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## **Active DNA Repair on Interplanetary Transport of Microbes (ADRoIT-M)**

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NASA's Strategic Plan in planetary science is to "ascertain the content, origin, and evolution of the solar system and the potential for life elsewhere." Key questions remain: "Is there life beyond Earth? Did life ever arise on Mars? Could it have been transferred to Earth (or vice-versa)?" Considerable evidence supporting the theory of interplanetary transfer of microorganisms by natural impact processes (lithopanspermia; literally "panspermia in rock") has been obtained from a combination of modeling, laboratory, and spaceflight experiments. However, with two exceptions (Biostacks I and II on Apollo 16 and 17), testing of the cruise phase of transport using long-term space exposure experiments has been limited to satellites in low Earth orbit (LEO) inside Earth's magnetosphere. Building upon our prior experience with nanosatellites and with long-term exposure of bacterial spores to the spaceflight environment, we propose the mission ADRoIT-M (Active DNA Repair on Interplanetary Transport of Microbes), an extended mission into deep space of a 6U (~12 kg) nanosatellite carrying spores of *Bacillus subtilis*, a spore-forming bacterium with an extensive (> 40-year) spaceflight heritage. The proposed experiment will deploy a positive-selection assay for error-prone repair of ionizing radiation-induced DNA damage, leading to germination and growth of mutant spores in a specific selective medium.

The experiment will be performed using a near-exact-copy of the 6U BioSentinel nanosatellite currently under development for deployment during NASA's EM-1 mission of the Space Launch System (planned for late 2018). Dormant spores of *B. subtilis* will be used as the test organism. A predetermined number of dried spores will be placed in microfluidic multiwell plates. At 6-month intervals during the experiment, subsets of wells will be flooded with growth medium containing the redox dye Alamar Blue and rifampicin (RFM), a potent inhibitor of RNA polymerase. Only cells that have suffered DNA damage leading to mutations the *rpoB* gene causing RFM-resistance will grow and convert Alamar Blue dye from the blue to pink form, which is measured by the onboard optical system. Single mutational events can be detected with this system due to their amplification by cell growth and metabolism. Wild-type spores and a series of isogenic strains defective in various DNA repair pathways, particularly (i) homologous recombination, (ii) double-strand break (DSB) repair, and (iii) translesion synthesis, will be tested in parallel to measure the relative contribution of different DNA repair systems to the overall induction of mutations. As in BioSentinel, biological dosimetry will be coupled to physical measurements of radiation rate and quality using on-board total ionizing dose (TID) sensors and a linear energy transfer (LET) spectrometer.

This research will build upon previous missions to LEO and will extend prior nanosatellite studies into interplanetary space. New insight into DNA damage and repair gleaned from this mission will also enhance understanding of space radiation hazards and their mitigation, both of which are relevant to the health and performance of human crew on long-duration exploration missions.