TERRESTRIAL BACTERIA TYPICALLY RECOVERED FROM MARS SPACECRAFT DO NOT APPEAR ABLE TO GROW UNDER SIMULATED MARTIAN CONDITIONS. Andrew C. Schuerger[1], Bonnie Berry[2], and Wayne L. Nicholson[3], [1]Dept. of Plant Pathology, University of Florida, Space Life Sciences Lab, Kennedy Space Center, FL 32899; acschuerger@my.ifas.ufl.edu; [2]Dept. of Biology, Univ. of Central Florida, Orlando, FL 32816; bberry@mail.ucf.edu [3]Dept. of Microbiology, University of Florida, Space Life Sciences Lab, Kennedy Space Center, FL 32899; wln@ufl.edu.

Introduction: Robotic and piloted spacecraft are launched from Earth with finite levels of microbial contamination that are composed of species similar to the cleanroom environments within which the vehicles are assembled. After entering the harsh environment of interplanetary space, the microorganisms on the exterior surfaces of spacecraft are subjected to biocidal factors that immediately begin to reduce the viable biomass and species diversity of the launched bioloads. The harsh conditions found on the surface of Mars are only slightly more conducive to the survival of terrestrial microorganisms than are found in interplanetary space. The reductions in biomass and species diversity of the launched bioloads on and within spacecraft are likely to simplify the forward contamination issues related to robotic and human mission to Mars by limiting the numbers of viable cells dislodged from spacecraft surfaces and dispersed onto the Martian terrain. However, because of the demonstrated long-term survival of bacteria in space [1,2], some viable cells or spores launched from Earth are likely to survive the cruise phase to Mars. Whether these viable cells or spores constitute a hazard to scientific missions or to the forward contamination of Mars will depend to a great extent on whether the viable microorganisms from Earth can grow and replicate under martian conditions. In a recent study [3,4], seven *Bacillus* spp. were tested for their sensitivity to growth at low pressures. Endospores of all seven species were unable to germinate and grow in pure CO_2 atmospheres at 25 mb or lower. The primary objective of this study was to begin to determine if non-spore forming terrestrial microorganisms can grow and replicate under simulated martian conditions.

Methods for growth on solid media: Thirty-two strains of 22 species of spore-forming and non-spore forming mesophilic bacteria were dispersed separately onto trypticase soy agar (TSA) and the cultures immediately transferred to a small bell-jar hypobaric system (Fig. 1). The bell-jar system was capable of maintaining pressures down to 15 mb over extended periods. When required, the bell-jar system was configured with a CO_2 generation system to create pure CO_2 atmospheres. The bell-jar system was maintained at 30 C.

Vegetative cells of the 32 strians from 16-hr-old TSA cultures were streaked separately onto fresh TSA in 2 or 3 quadrants, while flaming the transfer loop between quadrants. Endospores were not present in the 16-hr-old cultures of all spore-forming *Bacillus* spp. tested (four strains of *B. subtilis* and one strain of *B. megaterium*). The vegetative cells of all species were incubated in the bell-jar system for 48 hrs at various combinations of pressure, temperature, and pure CO_2 or O_2/N_2 atmospheres. Earth-controls were maintained at 30 C, 1013 mb pressure, and a standard O_2/N_2 (21%/78%) atmosphere for all experiments. Vegetative cells for all species were rated for robustness of growth on TSA using a simple rating system.

Fig 1. The bell-jar system installed within a microbiological incubator.

Methods for growth in liquid media: *B. subtilis* strain 168 and *E. coli* strain K12 were inoculated from fresh overnight cultures into liquid Luria-Bertani medium (LB), or LB medium containing glucose and potassium nitrate to enable anaerobic growth (LBGN). Liquid cultures were cultivated at 30 C in the bell jar at different pressures down to 25 mb in Earth-standard O_2/N_2 (21/78%) or pure CO_2 atmospheres. Overnight growth was measured and compared to control cultures incubated identically but at an Earth atmosphere at 1013 mb. Growth was measured as the increase of optical density of the cultures using a Klett-Summerson colorimeter fitted with the #66 (red) filter (Manostat, NY)..

