National Aeronautics and Space Administration

Collecting Diverse Microorganisms from Rover Spacecraft

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Introduction

The idea and issue of planetary protection was first raised in the late 1950s and officially was taken up by the Committee on Space Research (COSPAR) in the early 60s. Planetary protection is, according to the NASA office, is the "practice of protecting solar system bodies (*i.e.*, planets, moons, comets, and asteroids) from contamination by Earth life, and protecting Earth from possible life forms that may be returned from other solar system bodies." Their mission is to promote such practices in order to keep to their objectives of "preserving [their] ability to study other worlds as they exist in their natural states", to avoid "the biological contamination of explored environments that may obscure [their] ability to find life elsewhere – if it exists; and to ensure that [they] take prudent precautions to protect Earth's biosphere in case life does exist elsewhere."

For JPL, and the office of Planetary Protection, the avoidance of forward contamination is paramount, keeping the bioburden levels and contamination of spacecraft at a minimum. In order to find out how much and what type of organisms there are on the assembled spacecraft, even while they are assembled in a cleanroom environment, samples of the surface area of both the spacecraft themselves as well as the room itself are taken and placed under extreme treatment for sections at a time. The surviving microbes are of intense interest as they have the largest potential to be able to survive space conditions. The surviving isolates are cultured and archived to later be revived for further future study. An electronic database is also being used to organize the archived and preserved specimens, which can be of great use to future investigations. The microbe samples are identified using two primary methods in 16S rRNA gene sequencing and MALDI-TOF mass spectroscopy.

Results

There are several diverse genera that have been isolated from rover spacecraft, most of them are spore-forming. The isolates are then

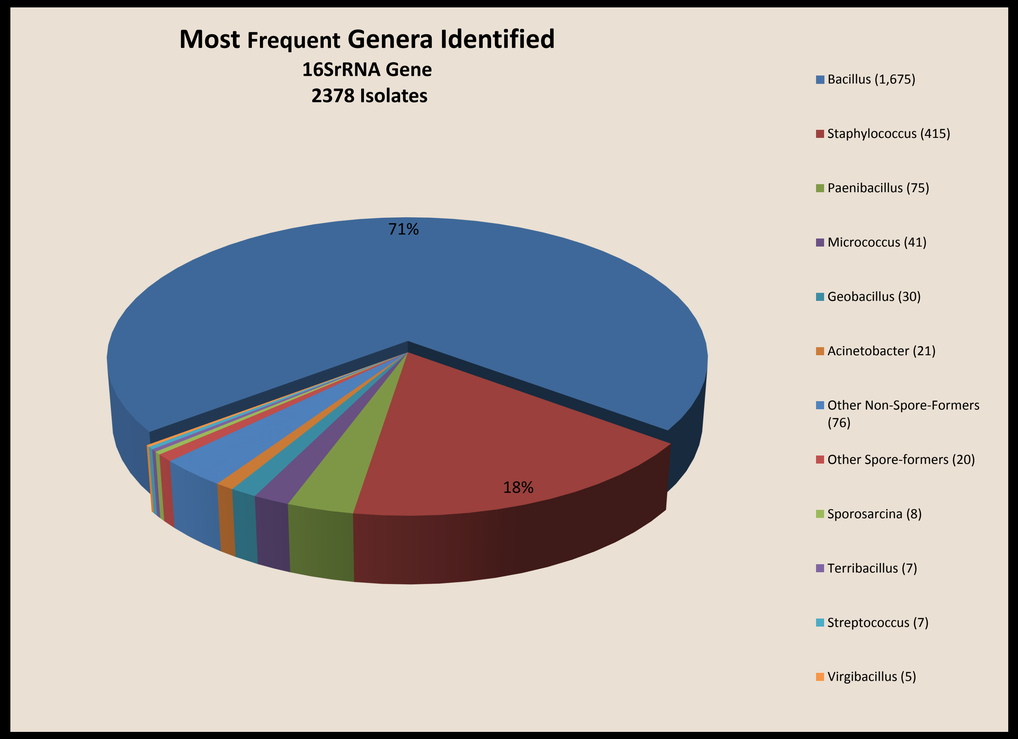

Figure 4. The majority of the genera that are forming the isolate collection is overwhelmingly seen as being in the genus *Bacillus*, making up more than seventy percent of the isolates identified.

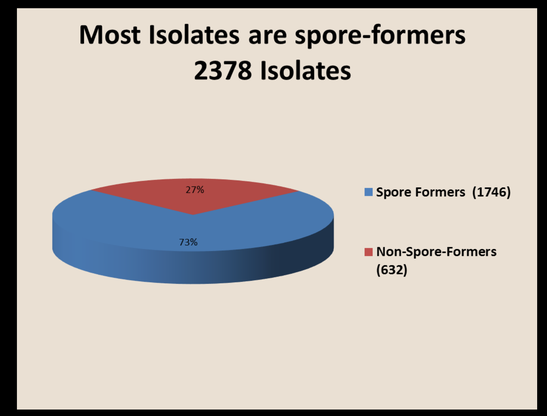

Figure 5. More than 70% of the isolates found from examining rover spacecraft, are spore-forming bacterial species, with those of the non-spore variety being mostly in the genus *Staphylococcus*.

Summary

The work on this project is still ongoing, as we look at several different isolate collections from different missions , the process is time consuming, with incidents of trial and error, in order to ultimately create a procedure that works.

Applications

A custom library would be able to flag organisms that have been isolated on more than one occasion. So as we see more and more similar isolates

As future missions come up, such as Mars 2020 and Insight, the need for this project of sequencing, identifying, and archiving microbial samples becomes even more important.

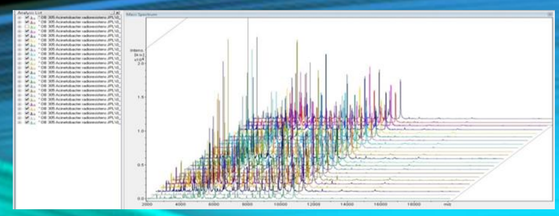

Figure 3. Multiple spectra are created from the MALDI-TOF Mass Spectral analysis of the organisms.

Methods

Figure 1. The Matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) Mass Spectrometer

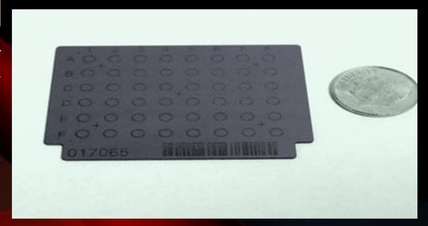

Figure 2. Isolate biomass is directly transferred onto target plates for MALDI-TOF Mass Spec analysis.

Procedure

1. Representative and non-representative isolates of particular 16SrRNA gene sequence OTUs (Operational Taxonomy Units) are revived and subcultured on Tryptic Soy Agar in order to provide healthy active cell biomass for the direct transfer procedure.
2. Directly transfer isolate biomass onto target plates and then treated with formic acid and then overlade with matrix.
3. A MALDI-TOF Mass Spectrometer is used as a rapid identification system for each microbial isolate on the target plate.
4. Multiple spectra are created for each target organism and are assembled into a single consensus spectrum creating an MSP (Mass Spectral Profile).
5. This spectrum is compared to the entire commercial database library and the JPL in-house database library for identification.
6. Direct transfer is also done to create RTC (Real Time Classification) analyses to compare to the created MSP.
7. If there is a correlation between the RTC and the MSP of all the representatives of an OTU, than that MSP is added to the planetary protection custom database library.

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