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, great attention is paid to the cleanliness of 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 future 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

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 generated from the isolates originating from the spacecraft microbial archive.

Preliminary results indicate that ~80% 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 pyrosequencing analysis. The core microbial populations belong to spore forming microorganisms such as Bacillus and Paenibacillus, as would be expected 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 analyses are planned to directly compare, in greater depth, the entire facility and modern downstream processing and analysis of these isolates. Notably, there were 48 OTUs from the NSA isolates that were not represented in the research pyrosequencing analysis. The core microbial populations belong to spore forming microorganisms such as Bacillus and Paenibacillus, as would be expected 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 analyses are planned to directly compare, in greater depth, the entire facility and modern downstream processing and analysis of these isolates.

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