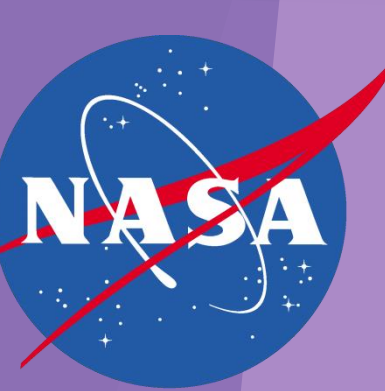


Creation of an In-House Mass Spectrometry Database using Matrix-Assisted Laser Desorption Ionization-Time-of-Flight



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Introduction

The MALDI Biotyper CA (Bruker corporation) is a fast, accurate and effective microbial identification system. The MALDI Biotyper identifies microorganisms using MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) Mass Spectrometry to measure a unique molecular fingerprint of highly abundant proteins that are found in all microorganisms. The characteristic patterns of these proteins are used to reliably and accurately identify a particular microorganism down to the species level by matching the respective pattern with a proprietary database. The Bruker MALDI Biotyper instrument and its associated database provide a fast and feasible technical path forward for the analysis of spacecraft microbial isolates. This Biotyper provides for a high-throughput and easy utilization into the current existing flow (e.g. can use the isolates that are directly growing from the NASA Standard Assay).

Rational Behind the Research

The current database is not comprehensive enough to encompass bacterial species found in the spacecraft assembly clean rooms. In absence of such database, we will be unable to get identification or get false positive identifications.

Mlx	Detected Species	Log(Score)
1	Bacillus muralis DSM 16288T DSM	1.579
1	Staphylococcus simiae DSM 17639 DSM	1.345
1	Flavobacterium flevense DSM 1076T HAM	1.313
1	Erysipelothrix rhusiopathiae EDQM Serotyp 2 FLR	1.297
1	Lactobacillus alimentarius DSM 20249T DSM	1.248
1	Staphylococcus simiae DSM 17638 DSM	1.216
1	Bacillus molaviensis DSM 9205T DSM	1.208
1	Bacillus atrophaeus DSM 675 DSM	1.202
1	Arthrobacter qandavensis DSM 15046T DSM	1.179
1	Lactobacillus gastricus DSM 16045T DSM	1.176

Figure 1. The space-craft associated microbe does not have a good log score when compared to the Bruker database.

Objectives

1. Create a Mass Spectrometry Profiles (MSP) in-house database for MALDI-TOF
2. Real Time Identification (RTC) of space-craft associated microbes using MALDI-TOF

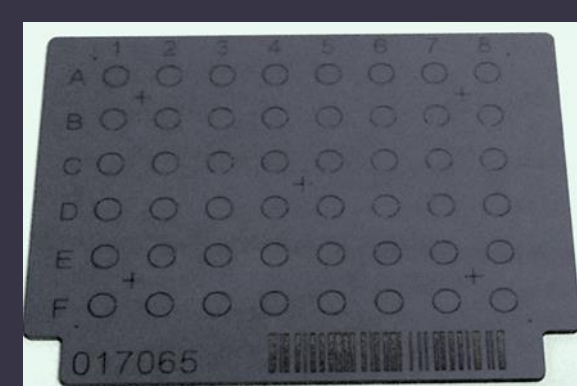


Figure 2. Example of a MALDI target



Figure 3. Bruker MALDI-TOF that was used for creation and validation of a database.

