Assessing Earth Based Contamination with Various Biomarkers in the Search for Extraterrestrial Life.

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The search for evidence of life on other planets, either by *in situ* exploration or sample return missions, will likely involve ultra-sensitive detection of biomarkers. Forward contamination of samples with cells or biomarkers from Earth would seriously compromise interpretation of results. Planetary protection technologists are constrained to utilizing existing quality control techniques to validate cleanliness of spacecraft involved in sample returned missions unlike life detection technologists who have several years to develop such technologies before extraterrestrial samples are returned and distributed. Accurately assessing the microbial burden associated with spacecraft surfaces involves the recovery of cells and/or molecules such as nucleic acids, proteins, lipids and carbohydrates. Real time microbial detection methods based on enzymatic activity (for general cleanliness), molecular presence (dipicolonic acid for spores), and the presence of specific genes (ribosomal RNA, Splyase and DPA synthase) are under evaluation to speed up the process of assembling immaculately clean spacecraft.

Characterizing the microbial communities on spacecraft and assembly facility surfaces is crucial in monitoring the cleanliness of these environments. Both conventional and molecular techniques were used to assess the microbial diversity and abundance associated with these environments. Culture-based techniques found species of *Bacillus* dominant, while direct DNA isolation, cloning and 16S rDNA sequencing analysis revealed the presence of many Gram-positive and Gram-negative microorganisms that could be both cultivable and non-cultivable. Several novel bacterial species were isolated, one of which, *Bacillus nealsonii* sp. nov., exhibited resistance to desiccation, H_2O_2, UV, and γ-radiation conditions while in spore-form. Various international space agencies have been actively researching the use of H_2O_2 vapor as a chemical sterilant. While assessing the cleanliness of several different spacecraft assembly facility surface locations, H_2O_2 resistant strains were repeatedly isolated. These *B. pumilus* strains were found in unclassified (entrance floors, ante-room, and air-lock) and classified (class 100K) (floors, cabinet tops, and air) areas.

Many isolates cultivated from both the Mars Odyssey Orbiter and its assembly and encapsulation facility were resistant to the conditions mentioned above. Populations, as inferred from clone libraries, were fairly consistent between the spacecraft and assembly facility surface samples. Surprisingly, a Gram-negative isolate *Acinetobacter radioresistens,* isolated directly from the spacecraft, was found to survive H_2O_2 and an appreciable dose of γ-radiation (>0.5 Mrad). The findings of this study improve our current understanding of the microbial community structure, diversity, and survival capabilities of microbes physically associated with spacecraft, hopefully leading to the development of suitable cleaning and sterilization technologies and effective construction of clean instruments for use in *in situ* experiments and sample return missions.