National Aeronautics and Space Administration

Phylogenetic Diversity of Microbial Isolates from the Mars Pathfinder

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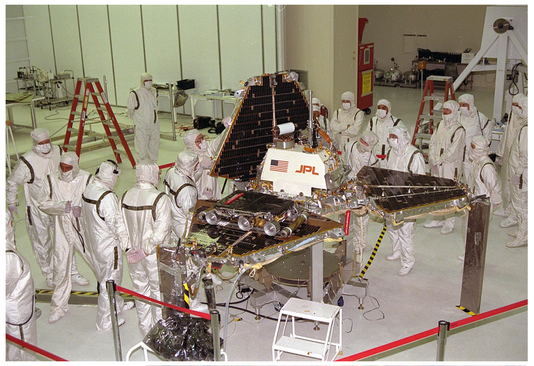
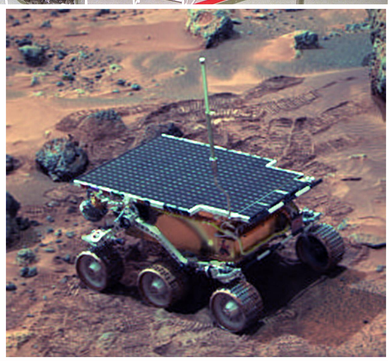
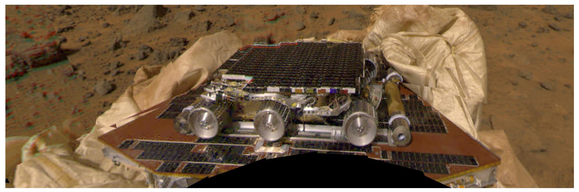
Introduction & Objectives

As we explore the universe and move beyond our terrestrial home, we are challenged with not contaminating the planets and other solar system bodies we explore. The Committee on Space Research (COSPAR) has outlined an international standard for cleanliness needed for space missions (COSPAR 2011). The National Aeronautics and Space Administration (NASA) has committed to these standards not only for compliance but also to ensure the integrity of our missions and future discoveries.

Cleanliness practices have been used in previous and current missions, specifically the Mars Science Laboratory (MLS) for both the landing craft and the Atlas V payload completed by the Biotechnology and Planetary Protection Group (Benardini et al., 2014). There is a need to create and maintain an archive of all possible microbes sent to space in order to be held accountable for any contamination and also to test and maintain the cleanliness protocols. Knowing what microbes were potentially sent will allow us to determine if microbes possibly found on other bodies are native or invasive. To maintain, and potentially exceed, the current standard for cleanliness it is necessary to inventory, catalog, and add to the database the samples collected from all missions, specifically the Mars Pathfinder (MPF).