Testing Approach

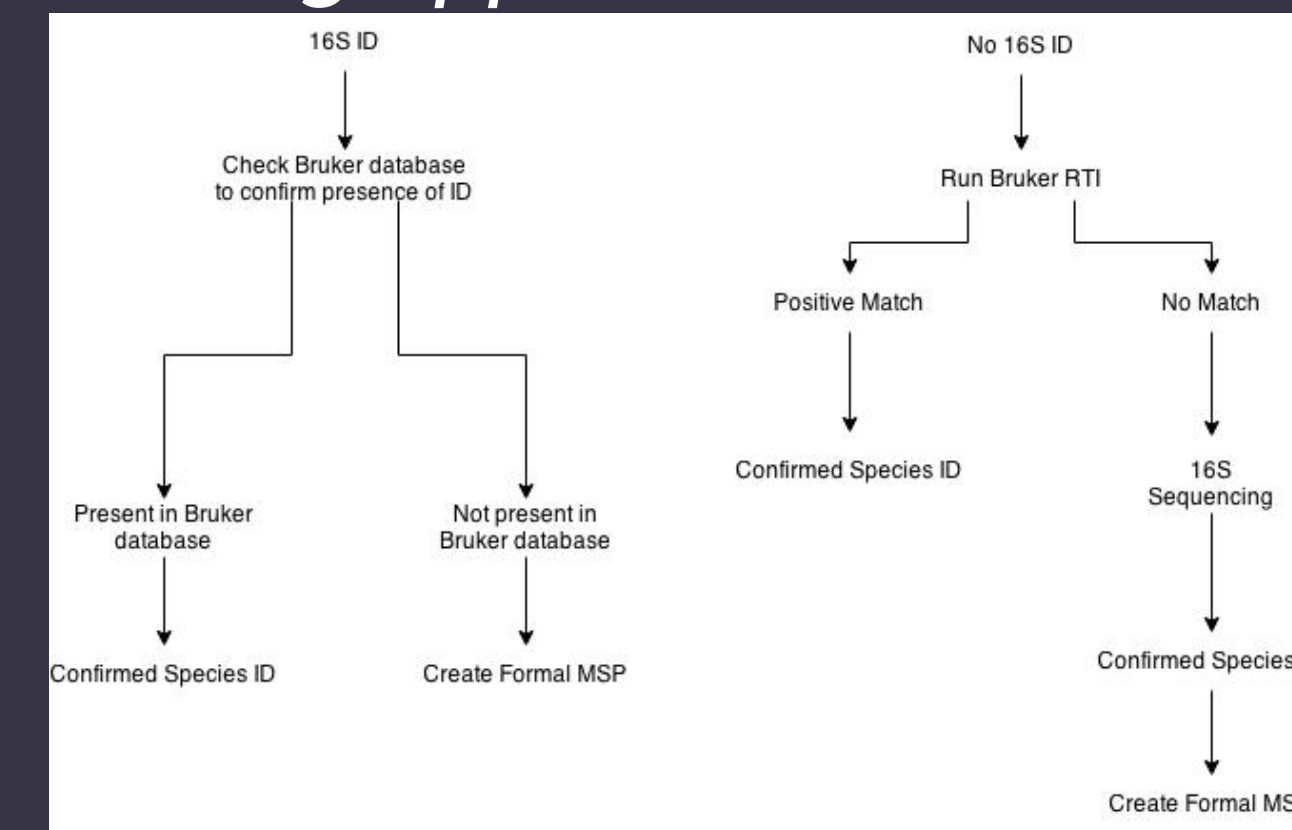


Figure 4. Flow chart for individual isolates.

Methods

When testing the identification of isolates with MALDI-TOF there are two possible methods to follow:

1. Isolates are stored in -80°C freezer
2. Isolates are revived from a glycerol or cryobead stock
3. Isolates are grown overnight in a 30°C incubator
4. Formic Acid/Ethanol Tube Extraction or Direct Transfer is performed
5. Samples prepared for MALDI-TOF target
6. Run samples using software dependent on whether it is an RTC or MSP being examined.
7. Analyze the Results

