Characterization of Hydrogen Peroxide Resistant Microbes Isolated from Spacecraft Assembly Facility[†].

K. Venkateswaran, S. Chung, C. Echeverria, C. Basic, and R. Koukol.
Planetary Protection Technologies, Jet Propulsion Laboratory, Pasadena, CA.

In ongoing investigations to map and archive the microbial footprints on various spacecraft components, we have examined the microbial populations of the Jet Propulsion Laboratory, Spacecraft Assembly Facility (JPL-SAF). The dimension of the JPL-SAF is 80' wide, 120' long, and 44' 4" high. Relative humidity was controlled at 40 ± 5% with a cap at 45% and the average temperature was maintained at 20 ± 5ºC. Personnel entry into this facility was minimal and carefully controlled by enforcement of a series of rigorous procedures. Because of controlled air circulation, desiccation, moderately high temperature, and low-nutrient conditions, the atmosphere of Spacecraft Assembly Facility (SAF) should be considered as an extreme environment where microbes might find difficult to thrive. Witness plates made up of spacecraft materials some painted with spacecraft qualified paints, were exposed for ~7 to 9 months and examined for the total cultivable aerobic heterotrophs, and heat-tolerant (80ºC for 15-min.) spore-formers. Many bacterial strains isolated from the JPL-SAF exhibited intense growth at 60°C (44%), and 10% NaCl (50%). This substantiated the fact the JPL-SAF is an extreme environment. Majority of these thermotolerant and halotolerant isolates were identified as *Bacillus* species. Based on the morphology, and physiology, 28 isolates were chosen for further study. Sequence analysis of nearly complete sequences of 16S ribosomal RNA revealed that these isolates formed seven clades: *Bacillus licheniformis, B. pumilus, B. cereus, B. circulans, Staphylococcus capitis, Planococcus citreus* and *Micrococcus lylae.* Among these 28 strains, 8 strains exhibited resistance to various doses (1 to 4 cycles) of hydrogen peroxide treatments (~6.0 mg H_2O_2 vapor/L per cycle). These strains were identified as *B. licheniformis* (4 strains), *B. pumilus* (3 strains) and *S. capitis* (1 strain). However, 3 strains each of *B. licheniformis* and *B. pumilus* showed resistance to 4 cycles of H_2O_2 treatment. *B. stearothermophilus,* the biological indicator of the H_2O_2 sterilizer efficacy, did not show any growth after one cycle of H_2O_2 treatment. Isolation of microbes that are resistant to H_2O_2 vapor has significant implications for the quality of products in the pharmaceutical and spacecraft industries that depend on low-heat sterilization technology. Contamination of extraterrestrial samples with cells or biomarkers from Earth would seriously compromise interpretation of results of a Space sample return mission.

[†] The research described in this paper was carried out by the Jet Propulsion Laboratory, California Institute of Technology, under contract with the National Aeronautics and Space Administration.