Phylogenetic and Phenotypic Diversity of Microbial Isolates from the Mars Exploration Rover

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ABSTRACT

Planetary protection is an international policy, which may require microbial reduction procedures and biological burden reports of space exploration missions launched, including NASA missions to Mars. The NASA Standard Assay verifies bioburden requirements by quantifying any aerobic, heterotrophic, heat-shock resistant isolates. Isolates taken from assayed spacecraft were then further streaked for purity and stored in glycerol stocks. During this study, over 350 isolates from the Mars Exploration Rovers (MER) implementation campaign were revived from glycerol stocks and analyzed via 16S rDNA sequence to further understand the diversity of the isolates. Upon identification, an Omnilog biochemical ID was employed as a rapid, first order screening method to understand the biochemical characteristics of the isolates. Concurrently, the isolates were preserved in both working stocks in a cryobead format and long-term frozen glycerol storage to allow for ease and further collaboration with the broad scientific community.

Many species surviving spacecraft microbial reduction belong to spore forming genera such as *Bacillus, Paenibacillus, Terribacillus, and Brevibacillus*. Isolate identification results show that 70% of the 318 organisms identified are related to members of the *Bacillus* genus and 13.8% belong to the *Staphylococcus* genus. The remaining 16.2% of the isolates were related to members of other genera such as *Oceanobacillus*, *Micrococcus*, *Streptomyces*, *Hydrogenophaga*, *Thermoactinomyces*, and *Carnobacterium.* The 16S analysis identified 31 novel species candidates, with less than 98.5% homology to the 16S sequence of their closest relatives, respectively. Preliminary results from the Omnilog plates for example, revealed that a majority (>80%) of the isolates could grow in 8% NaCl. Understanding the diversity of organisms isolated from spacecraft surfaces will enhance the cleaning and microbial reduction techniques and would provide a false-positive life detection library for use in a potential future Mars sample return campaign.

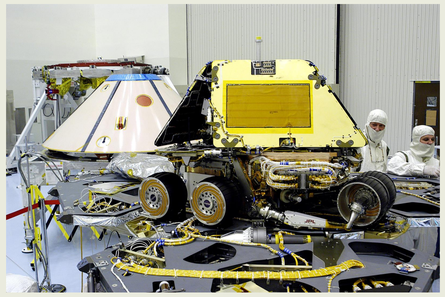

Figure 1: **Mars Exploration Rover during during assembly & test operations left panel), encapsulated in the aeroshell ready for launch operations (middle panel), and the Delta II launch of the rover (right panel). Photo Credits : NASA**

METHODS

Sample Collection: Samples were taken from the 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.

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 (or 1525R) 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, 518F and 1492R primers and for phylogenetic analysis, 16S rRNA gene sequences were analyzed using the rRNA analysis pipeline (www.ibest.uidaho.edu/tools).

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RESULTS

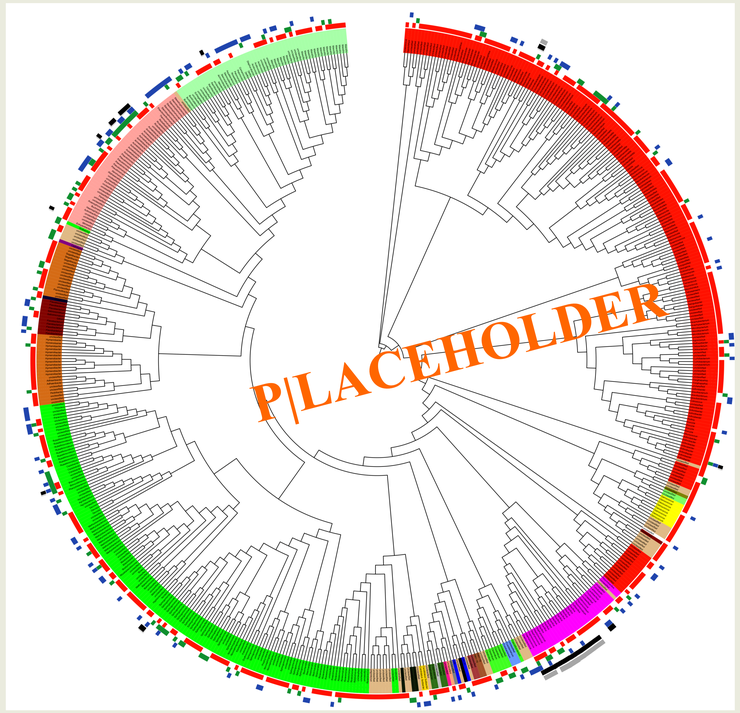

Fig 1: Phylogenetic correlation between Mars Exploration Rover NASA standard assay isolates.

DISCUSSION / CONCLUSIONS

NASA has sent numerous spacecraft to explore Mars in order to observe many properties of the planet, including its potential to sustain life either past or present. Planetary protection policy protects other planets and our own from both forward and backward contamination – the transfer of terrestrial life to another body (forward), and the transfer of extraterrestrial life to Earth should a sample be returned in the future (backward) – protecting the science of the exploration, and the natural integrity of other planets and moons. Mars Exploration Rover (MER) Spirit and Opportunity are Class IVa missions as landers not carrying instruments to investigate life on Mars thus were subjected to microbial reduction modalities including alcohol cleaning and dry heat microbial reduction. Despite the procedures enacted by NASA for maintaining cleanliness and reducing the microbial bioburden on the spacecraft and assembly and testing equipment, a wealth of diverse bacteria are still present on the spacecraft and within the associated assembly rooms[1]. The bacteria present on spacecraft and found in Jet Propulsion Laboratory cleanrooms typically contain a high incidence of bacteria within the Firmicutes phyla, such as *Bacillus* and *Paenibacillus* genera[2]. These genera are ubiquitous organisms that are known for their ability to survive extreme heat conditions due to their ability to form stress-resistant spores. The extreme heat and cleanliness measures also selects for the most stress resistant bacteria[3], and therefore may be highly tolerant to other stresses that damage DNA by using conserved repair mechanism pathways[4, 5]. Additionally, these spores can withstand further space environment stresses including full exposure to open space[6], thus demonstrating a potential to survive transit and conditions on life-supporting foreign bodies such as Mars[7].

In this study, a total of 353 isolates were revived from frozen stocks generated during the initial sampling of the spacecraft, and then refrozen to update the archive. DNA from all the isolates were extracted, amplified, and purified, then sequencing was attempted. Of these 165 revived isolates, 326 of them were successfully sequenced and identified via sequence identity against the National Center for Biotechnology (NCBI) Basic Local Alignment Search Tool (BLAST) database. A total of TBD isolates were identified as *Bacillus* species, TBD were identified as *Staphylococcus* species, and the other isolates are from a variety of different species including TBD *Paenibacillus*. Additionally, 7 putative novel species were identified based on the closest sequence match (<97.5%). This diversity distribution directly represents the organisms isolated from the NASA standard assay as collected from spacecraft surfaces during the planetary protection NASA requirements verification process. Thus, a false-positive life detection libraray from the MER contribution of isolates has been nearly completed should NASA initiates a Mars Sample Return campaign. Additionally, these isolates have been further preserved for future study for interested collaborators in working CryoBank beads and in glycerol stocks. Further understanding the tolerances and metabolic capabilities will enhance the cleaning and microbial reduction techniques conducted on spacecraft as well as assess the potential for these organisms to tolerate spaceflight and the martian environment.

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