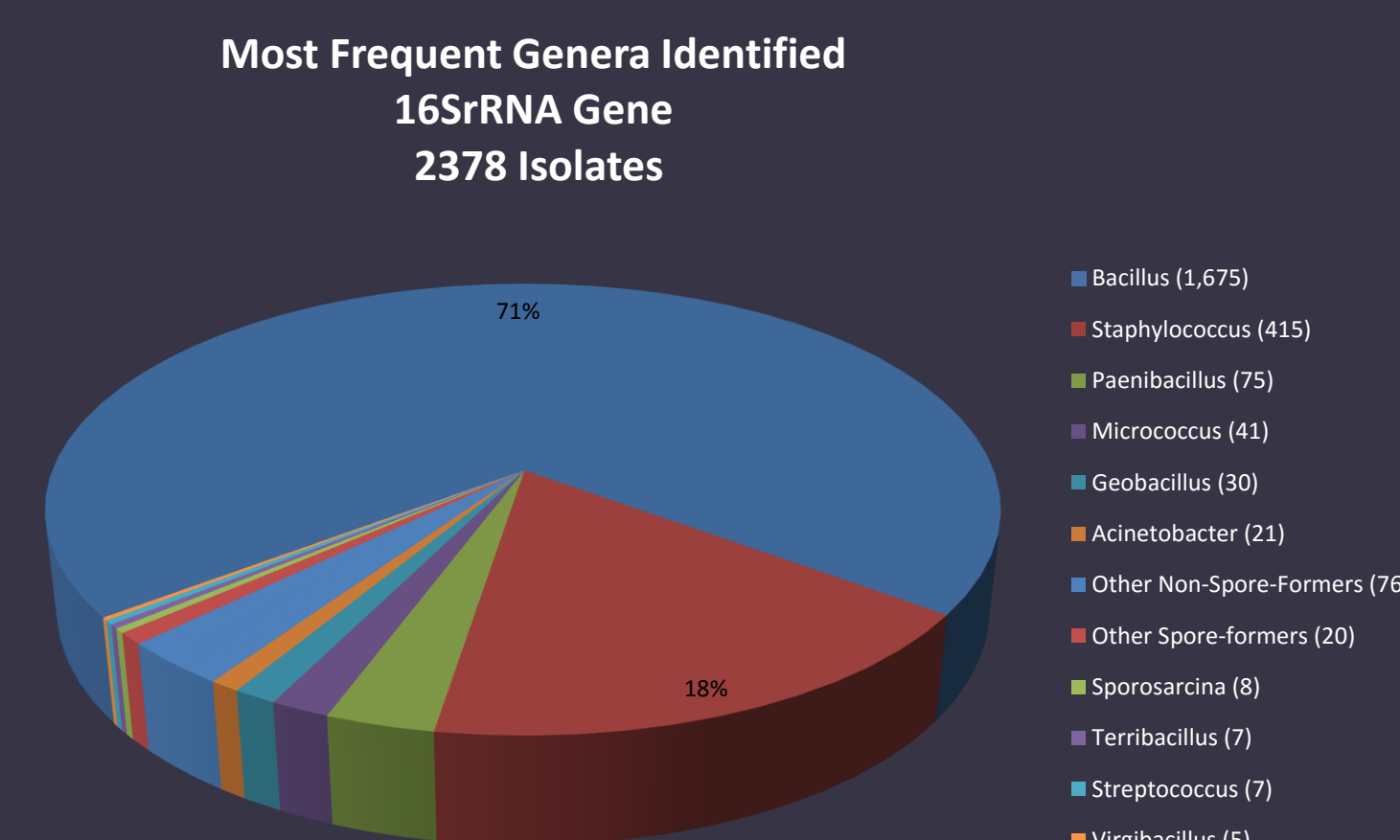


Figure 5. The Venn diagram depicts the abundance of the most dominant genera as identified by 16S rRNA gene sequencing.

- Based on 16S rRNA Sequencing information isolates were clustered into Operational Taxonomic Units (OTUs) at 99% sequence similarity.
- A representative from each OTUs was used to create MSP (figure 6).

