SPACECRAFT MICROORGANISMS AND THEIR IMPACT ON PLANETARY PROTECTION – ARE THEY ABLE GROW AT SIMULATED MARTIAN ATMOSPHERIC PRESSURE? P. Schwendner[1] and A. C. Schuerger[1], [1]Department of Plant Pathology, University of Florida/Space Life Sciences Laboratory, 505 Odyssey Way, N. Merritt Island, 32953, FL, USA; email: petra.schwendner@ufl.edu.

Introduction: When launching spacecraft and rovers to potential habitable zones, the protection of solar system bodies from contamination by Earth life and *vice versa* is essential. Planetary protection not only requires the preservation of extraterrestrial habitats in their natural state but also is a precaution to avoid contamination in places where life might exist or might have existed. As a consequence, microbial contamination on spacecraft and within the surrounding clean rooms is strictly regulated and monitored prior to launch. Once launched, microorganisms hitchhiking on the spacecraft might survive cruise-phase conditions to their target bodies. In addition, bacteria originating from Earth possess the metabolic range to grow in low pressures, low-temperatures, and anoxic atmospheres [1,2,3] that are similar to the Martian surfaces. In fact, currently more than 30 species from 10 bacterial genera are known to grow and thrive under these conditions [2]. Therefore, spacecraft microorganisms may pose a potential risk to the forward contamination of habitable zones on Mars. However, there is a paucity of data on the growth of bacteria recovered from actual Mars rovers or landers prior to their launch.

The goal of the current project was to expose and attempt to grow a broad range of bacteria obtained directly from spacecraft surfaces during the Viking, Pathfinder, Spirit, Opportunity, Phoenix, and Curiosity missions to simulated Martian conditions of 0.7 kPa, 0 °C, and CO_2-anoxic atmosphere (henceforth called *low-PTA* conditions). Furthermore, we sought to determine if specific anoxic redox couples increased metabolic activity and growth of chemoorganotrophic bacteria to simulated Martian conditions.

Material and Methods: The design and operation of the hypobaric chambers were described previously [1,2,3]. Briefly, double-thick agar plates were inserted into polycarbonate desiccators which were connected to low-pressure controllers. Anaerobic pouches were added to maintain anoxic atmospheres within the hypobaric chambers. The desiccators were placed in incubators set at 0 °C and the pressure reduced stepwise to reach 0.7 kPa. The conditions were maintained for 28 d and the hypobaric chambers were only opened to check for growth of the bacterial strains. A total of 125 microorganisms were tested. The six media investigated included the following: (1) 0.5x trypticase soy agar (TSA), (2) TSA + vitamins and minerals, (3) TSA + KNO_3 (nitrate reduction), (4) TSA + sulfates (sulfate reduction), (5) TSA + Fe^{3+}-citrate at pH 7.0 (1st iron reduction), (6) TSA + Fe^{3+}-citrate at pH 5.0 (2nd iron reduction). Bacteria were streaked on all 6 media in groups of 25 strains plus one positive control (*Serratia liquefaciens* ATCC 27592 [1]) and one negative control (*Bacillus subtilis* 168 [3]), and incubated at low-PTA conditions for 28 d. Three controls were run concurrently at 101.3 kPa with varying temperature and gas composition. (1) Plates were incubated at 101.3 kPa, 0 °C and a CO_2-enriched anoxic atmosphere. (2) Plates were incubated at 101.3 kPa, 0 °C under Earth-normal atmosphere (pN_2:pO_2 78:21). (3) Plates were incubated at 101.3 kPa, 30 °C under Earth-normal atmosphere (pN_2:pO_2 78:21).

Results: When grown on TSA only, none of the 125 tested strains revealed visible growth after 28 d of incubation at low-PTA conditions. Based on that result, the effects of different redox couples on bacterial growth were tested. Few changes were noted among the various strains and the five different media. One example was *Kocuria rosea* which grew better at 101.3 kPa, 0 °C, and Earth pO_2 on standard TSA when NO_3^- or SO_4^{2-} was added, compared to standard TSA or TSA + vitamins. However, *K. rosea* was not able to grow under low-PTA conditions. Results suggest that most of the bacteria present on spacecraft at launch may not pose a serious forward contamination risk during upcoming Mars lander or rover missions.

Conclusion: Data on microbial metabolism and growth under simulated Martin conditions near 0.7 kPa are critical for modeling the potential risks of forward contamination in habitable zones on Mars. Thus, results from these experiments will help to protect Special Regions on Mars, and will prepare for future human missions.

References: [1] Schuerger A. C. and Nicholson W. L. (2006) *Icarus, 185,* 143–152. [2] Schuerger A. C. and Nicholson W. L. (2016) *Astrobiology, 12,* 964-976. [3] Schuerger A. C. et al. (2013) *Astrobiology, 13,* 115-131.

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