

Genetic and Metabolic Profiles of Microbial Isolates from the Mars Exploration Rovers

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ABSTRACT

To minimize the risk of contaminating celestial bodies and to adhere to international law, strict planetary protection measures are implemented during spacecraft assembly, testing, and pre-flight launch operations. For Mars-based landers and rovers, all associated material such as components, surfaces, tools, paint, lubricant, etc. are sampled regularly with the NASA Standard Assay. Any spores (i.e. heat-shock resistant isolates) recovered are quantified, isolated, and stored. The work presented here is a study of the microorganisms isolated during the Mars Exploration Rover mission in 2003.

Of the 350 samples collected, 171 samples were reconstituted for characterization via 16S rRNA fingerprinting and 99 of them underwent phenotype characterization employing multiple carbon sources and ion concentrations in an automated microarray format using the Omnilog system.

The preliminary evaluation of the Omnilog rapid biochemical ID method demonstrated that 38% of isolates monitored by the Omnilog required repeated cultures and 34% were significantly different from the growth profiles in the Omnilog Database. It was also notable that 5.3% of samples demonstrated some degree of antibiotic resistance. The 16S analysis identified 5 novel species candidates and 18 novel subspecies candidates, with less than 97% or 98.5% homology to the 16S sequence of their closest relatives, respectively.

By screening the metabolic capacities and evolutionary distances of microbes isolated from clean-room materials, we have been able to identify novel isolates and estimate the extreme tolerances they may be capable of withstanding. Deeper investigation into desiccation resistance, Martian electron acceptor usage, and UV tolerance will be focused on the subset of novel or resistant isolates. Based on our experience culturing MER isolates, developing a custom environmental database of growth profiles of spacecraft-associated microbes in user-defined media components would make more efficient use of the Omnilog system for planetary protection purposes. The overall outcome of this study could benefit emerging cleaning and sterilization technologies for preventing forward contamination that could potentially impact life detection or sample return missions.

METHODS

Generation of Working and Stock Cultures

Of approximately 350 isolates collected from the MER spacecraft archive, 188 microorganisms were thawed in ice, vortexed, & streaked on a Tryptic Soy Agar plates incubated at 33°C for 24 hrs. Isolates were streaked out multiple times to ensure purity by visual inspection. Isolated colonies were then harvested for A) stock cultures (5 cryovials replicates in 1 mL of 30% glycerol) and B) working stock (Cryobeads [bioMérieux sa]).

DNA Extraction, PCR & Sequencing:

Pure isolates were inoculated into 15 mL of Tryptic Soy Broth and incubated overnight at 32°C. One mL of overnight broth was then used for DNA extraction using Tissue LEV Total RNA Purification Kit cartridge for the Maxwell 16 MDx system (Promega). 8F and 1512R primers were used with GoTaq (Promega) and MolBio 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, 512F, and 1492R primers and contigs were assembled using Vector NTI (Life Technologies) and aligned using a batch BLAST tool.¹

Phylogenetic Analysis:

Annotated sequences were selected based on sequence identity from the NCBI database and aligned with sample sequences using Clustal Omega.² No chimera sequences were detected by the DECIPHER tool.³ Phylogenetic relationships calculated using MEGA 5 software and annotated using iTOL (itol.embl.de).

Biochemical Characterization:

The Omnilog system (Biolog, Hayward, CA) was used to generate kinetic metabolic profiles for each sample. Each profile consists of the amount of metabolic activity over time, which is assayed by colorimetric dye, and measured by the pixel value of images taken at 15 min. intervals. Metabolic activity for each plate was normalized to a negative control well by the software, and compared to a reference database of >2650 species.

RESULTS

- 171 high-quality, chimera-free 16S sequences were obtained
 - 166 were positively matched to a species (> 97% identity)
 - 5 are novel species candidates (<97%). 18 isolates shared between 98.5% and 97% identity to published sequences and are considered novel subsp. candidates.
 - MER-111 and MER-91 are being further characterized as a novel *Paenibacillus* sp.
- The Omnilog system was tested on 91 organisms
 - 35% of samples classified by the system as either Low Similarity or No Match to members of the database.
 - 67% 16S fingerprints and Omnilog IDs matched at the level of genera, and 44% matched at the level of species. (Fig 2)

Fig 2: Phylogenetic relationships between type strains and isolates: The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. The bootstrap consensus tree inferred from 500 replicates. All positions containing gaps and missing data were removed. Genera are grouped by color. Type strains are labeled radially with Gen Bank accession no. Black dots indicate that genera was confirmed by Omnilog. Empty dots indicate no match or mismatch.⁴