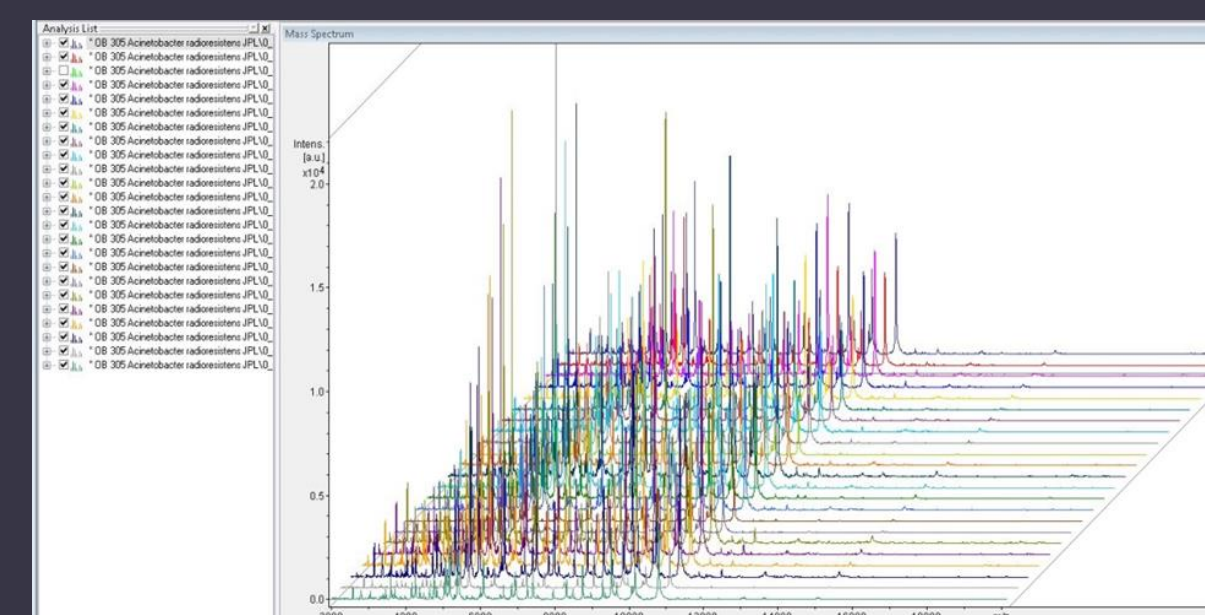


Figure 6. Multiple spectra (48) are obtained for each isolate that is then processed into a consensus spectra to form an MSP.

- Taxonomic affiliation of members of each OTUs were confirmed using RTC.
- Identification based on RTC (log score >2.0) was in agreement with 16S rRNA sequencing (figure 7)

A1	
PF3-1.5 2015-07-17T10:49:28.314 BDAL, Phoenix DT	
Matched Pattern	Score Value
Bacillus oceanisediminis PF4F_2.2	2.265
Bacillus firmus DSM 12T DSM	1.597

Figure 7. Example of an isolate matching to the MSP created along with the German and United States database. All three have log scores above 2.0 which is the minimum criteria to confirm a match.

Results

- Development, construction, and validation of an in-house MALDI biotyper profile database of spacecraft and associated microbes.
- In-house database creation efforts are in progress.
- RTC identification

Discussion

The in-house database creation will provide rapid identification of microbes that otherwise would take a couple weeks to process.

Benefits

1. Fast and reliable identification of bacterial isolates: Currently, the 16S sequence-fingerprinting takes about 2-4 days for identification, whereas a MALDI biotyper profiling can fully analyze and identify 96 samples within 1 hour.
2. Real-time application: MALDI biotyper will be used for real-time planetary protection implementation activities. Bacterial colonies produced during the NASA standard spore assay can be directly processed and identified on the very same day. Thus, we can apply MALDI biotyper profiling to discern identity of the contaminants, during flight project life cycles that are responsive to day-to-day ATLO operations.

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