Developing Planetary Protection Technology: Recurrence of Hydrogen Peroxide Resistant Microbes from Spacecraft Assembly Facilities.

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Hydrogen peroxide vapor is currently the sterilant-of-choice for flight hardware because it is a low-heat sterilization process suitable for use with various spacecraft components. Hydrogen peroxide is a strong oxidizing agent that produces hydroxyl free radicals (·OH) which attack essential cell components, including lipids, proteins, and DNA. Higher concentrations of hydrogen peroxide (10 to 30%) and longer contact times are required for sporicidal activity. The sporicidal activity is significantly increased when hydrogen peroxide is in the vapor phase. Planetary protection research efforts at the Jet Propulsion Laboratory (JPL) are focused on developing cleaning and sterilization technologies for spacecraft preparation prior to launch. An understanding of the microbial diversity of spacecraft assembly areas, and any extreme characteristics these microbes might possess, is necessary to develop these validation technologies.

Previous studies have shown that some heat-tolerant *Bacillus* species isolated from the JPL spacecraft assembly facility (SAF) are resistant to recommended hydrogen peroxide vapor sterilization exposures. Air samples (particles trapped on witness plates) and surface samples (25 cm^2) from floors and cabinets, collected at 3 different periods, were studied for their microbial diversity. A *Bacillus* species, which was related to a hydrogen peroxide resistant strain, was repeatedly isolated from various locations in the JPL-SAF. This species was found in both unclassified (entrance floors, ante-room, and air-lock) and classified (class 100K) (floors, cabinet tops and air) areas. The phylogenetic affiliation of these strains was carried out using biochemical tests and 16S rDNA sequencing. The 16S rDNA analysis showed >99% sequence similarity to *Bacillus pumilus*. In order to understand the epidemiology of these strains, a more highly evolved gene (topoisomerase II β–subunit, *gyrB*) was also sequenced. Among 4 clades, one cluster, comprised of 3 strains isolated from the air-lock area, tightly aligned with the *B. pumilus* ATCC 7061 type strain (97%). The *gyrB* sequence similarity of this clade was only 91% with the 3 other clades. The genetic relatedness of these strains, as per pulse field gel electrophoresis patterns, will be presented.

The vegetative cells and spores of 11 isolates were tested for their hydrogen peroxide resistance. Cells and spores were separately treated with 5% liquid hydrogen peroxide. After 60 minutes of exposure, the samples were diluted in tryptic soy broth and incubated at 32°C. Vegetative cells of one of the isolates, FO-036b, were the only cells to survive the exposure to hydrogen peroxide. In contrast, spores of several of the isolates survived exposure to hydrogen peroxide. Spores of these isolates do not appear to have any obvious morphological changes. We are in the process of analyzing these hydrogen peroxide resistant spores and comparing them to spores of microbes that are not hydrogen peroxide resistant. The impact and implications of the identification and recurrence of these hydrogen peroxide microbes, and their spores, will be discussed.