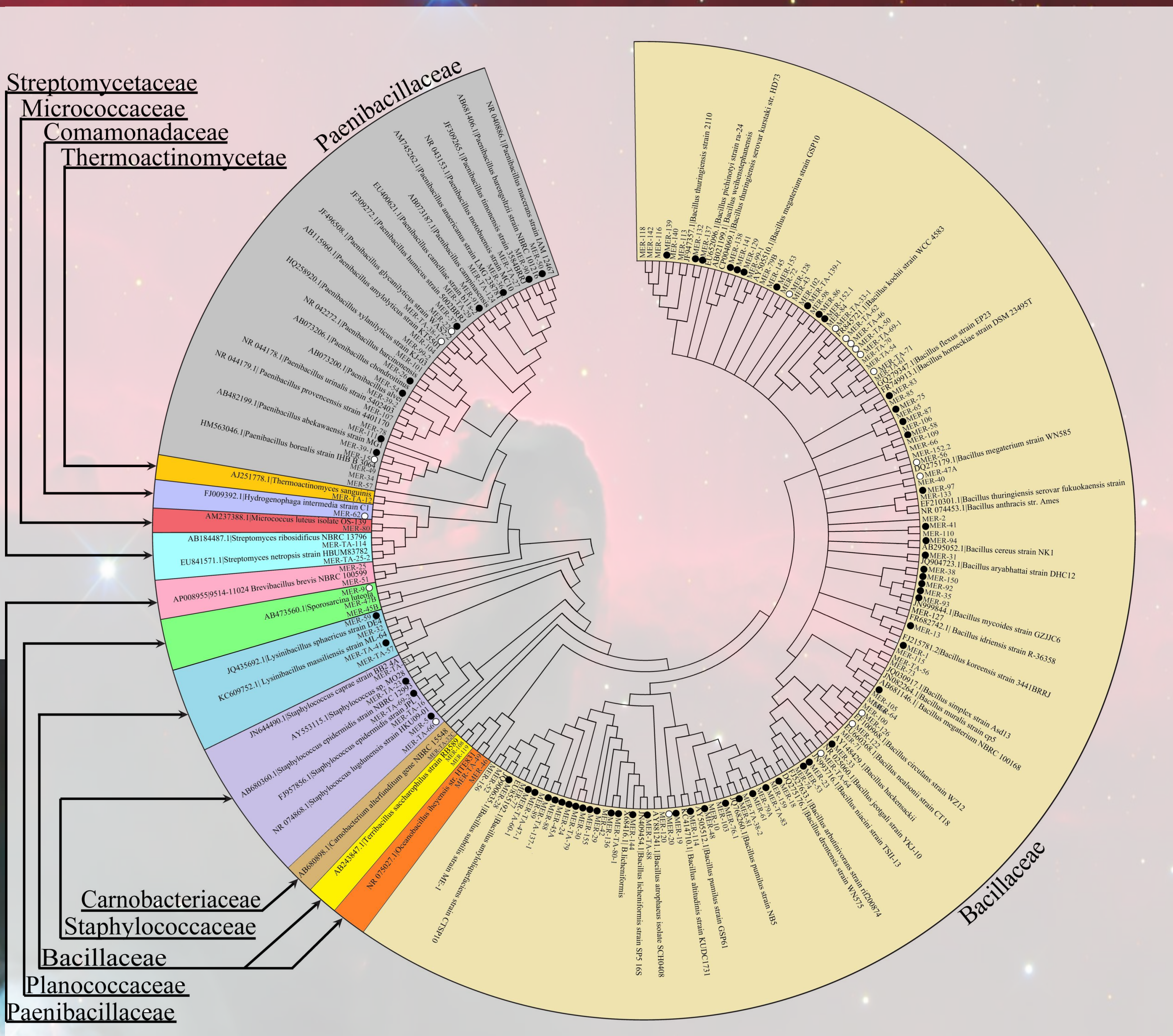
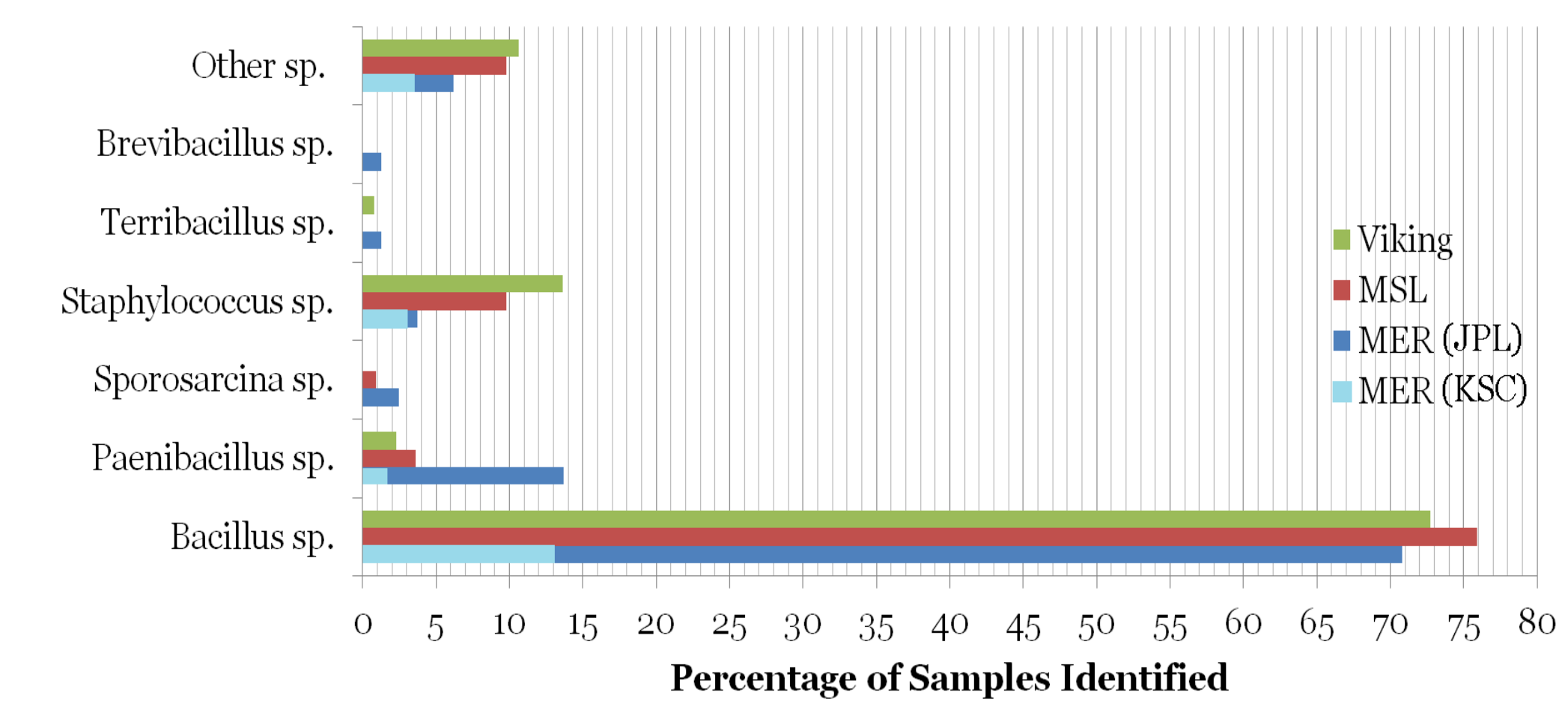


