L480	2.2017
6	<i>Acinetobacter radophila</i> L480_10613 L480	2.2017
7	<i>Acinetobacter radophila</i> L480_10614 H488	2.2012
8	<i>Acinetobacter radophila</i> B 381 L47L	1.1853
9	<i>Acinetobacter radophila</i> CC219_19504K C219S	1.0689
10	<i>Acinetobacter baumannii</i> DSM 300077 H484	1.0603

Microbial isolate spectra are compared to a ~5,800 organism commercial database. This Viking mission isolate has close matches (green) in this database.

Hit	Detected Species	Log(Score)
1	V41-32-076	0.7753
2	GA_1338 <i>Acinetobacter schroederi</i> strain YNB103 JPL	0.744
3	V42-15	0.6205
4	V42-15	0.6209
5	024_06_13c	0.618
6	B25-02b	0.574
7	0101-05	0.559
8	Fin V37-24 JPL	0.559

Many of the microbial isolates do not have close matches (red). A custom database consisting of microbial isolates from spacecraft assembly areas is being assembled. In this example the microbial spectra self-matches (green).

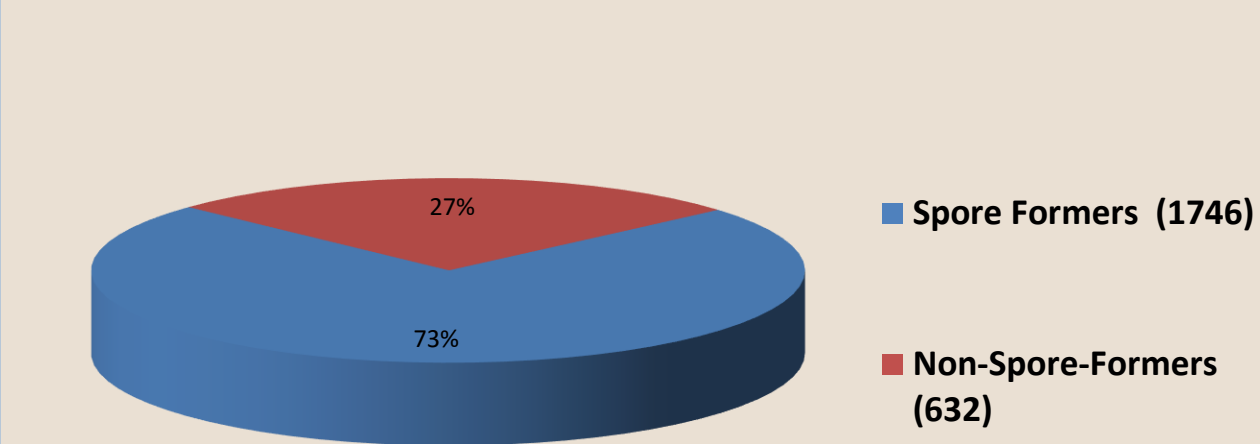


Dendograms illustrating the relatedness of the spectra that can be constructed. In this case, a Viking mission isolate (highlighted) is compared to both the commercial and our custom database, with top 10 hits shown.

Early results indicate that a substantial fraction of isolates run on the MALDI-TOF from the Planetary Protection Archive are not found in the commercial database. Therefore, a unique planetary protection spectral library is being constructed to catalog isolates from robotic spacecraft, and clean room environments. A custom library can flag organisms that have been isolated on more than one occasion.

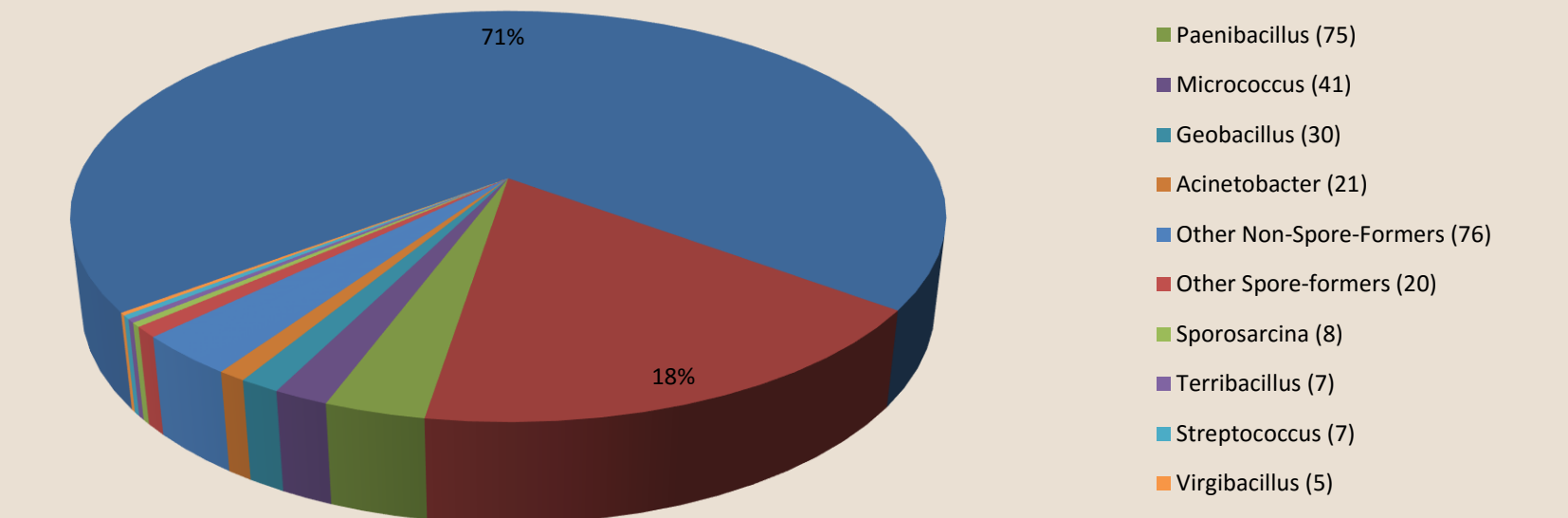
## RESULTS

### Most Isolates are spore-formers 2378 Isolates



An estimate based on the 16S rRNA gene identifications indicates that 73% 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.

### Most Frequent Genera Identified 16SrRNA Gene 2378 Isolates



This diagram 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. Most genera are known to have member species that are desiccation tolerance or form spores.

## CONCLUSIONS

- The microbial isolates originate from unique environments that are strongly influenced by human intervention despite elaborate measures to reduce the number of microorganisms. These measures include: dry heat, alcohol wipes, precision cleaning, HEPA-filtered air and cleanroom attire.
- 71% of the 16SrRNA gene sequences are from the genus *Bacillus*.
- 73% are spore-formers.
- A sub-collection selected for dry heat resistant microbes, had 30% (55/174) of it's isolates exhibiting homologies <97.5% with any described type species. These unique sequences are potential novel species.
- MALDI-TOF Spectrometry can provide similar microbial ID matches to conventional sequencing, if the organism is already in a database.
- MALDI-TOF analysis of new isolates using an in-house user database, can flag similar strains previously analyzed, and in comparison, isolates not previously observed.

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### IMAGE Credit:

- Background Photo: IA16768\_MtSharpMosaic-sol45-WB\_annotated-ful Image Credit: NASA/JPL-Caltech/MSSS. Mission public website

- PIA14126\_SAF\_MSL\_20110512-br2 clean room, NASA/JPL-Caltech/MSSS
- msl20110715\_2011-5878-full cruise stage & aeroshell, NASA/JPL-Caltech/MSSS

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