Genomic Characterization of Novel Extremophiles Associated with the Mars 2020 Mission Assembly Environments

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A comprehensive microbiological investigation was conducted to measure both cultivable and shotgun metagenomics-based microbial diversity analysis in NASA cleanrooms where Mars 2020 mission components were assembled. Genomes of 182 extremophilic strains were sequenced identifying 25 novel strains representing 14 previously unknown bacterial species. These strains underwent detailed physiological and phylogenomic analyses to uncover their taxonomic and phylogenetic affiliations. Additionally, metagenomic reads generated from this cleanroom in 2016 were analyzed to understand the abundance, prevalence, and persistence of these novel species.

Functional profiling revealed an increased number of genes for carbohydrate and amino acid transport and metabolism, along with genes for defense mechanisms. Antimicrobial resistance profiling predicted resistance against various drug classes, notably glycopeptide and phosphonic acid antibiotics, through mechanisms such as antibiotic target alteration and efflux. Analysis of biosynthetic gene clusters identified include ε-Poly-L-lysine from *Cellulomonas* species, which serves as an antimicrobial and biodegradable homopolymer. It also produces desferrioxamine B for iron uptake, crucial for medical and bioremediation

applications. Ectoine, produced by spore-forming *Bacillus* species, protects against osmotic stress using the ectABC gene cluster. Petrobactin, a siderophore from *Brevibacillus jpeli*, aids iron acquisition under iron-limited conditions through the asb gene cluster. Schizokinen, produced by *Bacillus pasadenensis*, facilitates iron uptake via the schiz gene cluster. Ulbactins F and G from Bacillus also aid iron acquisition. Tyrocidine, a cyclic decapeptide antibiotic from Bacillus, disrupts bacterial membranes. Bacilysin, another antibiotic from *B. pasadenensis*, inhibits cell wall biosynthesis. Carotenoid biosynthetic gene clusters in *Aeromicrobium jpeli* produce pigments with antioxidant properties, offering biotechnological applications in UV protection and health supplements. Paeninodin, a cyclic lipopeptide with potent antimicrobial properties, is found in *Ferdinandcohnia jepli*.

The genomic insights of these extremophiles significantly impact future spacecraft cleaning and decontamination strategies, reducing microbial burden. The findings also enhance practices and regulations in industries like pharmaceuticals and healthcare.