National Aeronautics and Space Administration

Development of an approach for bacterial source tracking using MALDI-TOF MS

Principal Investigator: Arman Seuylemezian (353)

Program: FY21 R&TD Innovative Spontaneous Concepts

Objective: The objective of this task was to establish a JPL NASA-unique approach for performing source tracking of highly related bacterial species in assembly and flight hardware environments by comparing MALDI-TOF mass spectrometry-based techniques with the industry standard whole genome sequencing approach.

Background: Microbial source tracking is a technique by which cell lineages are linked to a particular source point in comparison to other closely related lineages with the intent to discern differences between these closely related strains to identify unique contamination sources. There are currently no approaches for performing source tracking, or for discerning differences between unique microbial strains which belong to the same species. The industry standard for performing source tracking is to use a whole genome sequencing based approach to identify SNP (single nucleotide polymorphisms) which are characteristic genomic features that can be used to identify a unique strain. This approach is however limited in that it is costly and has a long turnaround time. JPL currently utilizes MALDI-TOF MS for performing species level identification of isolates generated from planetary protection sampling efforts. Due to the high sensitivity of this instrument, we intended to evaluate its usage in performing source tracking and discerning differences amongst closely related strains of organisms which all belong to the same species. The utilization of this technique in performing microbial source tracking would allow for the traceability of new strains uncovered from planetary protection sampling events to those currently existing in the MALDI-TOF MS library.

Approach; Microbial source tracking is a technique by which cell lineages are linked to a particular source point in comparison to other closely related lineages with the intent to discern differences between these closely related strains to identify unique contamination sources. There are currently no approaches for performing source tracking, or for discerning differences between unique microbial strains which belong to the same species. The industry standard for performing source tracking is to use a whole genome sequencing based approach to identify SNP (single nucleotide polymorphisms) which are characteristic genomic features that can be used to identify a unique strain. This approach is however limited in that it is costly and has a long turnaround time. JPL currently utilizes MALDI-TOF MS for performing species level identification of isolates generated from planetary protection sampling efforts. Due to the high sensitivity of this instrument, we intended to evaluate its usage in performing source tracking and discerning differences amongst closely related strains of organisms which all belong to the same species. The utilization of this technique in performing microbial source tracking would allow for the traceability of new strains uncovered from planetary protection sampling events to those currently existing in the MALDI-TOF MS library.

Significance/Benefits to NASA: In this study, we demonstrated the application of MALDI-TOF MS in performing source tracking. In particular, data acquired using MALDI-TOF MS based approaches and those generated using the gold standard whole genome sequencing approach were compared. In total, 19 isolates were used representing 3 unique species. Clusters formed using MALDI-TOF MS data were in high concurrence with those formed using whole genome sequencing. With the additional cost, and turnaround time associated with performing whole genome sequencing, the demonstration of the high accuracy of MALDI-TOF MS facilitates the usage of this instrument in performing strain level characterization and source tracking. In addition to the cost and time advantages, MALDI-TOF MS additionally provides a capability to discern differences amongst novel species which are highly prevalent on spacecraft associated surfaces. In demonstrating the capability of MALDI-TOF MS to discern differences amongst highly related strains, this work allows for the application of this technology to future missions implementing PP. This will allow planetary protection engineers to accurately assess the relatedness of newly recovered strains to those currently existing in our MALDI-TOF MS database at the strain level and perform source tracking based on the origin of currently characterized strains.

National Aeronautics and Space Administration

Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California

www.nasa.gov

Results:

As can be seen in figure 1a, there are distinct clusters forming for the quadruplicate profiles from each isolate. In fig 1b which is based upon WGS and fig 1c built from MALDI-TOF MS profiles, there are high similarities in the overall tree topology which demonstrates the accuracy of MALDI-TOF MS in discerning strain level differences. In addition, these figures suggest that the two strains originating from the MSL mission are more closely related to one another than either are to the strain originating from the MER mission.

Spectral Number	Isolate Name
1-4	MER 505
5-8	KSC 386
9-12	MSL 145.2

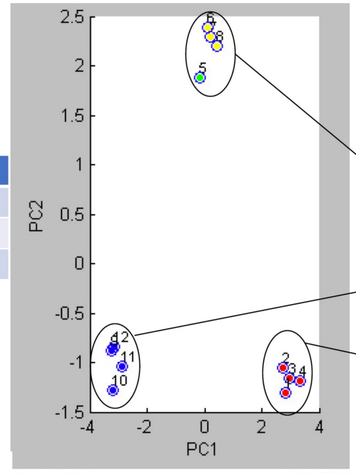

Fig 1a. A PCA (principal components analysis) plot of MALDI-TOF MS profiles of 3 unique strains (in quadruplicate).

