

Unveiling Hidden Extremophiles: Metagenomic Insights from NASA Mission Critical Cleanroom Environments

Kasthuri Venkateswaran
NASA Jet Propulsion Laboratory, Pasadena, CA, USA

Metagenomic sequencing in NASA mission critical cleanroom environments encountered challenges in detecting extremophiles due to their resilience and low abundance (<0.1% of total microbial burden). Stringent decontamination measures and nutrient scarcity resulted in less metabolically active extremophiles, leading to underrepresentation in shotgun metagenome sequencing data. The robust nature of extremophiles, particularly spores, protected their DNA, complicating extraction and potentially compromising genetic material quality. Additionally, their genetic material was often overshadowed by more abundant organisms, complicating detection.

Metagenomic sequencing's reliance on comprehensive reference databases for species identification posed a limitation; if certain extremophiles' genetic sequences were not cataloged, they remained undetected, underscoring the need for continuous database updates with newly sequenced extremophiles. Despite these challenges, the detection and study of extremophiles in cleanrooms were crucial in medical, pharmaceutical, and aeronautical industries, where contamination by even low-abundance microorganisms could have serious implications. To improve detection, targeted enrichment techniques and advanced DNA extraction methods were employed, along with the expansion of reference databases with new extremophile sequences.

Complementing metagenomic data, the study of cultivable microorganisms provided a direct assessment of living bacteria that could be grown in laboratory settings, aiding in disinfection protocol testing and practical applications such as resistance testing and genetic studies. Over six months (n=98 samples), a comprehensive microbiological investigation at the Spacecraft Assembly Facility (SAF) aimed to sequence the genomes of 182 strains, uncovering 25 strains representing 14 novel species. This included extensive examination of their physiological properties and in-depth phylogenomic analysis. Metagenomic signatures from the cleanroom environment (n=263 samples) were also investigated to determine the prevalence, incidence, and persistence of these novel species during the Mars 2020 mission assembly. Additionally, genomic functions of these novel bacteria were probed, focusing on unique enzymes and secondary metabolites that provided insights into their adaptive mechanisms in nutrient-scarce conditions.

This study enriched understanding of microbial resilience and adaptability, with significant implications for planetary protection and astrobiology. Improvements in DNA extraction protocols, increased sequencing depth, and minimized amplification biases were essential for comprehensive microbial community characterization, particularly in environments where rare microbes played significant ecological roles.