

Developing Planetary Protection Technology: Microbial Diversity and Radiation Resistance of Microorganisms in a Spacecraft Assembly Facility.

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Europa has attracted much attention as evidence suggests the presence of a liquid ocean beneath this Jupiter moon's frozen crust. Such an environment might be conducive to the origins of life. Since robotic exploration of Europa is being planned, it becomes crucial to prepare for bio-burden reduction of hardware assembled for Europa missions to avoid contamination of Europa's pristine environment. In this study, we examined the microbial diversity of samples collected from two flight-ready circuit boards and their assembly facility. Also, because Jupiter's strong radiation environment may be able to reduce the viable microbial contamination on flight components, we have also studied the effects of radiation on microbial communities found to be associated with the space-flight hardware and/or present in the assembly facility.

Surface samples thought to be representative of considerable human contact were collected from two circuit boards and various locations within the assembly facility using polyester swabs (swab samples). Likewise, sterile wipes were used to sample a shelf above the workstation where the circuit boards were assembled and the floor of the facility (wipe samples).

The swab and wipe samples were pooled separately and divided into two halves, one of which was irradiated with 1Mrad gamma radiation for 5.5 hours, the other was not irradiated. About 1.2×10^4 and 6×10^4 CFUs/m² cultivable microbes were detected in the swab and wipe samples, respectively. Radiation proved effective in inhibiting the growth of most microbes. Further characterization of the bacterial colonies observed in the irradiated swab and wipe samples is necessary to determine the degree of the radiation resistance. The 16S rDNA sequence analysis of the cultivable microbes indicated that the assembly facility consists mostly of the members of actinobacteria, corynebacteria and pseudomonads. However, the swab samples that include the circuit boards were predominantly populated with *Bacillus* and *Staphylococcus*. Molecular microbial diversity was also studied by cloning the 16S rDNA PCR fragment from the samples. The non-irradiated swab samples were largely populated by species of *Exiguobacter* and *Bacillus* whereas the irradiated swab samples were dominated by *Bacillus* and *E. coli*. Radiation damage of microorganisms was also investigated by epifluorescence microscopy. In summary, our study has shown that gamma radiation can inhibit the growth of most of the cultivable microbes, but preliminary results suggest that radiation such as this has little adverse effect on the DNA molecules of these microorganisms.

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Europa, one of the four largest moons of Jupiter, has attracted much attention as evidence has postulated the presence of a liquid ocean beneath this moon's frozen crust. Such an environment might be conducive to the origins of life, and it has been further speculated that some form of life might exist in Europa's subsurface ocean. Since robotic exploration of such an environment seems inevitable, it becomes crucial to both enhance our understanding of the bio-burden of instruments assembled for such a mission, and develop suitable methods of eliminating contamination of such pristine environments. In this study, we examined the microbial diversity of samples collected from two flight-ready circuit boards and its assembly facility, BAE Systems Manufacturing Laboratory and Assembly Area, Manassas, Virginia. Since evidence has suggested that Europa's atmosphere is highly oxidizing and radiation intensive, we have also studied the effects of radiation on microbial communities found to be associated with the space-flight hardware and/or present in the assembly facility. Eleven surface samples thought to be representative of considerable human contact were collected from two circuit boards and various locations within the assembly facility using polyester swabs. Likewise, clean room certified sterile wipes were used to sample a shelf above the workstation where the circuit boards were assembled and the floor of the BAE facility. The pooled swab and wipe samples were divided into two halves, one of which was irradiated with 1Mrad gamma radiation for 5.5 hours, the other was not irradiated. The cultivable, aerobic, heterotrophic bacterial populations were enumerated by pour plate assays on Trypto-Soy Agar. About 5×10^1 CFUs/25 cm² were detected in the swab samples, and 6×10^4 CFUs/m² were detected in the wipe samples. Radiation proved effective in destroying the cultivability of all microbes from both the swab and wipe samples as no cultivable microbes were observed in any of the irradiated samples. However, PCR amplification and cloning of 16S rDNA PCR fragments showed that the 16S rRNA gene fragments of the irradiated samples were not destroyed by the 1 Mrad radiation treatment. Based on colony morphologies, sixteen and fifteen representative colonies were picked from swab and wipe samples, respectively. The 16s rDNA sequence analysis of the cultivable microbes indicated that the BAE facility consists mostly of members of actinobacteria, corynebacteria and pseudomonads. However, the swab samples that include the circuit boards were predominantly populated with *Bacillus* and *Staphylococcus*. Molecular microbial diversity was also studied by sequencing cloned 16s rDNA PCR fragments from each sample. The non-irradiated swab samples were largely populated by species of *Exiguobacter* and *Bacillus*. Similar diversity was also shown in irradiated swab samples, the most abundant bacteria being species of *Bacillus* and *E. coli*. Radiation damage of microorganisms was also investigated by epifluorescent microscopy. In non-irradiated samples, a large variety of microbes were observed. However, in irradiated samples, the dominant population was eukaryotes, and their morphologies were distinct from eukaryotes seen in the non-irradiated sample. In summary, our study has shown that gamma radiation can inhibit the growth of cultivable microbes, but radiation such as this

seems to have little adverse effect on the DNA molecules of these microorganisms. It is possible that sub-populations of these microbes might have undergone mutations due to radiation and have entered into a viable but non-cultivable state and needs to be explored further.