

GROWTH OF HYPOBAROPHILE BACTERIA FROM SEVEN GENERA UNDER MARS CONDITIONS OF 7 mbar, 0 °C, AND CO₂-ENRICHED ANOXIC ATMOSPHERES. Andrew. C. Schuerger¹ and Wayne L. Nicholson², ¹Dept. of Plant Pathology and ²Dept. of Microbiology & Cell Science, University of Florida, 505 Odyssey Way, Exploration Park, N. Merritt Island, FL 32953; email: schuerg@ufl.edu; WLN@ufl.edu.

Introduction: The search for life on Mars may be assisted by characterizing the limits of growth for terrestrial microorganisms under martian conditions. Identifying microbial species present on spacecraft prior to launch that can grow under Mars-surface conditions will help develop appropriate rover sterilization protocols to mitigate against the forward contamination of Mars. Recent publications have demonstrated the growth of *Serratia liquefaciens* [1] and several *Carnobacterium* spp. [2] under simulated martian conditions of 7 mbar, 0 °C, and CO₂-enriched anoxic atmospheres. Here we present data that extends to seven genera with hypobarophile species capable of growth under martian conditions near 7 mbar.

Methods. First, type-strains of the genera *Serratia* (8 species) and *Carnobacterium* (10 species) were grown on double-thick trypticase soy agar (TSA) or R2A agar plates in hypobaric chambers [1] maintained at 7 mbar, 0 °C, and CO₂-enriched anoxic atmospheres for 35 days. Second, nine samples from extremophile and non-extremophile soils, plus samples from seven non-soil niches around the Kennedy Space Center, FL were assayed on TSA and R2A agars for the presence of species capable of growth under the simulated martian conditions listed above.

Results. Besides *S. liquefaciens* [1], five additional species of *Serratia* exhibited growth at 7 mbar including *S. ficaria*, *S. fonticola*, *S. gremesii*, *S. plymuthica*, and *S. quinivorans*. Only *S. marcescens* and *S. rubidaea* failed to grow at 7 mbar. Controls for the *Serratia* spp. assays grown at 7 mbar, 0 °C, and CO₂-enriched anoxic atmospheres indicated that the failure of growth for *S. marcescens* and *S. rubidaea* was likely due to sensitivities to low temperature and not, per se, to low pressure. Several *Carnobacterium* spp. exhibited faster growth than the *Serratia* spp. tested at the above conditions forming observable colonies within 7-10 days [2] instead of 14-21 d for *Serratia* isolates.

Second, experiments have recovered additional hypobarophiles from diverse ecological niches including Arctic soils, alpine soils, seawater, and human saliva (e.g., Fig. 1). The densities of bacterial hypobarophiles in soils ranged between a low of 1.9×10^2 cfu/g for an alpine soil from Mt. Baker, WA to a high of 5.1×10^4 cfu/g of soil from an arctic site near Colour Lake, Axel Heiberg Island, Nunavut, Canada. All other bacteria tested (i.e., 50+ mesophilic species from spacecraft and hundreds of species from Siberian permafrost, Arctic soils, etc.) failed to grow under mar

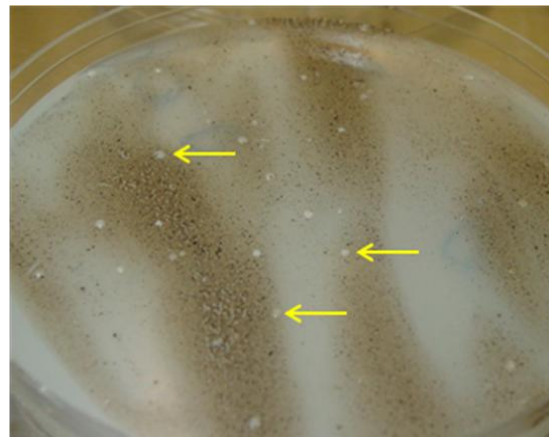


Fig. 1. White translucent colonies of the hypobarophiles, *Paenibacillus* spp. (arrows), isolated from soil collected near Colour Lake, Axel Heiberg, Nunavut, Canada. Colonies were observed after 21 days on R2A incubated under simulated martian conditions of 7 mbar, 0 °C, and CO₂-enriched anoxic atmosphere.

tian conditions. 16S sequencing of recovered bacterial hypobarophiles indicates that tolerance to 7 mbar is widespread in bacteria including species from the following genera: *Carnobacterium*, *Cryobacterium*, *Exiguobacterium*, *Leuconostoc*, *Paenibacillus*, *Serratia*, and *Streptomyces*. In soil assays for hypobarophiles at 7 mbar, between 10^4 to 10^8 total culturable bacteria and 10^2 to 10^5 total culturable fungi were recovered per gram of soil. And lastly, no fungal colonies were observed on TSA or R2A agars incubated at 7 mbar.

Discussion. The metabolic, genomic, or proteomic mechanisms responsible for the observed growth of these bacteria at 7 mbar are, as yet, unknown. Results suggest that some terrestrial microorganisms have the metabolic capabilities to grow on Mars at 7 mbar if directly transported to a hydrated and nutrient rich niche without requiring evolutionary adaptation to intermediate hypobaric environments prior to dispersal on Mars. Thus, it is plausible that terrestrial microorganisms on spacecraft may be capable of growth on or near the surface of Mars. Future research can now be designed to explore the effects of low water activity, high salts, and oligotrophic conditions on the growth of terrestrial microbes at low pressures under simulated martian conditions.

References: [1] Schuerger et al., 2013, *Astrobiology*, 13, 115-131. [2] Nicholson et al., 2013, *PNAS*, 110, 666-671.