Poster # Sunday-172

Identification Of Spacecraft-Associated Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry

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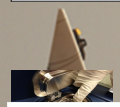
ABSTRACT

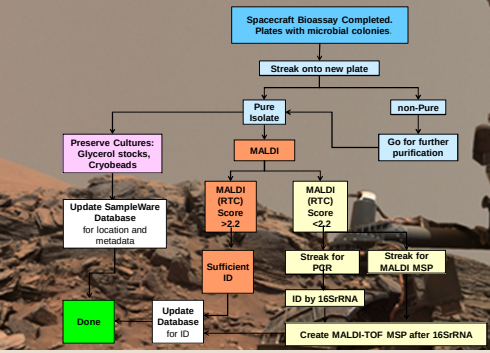
Microbes collected from robotic spacecraft and cleanroom assembly areas are archived in JPL's microbial culture collection. The microbial isolates that have been identified by 16S rRNA gene sequence (2318) most frequently belong to the following major genera: *Bacillus* (64%), *Staphylococcus* (18%) and *Paenibacillus* (3%) with other genera composing the remaining 15%. As an alternate, rapid and more sensitive approach, a subgroup of these isolates were identified by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) and compared to their 16S rRNA gene sequence identifications. An in-house database was created using bacterial isolates with known 16S rRNA identifications collected from Phoenix Mars lander hardware and was used to validate the correlation between 16S rRNA-based identifications and MALDI-TOF identifications. Similar gene sequences at the 99% homology level were grouped into operational taxonomic units (OTUs), and MALDI-TOF spectral profiles (MSP) were created for one representative isolate from each OTU and added to the in-house database. Fifty-three unique OTUs were produced from 147 Phoenix isolates based on 16S rRNA gene sequencing. Using MALDI-TOF based real time classification (RTC), 74% of Phoenix isolates matched their 16S rRNA gene identifications. RTC analysis was able to detect sub-species variations that were not possible by 16S rRNA sequencing. Creation of a more comprehensive database is required in order to correctly identify the cleanroom microbial isolates.

BACKGROUND

- Identifying our isolates with the commercial database was unsuccessful because the cleanroom communities are significantly different from the clinically-oriented database useful for diagnostic purposes.
- Bruker's database contains 5,989 microorganism profiles.
- Only 6.87% of our isolates were identified in the Bruker database (Phoenix and MSL trial sets.)
- The low rate of identification establishes the need for an in-house database.

Definitions
MALDI-TOF = Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry
RTC = Real Time Classification, a MALDI-TOF approach
MSP = Main Spectrum, an averaged consensus spectrum
OTU = Operational Taxonomy Unit



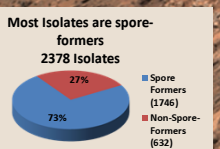


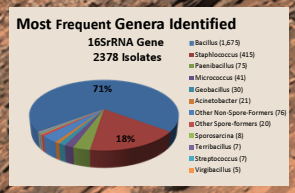
The overall approach for the spacecraft microbial archive. The identification by MALDI-TOF is illustrated in the orange and yellow pathways. This is the processing flow for new individual isolates added to the collection.

THE COLLECTION OF SPACECRAFT ISOLATES

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.

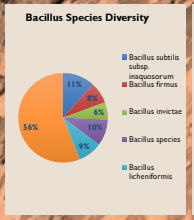
- To further understand how to avoid contamination of spacecraft hardware.
- To understand what organisms may be refractory to decontamination procedures such as, routine cleaning, heat microbial reduction (dry, ambient or partial humidity) or vapor phase hydrogen peroxide.
- To aid in understanding 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.

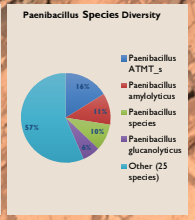


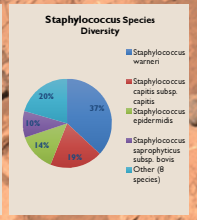


An estimate based on the 16S rRNA gene identifications indicates that 73% of the isolates are from spore-forming genera. Two sub-collections had higher frequency of non-spore-formers: the fairing of the Phoenix mission and the Viking collection. Half of the Viking samples were not heat shocked prior to bioassays, permitting the recovery of less heat tolerant organisms.

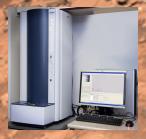
This diagram depicts the abundance of the most prominent genera as identified by 16S rRNA gene sequencing. Data from several sub-collections were compiled. The collection is dominated by the genus Bacillus and other spore-forming genera. Most genera are known to have member species that are desiccation tolerance or form spores.



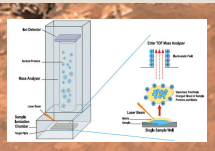


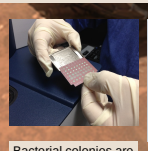


Identification by MALDI-TOF Mass Spec

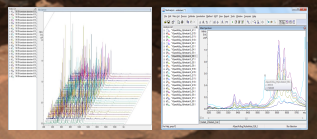


A MALDI-TOF Mass Spectrometer is used as a rapid identification system for microbial isolate.





