PERSISTENCE OF BIOMARKER ATP IN CELLS AND SPORES ON MARS: IMPLICATIONS FOR ASTROBIOLOGY AND PLANETARY PROTECTION Fajardo-Cavazos[1], Andrew C. Schuerger[2], and Wayne L. Nicholson[1], Departments of [1]Microbiology and Cell Science and [2]Plant Pathology, University of Florida, Space Life Sciences Laboratory, Kennedy Space Center, FL 32899 (WLN@ufl.edu)

Introduction: The search for life on Mars has been identified as a NASA Exploration Program goal of the highest importance [1]. Exactly what to look for as evidence of life is currently unknown because no martian life has yet been detected, but careful consideration of the commonalities in all life from Earth permits some general assumptions about putative martian life [1]. One such presumed characteristic is the use of organic molecules in biochemical processes, which may or may not have the same chemical structure as terrestrial biomolecules. Testing for biosignatures that are found ubiquitously, such as nucleic acids, lipids, central metabolic components, etc., which can be quantified with sensitive analytical methods amenable to automation is a natural starting point into the search for martian life.

Adenosine-5'-triphosphate (ATP) exhibits a number of properties lending it importance as a top-priority (Category A) biosignature in life detection experiments [2]. First, all living organisms on Earth transform energy, regardless of the source, into chemical energy by synthesizing ATP. Second, ATP is ubiquitous in living cells, is present in growing cells, and is released from cells when they are killed by heat, disinfectants, or other treatments that disrupt the integrity of the cell envelope [3]. Third, ATP has long been used as an extant biomass marker, largely because a sensitive luciferin/luciferase assay has been widely available for more than 30 years [4] that is amenable to automation for use during robotic *in situ* or sample return missions.

It has been supposed that one drawback of ATP as a biosignature molecule is that it does not persist in the environment outside cells, due to the intrinsic lability of its high-energy bonds [2]. However, we observed that purified ATP applied to spacecraft-qualified materials and exposed to simulations of the Mars environment was surprisingly stable, persisting with a half-life of 22 martian sols at -10˚C even when exposed to full-spectrum UV-visible-IR radiation characteristic of the martian surface [5]. In addition, a Mars surface irradiance model [6] was used to estimate residence times for ATP on the upper and lower surfaces of rovers and landers at any martian latitude; it was found that pure ATP could persist on Mars landers and rovers for extended periods, up to decades [5].

Bacterial spores are found both in endolithic environments and associated with spacecraft, thus are good candidates for interplanetary passengers [7-9]. Such organisms are not only more likely to contaminate Mars-bound spacecraft, but also to survive the Earth-to-Mars transit [8]. Given these considerations it is important to ascertain the permanence of detectable ATP biomarker when situated inside microbes themselves which are exposed on spacecraft surfaces to the surface environment of Mars. In addition, bacterial spores are actually pre-programmed to produce a burst of ATP if germination is triggered. Therefore, the possibility of Mars-exposed bacterial endospores still being capable of generating ATP during the initial stages of germination must also be investigated. In the present study we have concentrated on spores of *Bacillus subtilis* 168, spores of the hardy spacecraft contaminant *Bacillus pumilus* SAFR-032, and cells of the spacecraft contaminant *Acinetobacter radioresistens*, to study the longevity of ATP and ATP-generating ability upon exposure simulations of the Mars surface environment.

Materials and Methods: Vegetative cells were cultivated and spores produced and germinated as described previously [10-13]. The Mars Simulation Chamber (MSC) has been described in detail elsewhere [12, 14]. Cells of *A. radioresistens* 50v1, and cells or spores of 168 and SAFR-032, were deposited on sterile spacecraft-qualified aluminum coupons, placed in the 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 [10-13]. ATP was assayed as described previously [14].

Results and Discussion: It was observed that although spores and cells were inactivated rapidly (within minutes) in the MSC, their *in vivo* ATP levels remained relatively unaffected for up to 21 days of continuous UV irradiation, the equivalent of ~115 martian sols. The degradation rate of intracellular ATP was calculated and compared to the degradation rate of purified ATP previously determined under similar conditions [14]; the half-life of intracellular ATP was 3.2-fold longer than purified ATP in the MSC.

Ability of MSC-exposed bacterial spores to generate ATP during subsequent germination was measured with respect to spore viability. It was determined that the Mars UV dose required to inactivate 90% of germination ATP generation (LD_{90}) varied from 6 to 35 times the LD_{90} value for spore viability.

Therefore, even long-dead cells or spores still retain a significant amount of detectable biosignature ATP. Furthermore, the ability of spores to generate ATP given the appropriate germination conditions persists long after the spores themselves have been rendered non-viable by exposure to Mars surface condi-

tions. The results have profound implications for the search for life on Mars using ATP as a biosignature, and for the prevention of the forward contamination of Mars.

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