A Proteomics Approach to Analyze Hydrogen Peroxide Resistant Strains Isolated from a Spacecraft Assembly Facility

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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.

An understanding of the microbial diversity of spacecraft assembly areas, and any extreme characteristics these microbes might possess, is necessary to develop technologies for the validation of spacecraft cleaning and sterilization. Previous studies have shown that a hydrogen peroxide resistant *Bacillus* species (FO-036b), which was related to *Bacillus pumilus*, 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. Spores of this strain exhibit resistance to liquid hydrogen peroxide (5%) and to vapor hydrogen peroxide (16 mg/L). In contrast, spores of the *B. pumilus* Type strain, ATCC 7061, are not resistant to liquid or vapor hydrogen peroxide.

Spores proteins were isolated from FO-036b, *B. pumilus* ATCC 7061 (Type strain) and *B. subtilis* 168 using urea, DTT, SDS, etc. The isolated proteins were subjected to two-dimensional (2-D) gel electrophoresis and analyzed. We have identified several unique proteins of FO-036b that might be responsible for the H_2O_2 resistance observed in this species. We are in the process of sequencing these unique proteins and comparing them to proteins from *Bacillus* species that are not hydrogen peroxide resistant. The sequences and potential functions of these proteins will be presented. The impact and implications of the identification of these spore proteins in a hydrogen peroxide resistant *Bacillus pumilus* species, FO-036b, will be discussed.