Results: Results indicated that most bacteria were unable to grow under pure CO_2 atmospheres reagard-

less of pressure due to their obligate aerobic physiologies. However, six non-spore forming species were able to grow in pure CO_2 atmospheres down to 25 mb. The most robust growth on TSA at 25 mb under CO_2 was obsereved for two strains of the bacterium *Escherichia coli*. Other species that exhibited growth on TSA at 25 mb in pure CO_2 atmoshperes were: *Enterococcus faecalis, Proteus mirabilis, Serratia liquifaciens, Staphlococcus aureus, and Paenibacillus pabuli*, although in all cases growth was much weaker than controls incubated at 1013 mb. One interesting observation was that of the six species that could grow under CO_2 atmospheres at 25 mb, only one species (*Serratia liquifaciens*) could grow and replicate at 25 mb under both an Earth-normal O_2/N_2 and pure CO_2 atmosphere. The other five species grew only under CO_2 atmospheres at 25 mb.

Growth of submerged liquid cultures of two common laboratory bacteria, *B. subtilis* 168 and *E. coli* K12, showed that below ~100 mb growth was progressively inhibited (Fig. 2). *Escherichia coli* was less prone to low-pressure growth inhibition in liquid than was *B. subtilis*. It was postulated that a reduction in the concentration of dissolved oxygen at low pressure may have been the cause. To test this, both bacteria were cultivated at low pressures in LBGN medium, which allows anaerobic metabolism (i.e., glucose to allow fermentation and nitrate to allow anaerobic respiration, of which both strains are capable). Again, growth rate reductions at pressures below 100 mb were noted although not quite as severe for *E. coli* grown in LB alone at 25 and 50 mb (Fig. 2). Growth of *B. subtilis* 168 at 25 mb was near the lower limit of detection in this assay. The sensitivities to low pressures of both species were very similar between the TSA solid media and LB liquid media assays. We interpret these responses to be evidence that there may be a direct pressure effect on the two bacteria under hypobaric conditions.

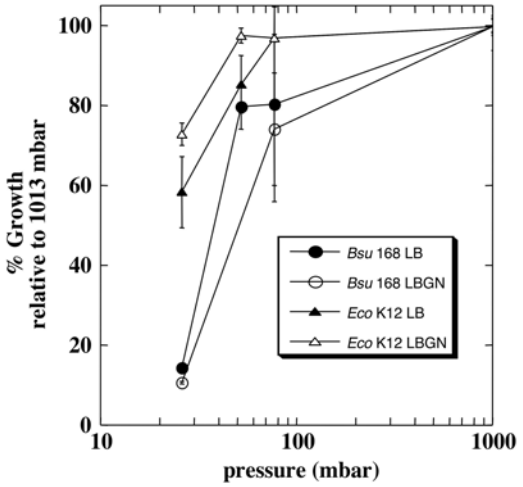

Fig. 2. Growth in liquid culture of *B. subtilis* 168 and *E. coli* K12 vs. pressure, relative to growth at Earth-ambient pressure.

Conclusions: These results indicate that of the bacteria tested (32 strains of 22 species) all microorganisms had significant difficulties growing at pressures that began to approach those found on the surface of Mars (6-10 mb). The results suggest that the microorganisms that remain viable on spacecraft surfaces after the 6-8 month transit time to Mars may not be readily capable of growth on the surface of Mars, even if exposed to a water and nutrient rich substrate. Furthermore, the observation that *E. coli* and *B. subtilis* were strongly inhibited near 25 mb in both the TSA and liquid-culture assays is interpreted to indicate that there may be a direct pressure effect on bacterial growth. Originally, we anticipated that both *E. coli* and *B. subtilis* would grow more easily in the liquid-culture assay than on TSA because we hypothesized that growth inhibition on TSA was due primarily to the desiccating conditions present at 25 mb and 30 C. However, we now question this hypothesis and conclude that there may be a direct pressure effect on bacterial cells that might inhibit their growth under simulated martian conditions. These results confirm and extend earlier reports [3,4] that indicated that seven *Bacillus* spp. were unable to grow at pressure below 25 mb under pure CO_2 atmospheres.

References: [1] Horneck et al., 1994, Adv. Space Res. 14(10):41-45; [2] Horneck et al., 2003, Adv. Space Res. 31(1):87-95; [3] Schuerger and Nicholson, (2005), LPSC Abstract 1366; [4] Schuerger and Nicholson, (2006), Icarus, (submitted).