IRRADIATION OF *BACILLUS SUBTILIS* SPORES UNDER EUROPAN CONDITIONS

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Introduction: Europa is a leading astrobiological target because of the possibility that its subsurface ocean is in contact with its rocky interior allowing for hydrothermal activity and the necessary conditions for life [1]. The technical challenges of remotely breaching an ice shell of unknown depth mean that for now potential missions to Europa will try to search for any clues of potential life that have made their way to the near-surface, where the unforgiving temperature, pressure, and radiation near-surface environments of Europa await. Therefore it is critical to understand the photochemistry and viability changes of biomolecules and microbes under these unforgiving conditions in order to develop plausible detection strategies and targets. For example, understanding the photochemistry of microbes and their associated biomolecules could help identify the type of organic compounds of biological origin a mass spectrometer might look for during a close flyby of Europa's surface.

Bacillus subtilis spores are a useful model organism for investigating the effects of irradiation under Europan conditions because of their relatively high resistance to a wide variety of extreme conditions in space [2], and their importance as an indicator species for reduction of forward contamination in planetary protection protocols. Furthermore, all microbial spores contain percent levels of dipicolinic acid (DPA) which is released upon germination or cell lysis, and is therefore a general high concentration biomolecule that might be detectable on Europa.

Experimental: *B. subtilis* spores and DPA were each irradiated in a high vacuum chamber at temperatures relevant to Europa. Spectra of both were taken with FTIR pre- and post-irradiation to observe photochemical changes after different amounts of irradiation. Temperature programmed desorption mass spectrometry was also performed to look for volatile fragments created during irradiation. Spores were recovered and cultured to measure changes in the rate of UV inactivation between 100K and room temperature. Irradiation experiments were also performed with micron layers of ice cover to explore the importance of shielding VUV photons.

Results and Discussion: FTIR spectra of *B. subtilis* and DPA are presented [3], both in good agreement with previous work [4, 5] The UV photolysis pathway of DPA is presented including its first step as a single decarboxylation to form pyridine carboxylic acid. However, no double decarboxylation to form pyridine was observed as might have been expected. Instead unidentified photoproducts were observed after long duration (16 hrs Ar-arc lamp) irradiation. The same novel photoproducts could also be observed in the spectra of irradiated spores confirming the importance of DPA as a spectral marker of spores and as a part of their UV resistance. Additional spectral evidence showed predominantly amide group destruction, most likely associated with spore coat proteins.

Spores remained viable under UV irradiation longer at 100 K than at room temperature in agreement with previous work [6, 7]. Ice layers covering the spores did not change their photochemistry or reduction in viability compared to uncovered spores.

This work begins to constrain the potential for forward contamination of microbes under Europan conditions, and observed a 99.9% reduction in *B. subtilis* viability after ~40 hrs of irradiation at Europa. More work is needed to explore the potential for sulfuric acid or hydrated minerals in the ice to increase the shielding of the ice and extend the viability of organisms at the surface. DPA is a useful marker of spores both within the cell and once released, but will also be photolyzed into smaller fragments under Europan surface conditions.

References:

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