Fig 3: Selected Kinetic Metabolic Profiles – The selected kinetic metabolic profiles show a description, reagent, and the percentage of organisms (n=46) able to metabolize in the presence of the reagent, indicated by a colorimetric dye, relative to the control well for 12 of the 96 profiles examined.

Gram Positive Antibiotic	Vancomycin	0%
	1% NaCl	63.2%
	5% NaCl	94.7%
Salinity Tolerance Assays	8% NaCl	86.8%
	1% Sodium Lactate	97.4%
Facultative Anaerobic Metabolite	Fusidic Acid	0%
	Lincomycin	0%
	Rifamycin SV or Minocycline	5.3%
Reactive Oxygen Species Production	Tetrazolium Blue	55.3%
	Potassium Tellurite	100%
	Sodium Butyrate	100%

Fig. 1: Preliminary bioburden genera between missions and locations: The graph is a comparison of isolates from the MSL, Viking and MER missions showing the percentage of samples distributed among the most common genera identified. It also shows the distribution between MER collection sites at Kennedy Space Center and the Jet Propulsion Laboratory



DISCUSSION

The microbial diversity identified by this work is consistent with comparable, culture-dependent methods used on MSL samples, apart from slight differences in Staphylococci & Paenibacilli quantities (Fig 1).

Within MER isolates, Bacilli and Paenibacilli isolates were evenly distributed between collection sites, while Planococcaceae were restricted to JPL and Streptomycetaceae to KSC. Staphylococcaceae were found 17x more frequently at KSC. Some of these differences can be attributed to variations in humidity and climate between locations.

While it is worthwhile to note that culture-independent cloning-based identification work carried out on MSL isolates failed to recover members of Thermoactinomycetes, Micrococcaceae, and Streptomycetaceae, they successfully identified samples from 5x the number of different bacterial families. Coupled with culture-independent, DNA microarray methods, diversity from a minimum of 33.5x more bacterial families, including members of the three families listed above, were recovered.⁵

Further development of a kinetic metabolic based profiling system as a rapid ID tool would benefit from assembling a Spacecraft-associated microbial database of custom metabolic profiles from aggregate data. In addition, modifying the group of colorimetric reagents used to generate profile would better adapt a primarily clinical system for use with environmental samples.

Of the environmental isolates positively identified, preliminary results of the isolates tested within this study suggest that microbial species could be identified within ~8-10 hours from spacecraft surfaces. Notably, if this system was optimized and a custom database generated this would provide a rapid identification that could be used in real-time (same shift) to provide feedback for spacecraft contamination events during assembly, testing and launch operations.

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