Phylogenetic Diversity of Microbial Isolates Associated with the Phoenix Mission

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Abstract

Planetary protection is concerned with preventing the transfer of biological material between planets. Flight projects are allocated limits on total bioburden and bioburden density. Compliance is currently determined by a culture-based method that selects for heat-tolerant, heterotrophic aerobes. Microbial contaminants of the Phoenix spacecraft and associated hardware were identified to species level based on 16S rDNA similarity. Most (73%) of the 211 identified isolates were spore-formers, belonging to the genera *Bacillus* (67%) or *Paenibacillus* (6%). Non-spore-formers were predominately *Staphylococcus* (20%). Several extreme-associated species were identified, including stratospheric *Bacillus aryabhatti* and halophilic facultative anaerobe, *Sporosarcina aquimarina*. Five potentially novel *Bacillus* species were identified.

Methods

Sample preparation

The spacecraft components were regularly sampled with swabs and wipes during assembly, test and launch operations. Cultivation followed the NASA Standard Assay method. Microbes were sonicated off of the swab into DI-water, heat-shocked at 80^{o} for 15 minutes, and incubated in Tryptic Soy agar at 32^{p} for 3 days. Isolates were subcultured and stocks frozen in glycerol at -80^{o} C. Frozen isolates were cultured onto Tryptic Soy Agar plates and incubated at 32^{o} C for at least 48 hours.

DNA processing and sequencing

DNA was extracted using the Maxwell 16 MDx system (Promega) with the Tissue LEV Total RNA Purification Kit. A portion of the 16S ribosomal RNA gene sequence (primers: 8F/1525R) was amplified by polymerase chain reaction (PCR) and sequenced.

Data analysis

To determine the species of a microbial isolate, its 16S rDNA sequence was compared, using BLAST, against homologous sequences from known species found in the NCBI GenBank and GreenGenes databases. Quality sequences were aligned and their phylogenetic relationships determined using MEGA 6.0, according to the neighbor-joining method with the Kimura 2-parameter substitution model.

Discussion

Mars exploration has revealed potential habitable environments, while research into life in extreme environments on Earth has expanded our concept of habitability. The next generation of missions may carry life detection instruments, and have targets with the potential to support life, such as Europa or the subsurface of Mars. But the scientific integrity of any life detection depends on our ability to avoid – or at least recognize – false positives that may result from terrestrial organisms carried by the spacecraft. Because of the necessary compromise between spacecraft material integrity and bioburden reduction methods, current technology does not allow for a mission to be completely sterile at launch. Characterizing spacecraft-associated microorganisms is of interest to the field of planetary protection for three reasons: 1) Information on their resilience to sterilization methods, as well as their potential to survive and reproduce under Mars or other planetary conditions, may guide technology development and mission planning. 2) New technologies are being developed to supersede the NASA Standard Assay, but must first be validated against the legacy method. An understanding of the diversity captured by the Standard Assay is therefore desirable for comparison. 3) The archive can serve as a false-positives library in case of candidate life detection, especially for a potential Mars Sample Return.

The isolates described here nearly complete the study of archived isolates from the Phoenix mission. In total, good-quality 16S rDNA sequences were obtained from 211 isolates. Based on BLAST identification, most of these were spore-forming species, such as *Bacillus* (67%) or *Paenibacillus* (6%). *Staphylococcus* was common, at 20%. Several extreme-associated species were identified. These included *Bacillus aryabhatti*, originally described from high-altitude aerial samples, and *Sporosarcina aquimarina*, a halophilic facultative anaerobe. Five potentially novel *Bacillus* species were identified on the basis of <97% sequence identity to their closest match, with two being highly divergent (93% identity). Future work will characterize the phenotypes of the potentially novel species identified here.