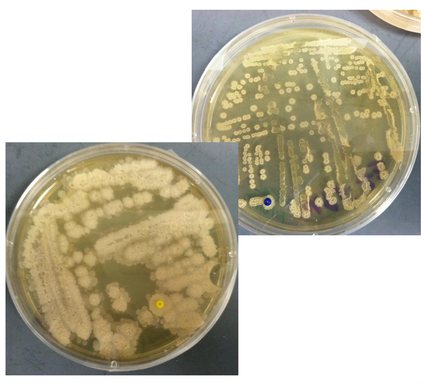






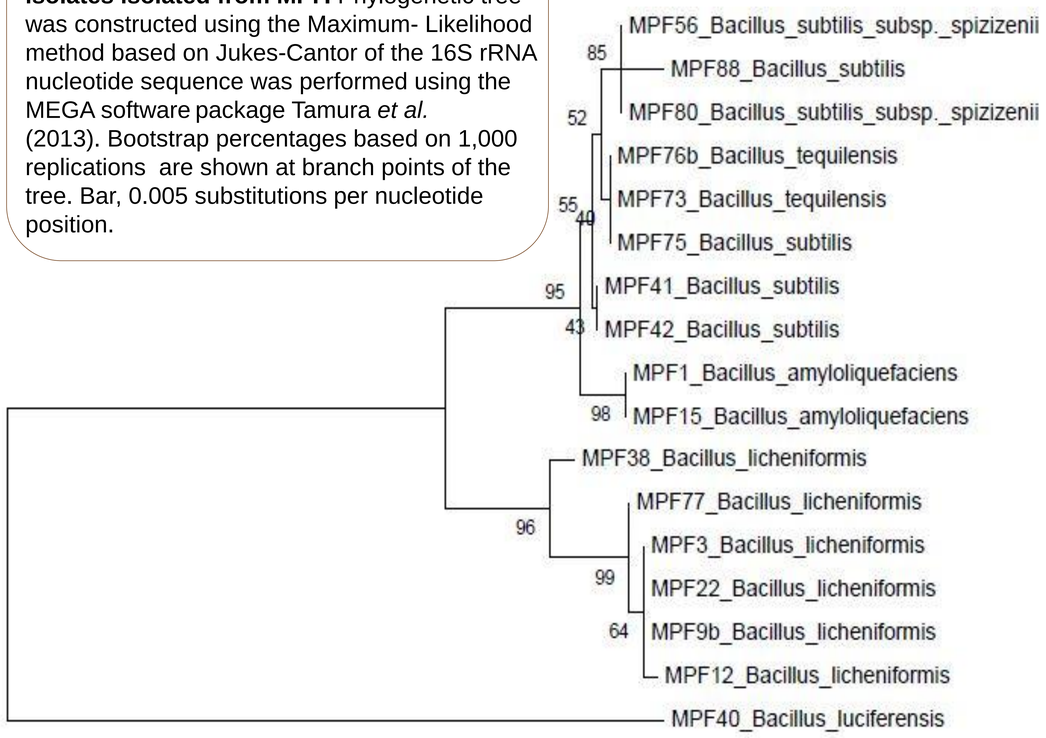
Methods

1. Ensure that the harvested samples are pure (a single microbe type).
2. Streak samples on a Tryptic Soy Agar so that individual colonies can be isolated. Individual colonies transferred to a second plate and grown again.
3. Colonies grown overnight to increase the biomass for DNA extraction.
4. The DNA from the cells in these colonies extracted using the Maxwell 16 MDx instrument (Promega Coporation, Madison, WI).
5. The 16S rRNA gene sequence amplified out of the genomic DNA. (Primers have been designed to capture the 16S gene that is ~1500 bp in length.) This region in particular was amplified using PCR. After PCR, the sample was run on an agarose gel (via gel electrophoresis) to verify that PCR successfully amplified the region.
6. The samples were then cleaned using the QIAquick PCR cleanup kit (Qiagen) and prepped for sequencing.
7. Sequencing performed by a commercial laboratory.
8. The assembly and analysis of the sequencing data completed using the DNASTAR Lasergene 11 software package (DNASTAR Inc, Madison, WI).



Results

Fig 1: Phylogenetic relationships between isolates isolated from MPF. Phylogenetic tree was constructed using the Maximum- Likelihood method based on Jukes-Cantor of the 16S rRNA nucleotide sequence was performed using the MEGA software package Tamura *et al.* (2013). Bootstrap percentages based on 1,000 replications are shown at branch points of the tree. Bar, 0.005 substitutions per nucleotide position.



- 17 high-quality 16S rRNA sequences were obtained
- Clusters indicate closely related or possibly the same organism
- All isolates are believed to be spore formers
- 1 Bacillus luciferensis showed noticeable differences in sequence from all other isolates

- *Isolates numbered by extraction date and location.*
- *Potential species names attached when a greater than 98% match was found*

Conclusions & Discussion

- Comparison to the Kennedy Space Center for isolate overlap with other mission sets is needed to determine a possible pattern in organisms over time
- No bacterial isolates exhibits less than 95% sequence similarity with any validly described bacterial type strain representing a potential novel genus/ species
- All of the bacterial strains from this study are members of the genus Bacillus
- MPF 40 showed noticeable sequence differences and should be re-tested to confirm results
- Given Bacillus' association with soil these results may indicate further need for cleanliness protocol

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References:

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