ANCIENT POLYEXTREMOPHILIC MICROBES WITH THE POTENTIAL FOR SURVIVAL ON MARS.
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Introduction: Few terrestrial microorganisms are likely to have the capability for survival on Mars due to multiple extremes, including low temperature, anaerobic, hypobaric, and desiccating conditions, ultraviolet and ionizing radiation, perchlorates, etc. Halophiles belonging to the third Domain of Life, Haloarchaea, have been proposed to potentially survive on the Red Planet due to their exceptional physiological and genetic properties [1]. These ancient microbes flourish in hypersaline brines where they encounter saturating salinity, solar radiation, and other extreme conditions [2].

We are studying effects of extreme conditions on a common laboratory strain, *Halobacterium* sp. NRC-1, isolated from a San Francisco Bay saltern and a novel environmental isolate, *Halorubrum lacusprofundi*, from Deep Lake, Antarctica, [3]. Our approach employs exposure of cells to stressors in the laboratory or in the environment and use of a combination of microbiology, genomics, transcriptomics, genetics, and biochemistry to determine the effects. These studies suggest that the molecular properties of Haloarchaea offer advantages for potential survival and adaptation on Mars.

Results and Discussion: In a series of studies, we tested effects of cold temperature, anaerobic conditions, perchlorate oxidizers, UV light, and ionizing radiation on *Halobacterium* sp. NRC-1 and *H. lacusprofundi* in the laboratory. We also tested their ability to survive launches into Earth's stratosphere, a Mars analog, characterizing their physiological and molecular responses.

Growth at low temperature, anaerobically, and with light. *Halobacterium* sp. NRC-1 grows in 3-5 M NaCl at moderate temperatures [4-5]. *H. lacusprofundi* also grows at cold temperatures, down to -2 ºC, where the media remains liquid due to freezing point depression. Both strains are also capable of anaerobic growth, with *Halobacterium* sp. NRC-1 respiring DMSO [6] and *H. lacusprofundi* respiring perchlorate [7]. In addition, both possess the light-driven proton pump bacteriorhodopsin in their purple membrane for phototrophic growth [8]. Purple membrane may be an early evolutionary invention on Earth with potential as a remote biosignature.

UV and ionizing radiation survival. *Halobacterium* sp. NRC-1 is highly UV-C tolerant, about 2.5 x as yeast, 5 x *E. coli*, and 50 x human cells [8]. Light repair of UV DNA damage is carried out by an efficient photorepair system using photolyase, and dark repair is carried out primarily by excision repair using UvrABC exinuclease, as shown by gene deletion, biochemical, and phenotypic analyses [9-11]. *Halobacterium* sp. NRC-1 derivatives are among the most highly ionizing radiation tolerant organisms known [12]. Mutants isolated with even higher ionizing radiation tolerance were selected and found to have up-regulated expression of a single-stranded DNA-binding complex, RPA, which was detected by transcriptomic analysis and verified genetically [13]. Ionizing radiation survival likely increased through improvement of recombinational or double-stranded break repair. The understanding of the critical roles that DNA repair systems play in radiation damage survival provides insights into mechanisms that may allow engineering better survival with high radiation levels encountered on Mars [14].

Survival in Earth's stratosphere: a Mars analog. *Halobacterium* sp. NRC-1 and *H. lacusprofundi* were launched to Earth's stratosphere using weather balloons, traveling to ~35 km altitude above sea level. Survival assayed after return to Earth was higher for the Antarctic isolate, resulting from its better freeze- thaw survival [15]. Genomic and transcriptomic analyses showed differences in the number and expression of cold-shock genes, and suggested that *H. lacusprofundi* may be a superior Haloarchaeon for survival on Mars [16].

Conclusions: Haloarchaea are remarkable microorganisms capable of growth at low temperature, anaerobically, with visible light and perchlorate, in hypobaric and desiccating conditions. They are UV and ionizing radiation tolerant and survive exposure to Earth's stratosphere, and represent excellent models for survival on Mars.

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