Comparative microbial diversity analysis between the NASA Standard Assay and molecular approaches for the Mars Exploration and Mars Science Laboratory Rovers

James Benardini[1*], Myron La Duc[1], Stephanie Smith[2], Wayne Schubert[1], Susan Childers[3], and Andrej Paszczsynski[2]

[1]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA [2]University of Idaho, Moscow, ID [3]Colby College Waterville, ME

*CORRESPONDING Author: J.Benardini@jpl.nasa.gov

Abstract

The NASA Standard Assay (NSA) is a procedure that is utilized to verify outbound spacecraft cleanliness during missions that address the International Policy requirements of biological contamination. During the assembly, testing and launch operations for a mission planetary protection efforts are comprised of collecting samples from spacecraft surfaces and processing the sampling matrices via sonication, heat shock (80C, 15 min), and aerobic growth (32C, 72h). The resulting colonies are then enumerated and bioburden densities calculated for spacecraft surfaces. In parallel, research endeavors were conducted to collect and analyze a broader breadth of microbes using 16S rRNA gene marker analyses from the spacecraft and associated cleanroom surfaces using clone libraries, phylochip, and 454 FLX tag-pyrosequencing. During the implementation campaigns for the Mars Exploration Rover (MER) and Mars Science Laboratory (MSL) isolates resulting from the NSA were preserved and archived for further study. Recently, these isolates have been revived from the freezer, further characterized using 16S rRNA gene sequencing. Upon sequencing, a direct comparison of these isolates to the non-culture based libraries were annotated and displayed using an interactive Phylogenetic tree (iTOL) with multi-value bar chart for comparison for both the MER and MSL missions. In general, both missions observed <10% of the isolates that were not spore formers. Molecular techniques exhibited much greater microbial diversity compared to NSA approach. Only 1 to 10% of the sequences obtained from molecular techniques shared phylogenetic relatedness with NSA based isolates. This core microbial population belongs to spore forming microorganisms such as *Bacillus and Paenibacillus*. The iTOL tree allows us to visually represent and quantifiably understand the diverse microbial population with varying abundance observed using molecular and NSA approaches. In conclusion, results of this study may aid NASA in understanding the strengths and weakness of the NSA as well as provide direct feedback on the types of organisms that are archived and technical approach on future missions.

RESULTS

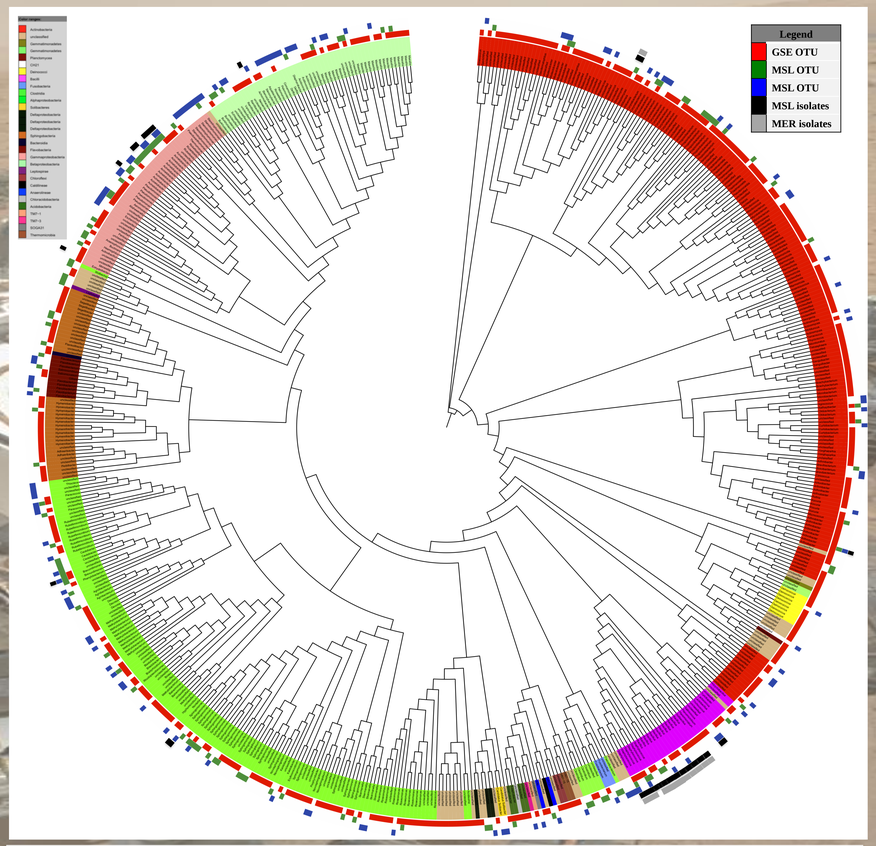

Fig 1: **Phylogenetic correlation between NASA standard assay isolates and 454 tag-encoded pyrosequencing operational taxonomic units (OTU)**. iTOL tree modified from La Duc et. al. (2012) AEM 78(16):5912-22 by manually adding MSL and MER isolates to the original tree.

