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ABSTRACT

Bacillus and Staphylococcus were the two most prominent genera identified by 16S rRNA gene sequencing. Data from several sub-collections were compiled. The collection is dominated by the genus Bacillus and other spore-forming genera. Most genera are known to have members that are desiccation tolerant or form spores. Microbes collected from robotic spacecraft and cleanroom assembly areas are archived at JPL’s microbial culture collection. The microbial isolates that have been identified by 16S rRNA gene sequence (18%) most frequently belong to the following major genera: Bacillus (21%), Staphylococcus (7%), and Paenibacillus (56%). A single MSP was created and 19 Bacillus subtilis isolates (ID by gene sequence) were tested by RTC. 13 isolates matched (green) and 3 had lower scores. MALDI-TOF detects sub-species variations that were not possible by 16S rRNA sequencing. Creation of a more comprehensive database is required in order to correctly identify the cleanroom microbial isolates.

RESULTS

1. The workflow was streamlined by the following bioinformatics approach:
   a. Update SampleWare curated database (EzTaxon) and create MSP.
   b. Update database (MLST) for all numbers.
   c. Multiple spectra are obtained for each microbe, that are processed into a consensus spectrum.
   d. Align sequences using multiple sequence alignment (Clustal Omega)
   e. Identify isolates using MALDI-TOF Mass Spectrometer

2. Align sequences using multiple sequence alignment (Clustal Omega)
   a. Create MALDI-TOF MSP after 16SrRNA
   b. Make 16SrRNA gene sequence for 100 numbers.

Bioinformatics Workflow

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   b. Make 16SrRNA gene sequence for 100 numbers.

3. MALDI-TOF analysis of new isolates using an in-house user database, can quickly flag similar strains to commercial databases.

4. The overall approach for the spacecraft microbial archive. The identification by MALDI-TOF is illustrated in the orange and yellow pathways. This is the processing flow for new individual isolates added to the collection.

5. This diagram depicts the abundance of the most prominent genera as identified by 16S rRNA gene sequencing. Data from several sub-collections were compiled. The collection is dominated by the genus Bacillus and other spore-forming genera. Most genera are known to have members that are desiccation tolerant or form spores.

Identification by MALDI-TOF Mass Spec

A MALDI-TOF Mass Spectrometer is used as a rapid identification system for microbial isolate.

Bacterial colonies are directly applied to a target plate and overlaid with a matrix compound.

Multiple spectra are obtained for each microbe, that are processed into a consensus spectrum.

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