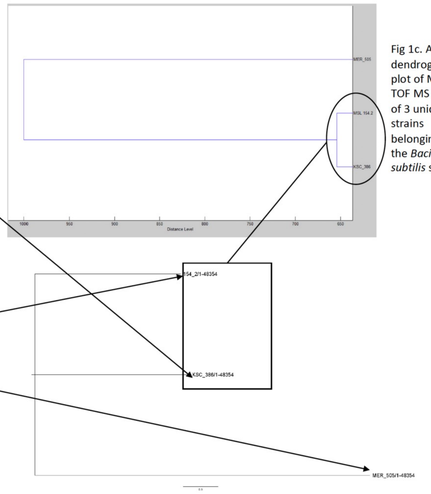

Fig 1b. A phylogeny of 3 unique strains belonging to the *Bacillus subtilis* species built from concatenated SNPs

Fig 1c. A dendrogram plot of MALDI-TOF MS profiles of 3 unique strains belonging to the *Bacillus subtilis* species

In fig 2a we see a close clustering between the replicate profiles of each unique strain. In addition, there is a direct correlation between the clusters observed in the PCA plot and those seen in the SNP based phylogenetic tree. Additionally, these clusters also suggest a closer relationship between strains originating from each unique spacecraft mission. However, interestingly one strain originating from Kennedy Space Center (a geographically distinct location) shares more similarity with the two strains originating from the MER mission than both strains KSC 645 and KSC 351.

Spectral Number	Isolate Name
1-4	KSC 283
5-8	KSC 351
9-11	KSC 645
12-15	Odyssey P107
16-19	P 121
20-23	MER 110
24-27	MER 128

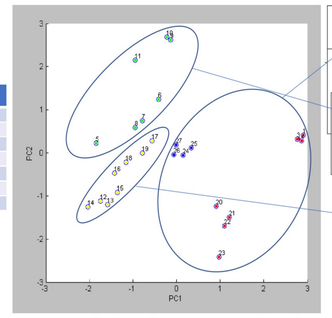

Fig 2a. A PCA (principal components analysis) plot of MALDI-TOF MS profiles of 7 unique strains (in quadruplicate).

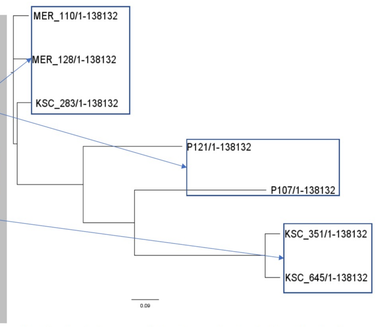

Fig 2b. A phylogeny of 7 unique strains belonging to the *Bacillus megaterium* species built from concatenated SNPs

In fig 3a we again see very close clustering of the quadruplicate profiles between the MALDI-TOF MS profiles. In addition, there is close concurrence with the overall tree topology observed in fig 3b. Similarly to figures 1 and 2 there is close relatedness amongst strains isolated from each unique spacecraft mission, however there is also close clustering between isolates originating from vastly different geographical distances such as those isolated at KSC.

Spectral Number	Isolate Name
1-4	Fairing 10M-2.2
5-7	Fairing 12A-4
8-11	TA 76
12-15	TA 172
16-19	MSL 128.1.2
20-23	KSC 640
24-27	KSC 657
28-31	TA 170
32-35	PF 24B.2

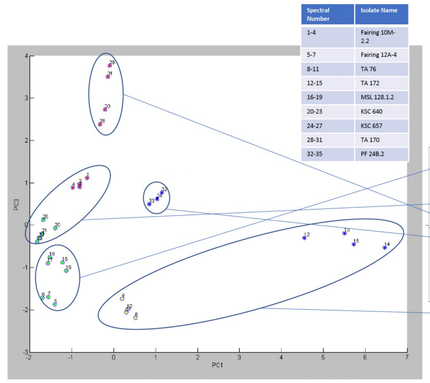

Fig 3a. A PCA (principal components analysis) plot of MALDI-TOF MS profiles of 9 unique strains (in quadruplicate).

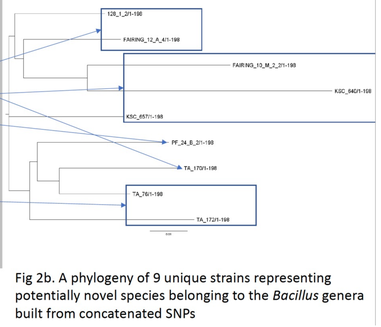

Fig 2b. A phylogeny of 9 unique strains representing potentially novel species belonging to the *Bacillus* genera built from concatenated SNPs

PI/Task Mgr Contact
Email: Arman.Seuylemezian@jpl.nasa.gov

Clearance Number:
RPC/JPL Task Number: R21245