

**DNA STRAND BREAKS, PHOTOPRODUCTS, AND REPAIR IN ANALOG SPACE AND MARS ENVIRONMENTS: IMPLICATIONS FOR MICROBIAL INTERPLANETARY TRANSFER** Wayne L. Nicholson<sup>1</sup>, Ralf Möller<sup>2</sup>, Thierry Douki<sup>3</sup>, José Robles<sup>4</sup>, Galen Bruno<sup>1</sup>, Patricia Fajardo-Cavazos<sup>1</sup>, and Andrew C. Schuerger<sup>1</sup>. <sup>1</sup>University of Florida, Space Life Sciences Laboratory, Kennedy Space Center, FL 32899 (WLN@ufl.edu), <sup>2</sup>German Aerospace Center (DLR), Cologne, Germany, <sup>3</sup>Atomic Energy Commissariat (CEA), Grenoble, France, <sup>4</sup>Mt. Stromlo Observatory, Australian National University, Canberra, Australia.

**Introduction:** Interplanetary transport of microorganisms between Earth and Mars can be envisioned to occur either by natural impact processes (i.e., lithopanspermia) or by human spaceflight activities [1, 2]. Transfer through space and residence on Mars would subject microbes to UV, ionizing radiation, high-energy (HZE) particles, ultrahigh vacuum (UHV), low pressure, extreme desiccation, and thermal extremes causing both lethal and mutagenic DNA damage to cells [1, 3-6].

Bacterial spores are found both in endolithic environments and associated with spacecraft, thus are good candidates for interplanetary passengers [1-3]. In the present study we have concentrated on spores of the model spore-former, *Bacillus subtilis* 168, and the hardy spacecraft contaminant *Bacillus pumilus* SAFR-032 to study the types of DNA damage induced in a hypothetical Earth-to-Mars transit and deposition on Mars, and the types of DNA repair mechanisms needed to survive the journey.

**Materials and Methods:** *B. subtilis* 168 and derivatives, and *B. pumilus* SAFR-032 spores were produced and cultivated as described previously [5-8]. Chambers providing simulations of space [5,6] and Mars surface conditions [7, 9] are described in detail elsewhere. Spores of 168 and SAFR-032, as well as naked DNA, were deposited on spacecraft-qualified aluminum coupons, placed in a Mars Simulation Chamber (MSC) replicating the environment on the Mars surface (Mars atmospheric gas mixture, 710 Pa pressure, -10°C) and exposed to solar UV-vis-near IR flux matching the spectrum and intensity of the martian surface. Spore survival was determined by standard viability assays [5-8]. DNA photoproducts were determined by HPLC/tandem MS as described previously [10]. DNA double-strand breaks (DSB) and single-strand breaks (SSB) were determined by neutral and alkaline agarose gel electrophoresis, respectively, as described previously [11].

#### **Results and Discussion:**

**DNA strand breaks.** Exposure of *B. subtilis* spores to parameters of the space environment (UHV, UV, X-rays, and HZE particles) has previously been shown to induce both SSB and DSB in DNA [12, 13]. When naked DNA was exposed to Mars conditions but shielded from solar radiation, no SSB or DSB were

observed after 2 hours of exposure. However, exposure of DNA to simulated Mars solar radiation for as little as 3 minutes resulted in rapid and dose-dependent induction of both DSB and SSB. When spores themselves were exposed to simulated Mars conditions but shielded from solar radiation, no SSB or DSB were observed after 72 hours. When treated with simulated Mars solar radiation, SSB and DSB were formed, but at a much slower rate than that observed with naked DNA.

**DNA photochemistry.** DNA photoproducts in naked DNA exposed to Mars simulated solar radiation were found to consist of mostly cyclobutane pyrimidine dimers and lesser amounts of 6-4 photoproducts; the spore photoproduct (SP) was not observed, in contrast to naked DNA films irradiated with 254-nm UV under UHV [14] or at 1-2 Pa [15]. Experiments to measure the *in vivo* photochemistry of DNA within spores is ongoing at this time.

**DNA repair.** DNA damage in spores is repaired during subsequent spore germination [1, 3]. UV photoproducts are repaired mainly by the dedicated enzyme SP lyase, nucleotide excision repair, and recombinational repair [1, 3]. Recently we have discovered that the Non-Homologous End Joining (NHEJ) DNA repair pathway is very important for the resistance of *B. subtilis* spores to UHV, X-rays, and HZE particle bombardment characteristic of the space environment, which produce mainly SSB and DSB [5, 6]. Future experiments are directed toward understanding the interaction of DNA strand breaks and photodamage with DNA repair systems in the survival of spores to the Mars surface environment.

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