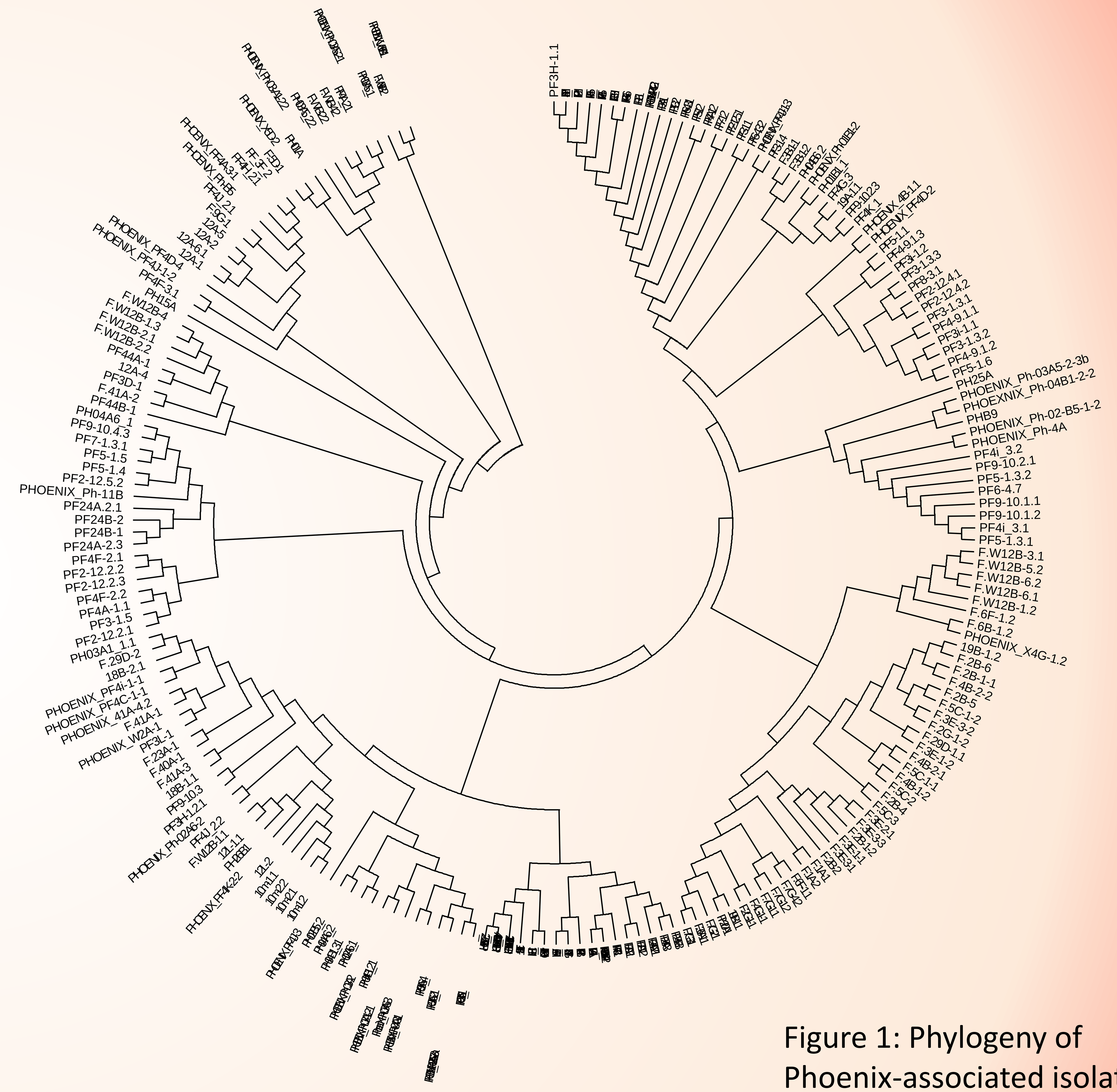

Figure 1: Phylogeny of Phoenix-associated isolates.

Acknowledgements

Funding for this work was provided by NASA through the JPL/Caltech Mars Program Engineering Office, and the California Institute of Technology Summer Undergraduate Research Fellowship (SURF). We would like to thank Aleksandra Checinska for advice and lab support. The authors would also like to acknowledge 2014 summer interns Ryan Hillary, Ryan Hendrickson, Erik McFarland and Kyla Bradylong for mutual support and discussion. The authors wish to acknowledge Garrett Smith, Nicholas Sanchez, Neil Schwalb, Farah Zerehi and Anabel Carigo for their laboratory and DNA sequence work for Phoenix microbial isolates.

The research described here was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.