Fig 2: **MER rover during assembly & test operations.**

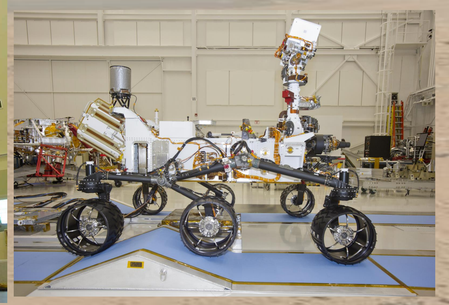

Fig 3: **MSL rover during assembly & test operations.**

Table 1: **MER and MSL spacecraft isolates not represented on the iTOL.**

Genus	Species	Spacecraft
Brevibacillus	brevis	MER
Carnobacterium		MER
Cohnella	phaseoli	MER
Gracilibacillus	dipsosauri	MSL
Hydrogenophaga		MER
Leclercia	adecarboxylata	MSL
Leclercia	sp.	MSL
Lysinibacillus	massiliensis	MER
Lysinibacillus	sphaericus	MER
Micrococcus	luteus	MER
Oceanobacillus	iheyensis	MER
Paenibacillus	abekawaensis	MER
Paenibacillus	alvei	MER
Paenibacillus	amylolyticus	MER
Paenibacillus	anaericanus	MER
Paenibacillus	barcinonensis	MER / MSL
Paenibacillus	barengoltzii	MER
Paenibacillus	camelliae	MER
Paenibacillus	campinasensis	MER
Paenibacillus	castaneae	MER
Paenibacillus	chondroitinus	MER
Paenibacillus	favisporus	MSL
Paenibacillus	glycanilyticus	MER

Genus	Species	Spacecraft
Paenibacillus	humicus	MER
Paenibacillus	lactis	MSL
Paenibacillus	lautus	MSL
Paenibacillus	macerans	MER
Paenibacillus	motobuensis	MER
Paenibacillus	mucilaginosus	MSL
Paenibacillus	mucilaginosus	MSL
Paenibacillus	polymyxa	MSL
Paenibacillus	provencensis	MER
Paenibacillus	sabinae	MER
Paenibacillus	sp.	MSL
Paenibacillus	timonensis	MER
Paenibacillus	urinalis	MER
Paenibacillus	xylanilyticus	MER
Sphingopyxis	alaskensis	MSL
Sporosarcina	aquimarina	MSL
Sporosarcina	luteola	MER
Sporosarcina	sp.	MSL
Streptococcus	mitis	MSL
Streptococcus	sanguinis	MSL
Terribacillus	saccharophilus	MER
Thermoactinomyces	sanguinis	MER
Xanthomonadaceae	bacterium	MSL

METHODS

Sample Collection: Samples were taken from the MSL and MER flight system hardware surfaces during the lengthy assembly process. Microbes from the swabs and wipes were extracted into buffer and 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 further archived. The remaining extraction buffer was pooled and for total DNA extraction for 454 tag-encoded pyrosequencing analysis. Similarly, macrofoam sponge sampling devices collected ground support equipment, extracted into PBS buffer and pooled for total DNA extraction

DNA Extraction, PCR & Sequencing: Nucleic acids were extracted from 1 mL of broth using Tissue LEV Total RNA Purification Kit cartridge for the Maxwell 16 MDx system (Promega). For the isolates, 8F and 1512R primers were used with GoTaq (Promega) and MoBio reagents in PCR amplification of the 16S rDNA gene. PCR products were confirmed electrophoretically in 1% agarose gel with SYBR Green (Life Technologies) and purified for sequencing using the QIAquick PCR Purification Kit (Qiagen). Sanger sequencing was performed by Macrogen Inc. (Rockville, MD) using 27F, 512F, and 1492R primers and for phylogenetic analysis, 16S rRNA gene sequences were analyzed using the rRNA analysis pipeline (www.ibest.uidaho.edu/tools). For the 454 tag-encoded pyrosequencing analysis, total DNA extracted from the NASA standard assay extraction fluids and ground support equipment was amplified and processed as per La Duc et.al. (2012) AEM 78(16):5912-22.

DISCUSSION

Microbial populations from NASA cleanroom facilities and spacecraft associated ground support equipment (GSE) have been extensively characterized. These populations have been identified through traditional culturing, clone libraries, phylochip analysis, and most recently 454 tag-encoded pyrosequencing. During the assembly, testing, and launch operations of MSL, the excess NASA standard assay extraction fluid was analyzed for phylochip and 454 tag-encoded pyrosequencing. Since launch, data has been generated to identify and further archive the isolates collected from the spacecraft surface samples. Therefore, the existing data can start to be compared to the data currently being generate from the isolates originating from the spacecraft microbial archive.

Preliminary results indicate that ~8-10% of the sequences obtained from molecular techniques shared phylogenetic relatedness with NSA based isolates. Notably, there were 48 OTUs from the NSA isolates that were not represented in the research pyrosequening analysis. The core microbial populations belongs to spore forming microorganisms such as *Bacillus and Paenibacillus*, as would be predicted due to heat-shock processing. The iTOL tree allows us to visually represent and quantifiably understand the diverse microbial population with varying abundance observed using molecular and NSA approaches. Future analysis are planned to directly compare, in greater depth, the entire facility and modern downstream analyses based OTUs with that of the final archive isolate data samples. In conclusion, results of this study may aid NASA in A) understanding the strengths and weakness of the NSA as well as provide direct feedback on the types of organisms that are archived and technical approach on future missions B) be helpful in risk assessments and C) understanding the entire microbial population for a genetic based spacecraft inventory.

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