ULTRASTRUCTURE OF THE BACTERIUM, *SERRATIA LIQUEFACIENS*, GROWN UNDER SIMULATED MARS CONDITIONS OF 0.7 kPa, 0 °C, AND CO_2-ENRICHED ANOXIC ATMOSPHERES.

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Introduction. Mars spacecraft are launched with low numbers of viable terrestrial microorganisms, some of which may be capable of growth under Martian conditions. Recent studies [1,2,3] have demonstrated that environmental bacteria from 10 genera can grow under hypobaric conditions similar to the surface conditions found on Mars (0.7 kPa, 0 °C, and CO_2 anoxic atmosphere). The bacterial generalist, *Serratia liquefaciens* ATCC 27592, was observed to form colonies on trypticase soy agar (TSA) in 10-14 days [1]. Other experiments [2,3] have recovered additional hypobarophiles from diverse ecological niches including Arctic and alpine soils, Siberian permafrost, and seawater. No fungi or archaea have yet been identified capable of growth under the conditions tested above [see 1,2,3].

The objective for the current study was to characterize the ultrastructural changes in *S. liquefaciens* grown under simulated martian conditions in order to better predict how low-pressure environments like Mars might affect the growth of hypobarophiles plausibly present on Mars spacecraft.

Methods. Vegetative cells of *S. liquefaciens* were grown on double-thick agar plates of TSA incubated for 28 d at 0.7 kPa, 0 °C, and CO_2-enriched anoxic atmospheres, or in Earth-normal lab conditions of 101.3 kPa, a O_2/N_2 gas composition, and 30 °C. Cells were harvested in 1 x phosphate buffered saline (PBS), fixed in 2% glutaraldehyde in PBS, washed three times in fresh PBS, fixed a 2nd time in 1% buffered osmium tetroxide, washed in PBS, dehydrated in a graded ethanol series, critical point dried, coated with gold-palladium, and imaged in a Hitachi Su5000 scanning electron microscope.

Results. Compared to lab controls (Fig. 1A), cells of *S. liquefaciens* grown in 0.7 kPa, 0 °C, and CO_2-enriched anoxic atmospheres (Fig. 1B) were observed to: (1) exhibit swollen blunt ends at sites of cell division tapering to unusually constricted points on the distal ends of progeny cells, (2) cell division appeared disrupted with division planes occurring at odd angles often forming right-angle oriented daughter cells, (3) some cells failed to form divisional planes resulting in long spiral and oddly shaped cells measuring up to 6-8 µm in total length, and (4) fimbriae were lacking. Results suggest that cells of *S. liquefaciens* grown at 0.7 kPa exhibited significant disruptions in assembly of cell walls that impaired normal cell division.

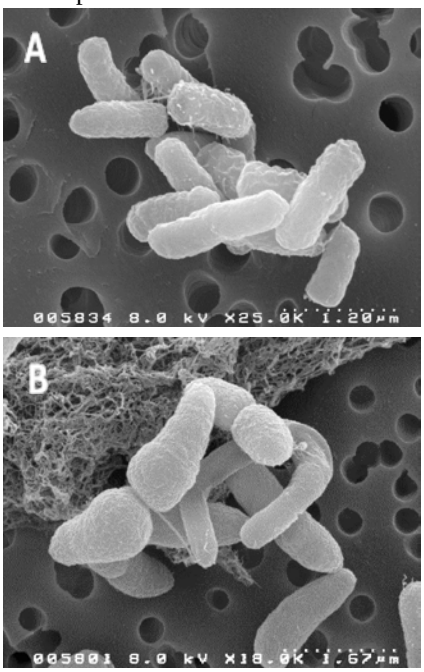

Fig. 1A. Cells of *S. liquefaciens* grown under lab conditions at 101.3 kPa. **Fig. 1B.** Cells of *S. liquefaciens* grown under simulated Mars conditions at 0.7 kPa (see text).

Discussion. The metabolic, genomic, and proteomic mechanisms responsible for the observed growth of *S. liquefaciens* at 0.7 kPa are, as yet, unknown. Results [1,2,3, and here] suggest that at least some terrestrial microorganisms have the metabolic range for metabolism and growth when directly transported to hypobaric conditions on Mars near 0.7 kPa without requiring adaptive steps at intermediate pressures. 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

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