STRATOSPHERE CONDITIONS INACTIVATE BACTERIAL ENDOSPORES FROM A MARS SPACECRAFT ASSEMBLY FACILITY. C. L. Khodadad[1], G. M. Wong[2], and D. J. Smith[3], [1]Sierra Lobo, Inc., Kennedy Space Center, FL 32899, christina.l.khodadad-1@nasa.gov, [2]Department of Geosciences, Pennsylvania State University, University Park, PA 16802, gkw5061@psu.edu, [3](corresponding author) NASA, Space Biosciences Division, Ames Research Center, Moffett Field, CA 94035, david.j.smith-3@nasa.gov.

Introduction: Preventing the forward contamination of Mars is required for United States and international space missions. Yet, spacecraft leaving Earth still carry microorganisms onboard, embedded within surfaces, instruments, electronics, and other inaccessible areas that cannot be readily cleaned. Currently-allowable microbial bioburden on spacecraft – while relatively low [1] – makes the pristine Mars environment vulnerable to contamination. Moreover, future life detection missions could be threatened by false positives without a better understanding of which microorganisms are most capable of persistence, growth or replication once delivered to the Red Planet. A recent analysis from the Second MEPAG Special Regions Science Analysis Group [2] identified major knowledge gaps associated with polyextremophiles (microorganisms resistant to more than one environmental stressor), particularly when shielded from ultraviolet (UV) light on Mars by global dust storms, regolith, or overlying dead microorganisms.

Methods: Smith [3] argued that the Earth's stratosphere would allow multiple Mars-like conditions to be simultaneously tested if polyextremophilic species could be exposed to the upper atmosphere and returned for analysis. Recent missions have demonstrated the feasibility of transporting biological samples to the stratosphere using large scientific balloons [4]. The pressure of the thin and dry stratospheric air around 25 to 38 km above sea level (ASL) is roughly equivalent to the surface pressure on Mars (0.5 to 1 kPa). The stratosphere is also a cold and extremely dry environment with elevated levels of ionizing and non-ionizing radiation [5]. Relative humidity levels can drop below 1%, and temperatures in the lower stratosphere regularly reach -100 °C. Stratospheric radiation is substantially higher than doses at other frequently-visited Mars analog environments, including the McMurdo Dry Valleys of Antarctica and the Atacama Desert. Laboratory environmental chamber experiments have been employed in the past to simulate martian conditions but artificial illumination sources do not realistically represent the dynamic nature of sunlight. Moreover, most ground-based simulation studies do not simultaneously create the full range of biological stressors present on Mars (i.e., hypobaria, desiccation, irradiation, nutrient-deprivation, oxidation, and low temperatures). Conveniently, Earth's upper atmosphere produces a natural combination of these extreme conditions. Measuring the response and survival of polyextremophilic species in the stratosphere can therefore be used to test Mars forward contamination scenarios.

Results: On 10 October 2015 we flew a balloon experiment to the stratosphere reaching an altitude of 31.4 km ASL [6]. The Exposing Microorganisms in the Stratosphere (E-MIST) payload carried known quantities of bacterial endospores to the Mars-like environment for 2, 4, 6, and 8 h exposures. We used a spacecraft assembly facility-isolated bacterial strain *Bacillus pumilus* SAFR-032 for the balloon flight (and subsequent ground experiments). We found that within 120 and 240 min, spore viability was significantly reduced by 2 and 4 orders of magnitude, respectively. By 480 min, < 0.001% of spores carried to the stratosphere remained viable. Unfortunately, an instrument malfunction prevented the acquisition of ultraviolet (UV) light measurements during our balloon mission. To make up for the absence of radiometer data, we calculated a stratosphere UV model and also conducted ground tests with a 271.1 nm UVC light source (0.5 W/m^2), observing a similarly rapid inactivation rate when using a lower number of contaminants (640 spores per sample). With the relatively few spores that survived the stratosphere, we performed a re-sequencing analysis and identified 3 single nucleotide polymorphisms compared to unexposed controls.

Conclusions: Our balloon flight results predict that most terrestrial bacteria would be inactivated within the first Sol on Mars if contaminated spacecraft surfaces receive direct sunlight. The starting concentration of spores and micro-configuration on hardware surfaces appeared to influence survivability outcomes in both experiments. Our re-sequencing results suggest the small portion of bacteria enduring radiation-rich environments (e.g., Earth's upper atmosphere, interplanetary space, or the surface of Mars) may be pushed in evolutionarily consequential directions.

References: [1] Benardini III et al. (2014) *Astrobiology*, *14*, 27-32. [2] Rummel et al. (2014), *Astrobiology*, *14*, 887-968. [3] Smith (2013) *Astrobiology*, *13*, 981-990. [4] Smith et al. (2014) *Grav. Space Res.*, *2*, 70-80. [5] Schuerger and Nicholson (2016) *Astrobiology*, *16*, 335-347. [6] Khodadad et al. (2017) *Astrobiology* (in press).