

Molecular Microbial Analyses of the Mars Exploration Rovers Assembly Facility

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During space exploration, the control of terrestrial microbes associated with robotic space vehicles intended to land on extraterrestrial solar system bodies is necessary to prevent forward contamination and maintain scientific integrity during the search for life. Microorganisms associated with the spacecraft assembly environment can be a source of contamination for the spacecraft. In this study, we have monitored the microbial burden of air samples of the Mars Exploration Rovers' assembly facility at the Kennedy Space Center utilizing complementary diagnostic tools. To estimate the microbial burden and identify potential contaminants in the assembly facility, several microbiological techniques were used including culturing, cloning and sequencing of 16S rRNA genes, DNA microarray analysis, and ATP assays to assess viable microorganisms. Culturing severely underestimated types and amounts of contamination since many of the microbes implicated by molecular analyses were not cultivable. In addition to the cultivation of *Agrobacterium*, *Burkholderia* and *Bacillus* species, the cloning approach retrieved 16S rDNA sequences of oligotrophs, symbionts, and γ -proteobacteria members. DNA microarray analysis based on rational probe design and dissociation curves complemented existing molecular techniques and produced a highly parallel, high-resolution analysis of contaminating microbial populations. For instance, strong hybridization signals to probes targeting the *Bacillus* species indicated that members of this species were present in the assembly area samples; however, differences in dissociation curves between perfect-match and air sample sequences showed that these samples harbored nucleotide polymorphisms. Vegetative cells of several isolates were resistant when subjected to treatments of UVC (254 nm) and vapor H₂O₂ (4 mg/L). This study further validates the significance of non-cultivable microbes in association with spacecraft assembly facilities, as our analyses have identified several non-cultivable microbes likely to contaminate the surfaces of spacecraft hardware.