Bacterial colonies are directly applied to a target plate and overlaid with a matrix compound.

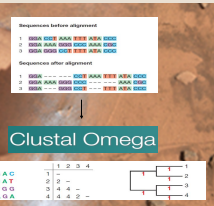


Multiple spectra are obtained for each microbe, that are processed into a consensus spectrum.

APPROACH

Bioinformatics Workflow

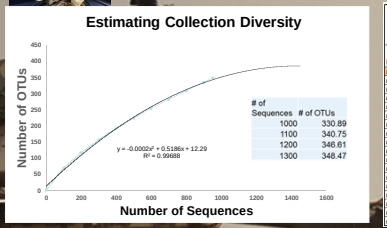
1. The workflow was streamlined by the following bioinformatics approach:
2. Align sequences using multiple sequence alignment (Clustal Omega)
3. Cluster sequences at 99% 16s rRNA similarity using a furthest-neighbor algorithm to form operational taxonomic units- OTUs (mothur)
4. Choose representative isolate, assign taxonomic identification using a curated database (EzTaxon) and create MSP.
5. Run RTCs for other members of the same OTUs cluster, to verify that they match to their representative MSP.



RESULTS

Database	MSPs	RTC Match Success
Commercial / Instrument	5982	7 %
Combined (JPL + Commercial)	6330	97 %

MALDI-TOF Metrics	Phoenix Spacecraft	MSL Spacecraft	Viking Spacecraft	Mixed Grouping 2016
Isolates tested	147	44	177	348
MSP's created	53	14	68	301
Grouped into OTUs	53	14	68	348
Correctly Identified	73.4%	61.3 %	46.9 %	97 %
Incorrectly Identified	3.5%	15.9 %	19.8 %	3 %
No Identification with database members	13.2%	20.45 %	18.62 %	-
Poor spectra (flat line)	9.7%	2.3 %	-	-



This graph is an estimate the collection diversity. Since the line has not reached a plateau, more gene sequences will be needed. If the curve continues to follow the fitted line, another 400 sequences will be required to capture "full diversity."

The points are based on 16S sequences. Perhaps an equal number of MSP's will be required for MALDI-TOF identification.

Sub-species Variation

Known 16S rRNA ID	Isolate name Tested	Result by MALDI-TOF RTC	RTC Score (3.0 is perfect)	Comment
Bacillus subtilis	V46-19*	*Bacillus subtilis* V46.19*	2.782	Starting MSP
Bacillus subtilis	V61-7	*Bacillus subtilis* V46.19*	2.171	
Bacillus subtilis	V43-20	*Bacillus subtilis* V46.19*	2.213	
Bacillus subtilis	V44-17b	*Bacillus subtilis* V46.19*	2.434	
Bacillus subtilis	V44-14*	*Bacillus subtilis* V46.19*	2.382	
Bacillus subtilis	V43-21	*Bacillus subtilis* V46.19*	2.422	
Bacillus subtilis	V44-16	*Bacillus subtilis* V46.19*	2.63	
Bacillus subtilis	T3-11	*Bacillus subtilis subsp. inaquosorum*	2.227	
Bacillus subtilis	M5-19	*Bacillus subtilis subsp. Subtilis V3.*	1.981	
Bacillus subtilis	V2-20	*Bacillus subtilis* V46.19*	2.45	
Bacillus subtilis	V44-Fa	*Bacillus axarhi* V44.Fb*	2.281	
Bacillus subtilis	V44-17b	*Bacillus axarhi* V44.Fb*	2.193	
Bacillus subtilis	V44-17a	*Jonbacter mobilis* V41.32	2.311	
Bacillus subtilis	V35-12*	*Terribacillus saccharophilus* V37.2	2.293	
Bacillus subtilis	M2-13	*Bacillus subtilis subsp. Subtilis V3.*	2.279	
Bacillus subtilis	T5-20	*Bacillus mojavensis* T5.24	2.242	
Bacillus subtilis	V44-27a	*Bacillus subtilis* V46.19*	2.402	
Bacillus subtilis	M'V37-11	*Bacillus subtilis subsp. Subtilis V3.*	1.982	

An illustration of sub-species variation is depicted in the table above. A single MSP was created and 19 Bacillus subtilis isolates (ID by gene sequence) were tested by RTC. 13 isolates matched (green) and 3 had lower scores. MALDI-TOF detects major cell proteins, which is a larger group than just the 16S portion of the ribosome.

Discussion

- Only 7% of spacecraft & cleanroom isolates match the commercial database.
- 97% self-matched to newly created MSP profiles
- 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 quickly flag similar strains previously analyzed.
- Distinguishing sub-species currently under investigation.

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IMAGE Credit : Background Photo & inset: NASA/JPL-Caltech/MSSS http://mars.nasa.gov/multimedia/images/?ImageID=7437. NASA/JPL-Caltech/MSSS. Mission public website

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