Characterization of perchlorate-resistant halophilic and halotolerant microorganisms from Big Soda Lake, Nevada, USA. T. Matsubara[1,2,3,4], K. Fujishima[1,5], C. W. Saltikov[3], S. Nakamura[4] and L. J. Rothschild[3,6], [1]Universities Space Research Association (USRA), NASA Ames Research Center, Moffett Field, CA 94035-1000, USA, [2]Advanced Studies Laboratories (ASL), University of California Santa Cruz – NASA Ames Research Center, Moffett Field, CA 94035-1000, USA, [3]Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA, [4]Department of Bioengineering, Tokyo Institute of Technology, Yokohama 226-8501, Japan, e-mail: matsubara.t.ag@m.titech.ac.jp, [5]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo 152-8551, Japan, e-mail: fuji@elsi.jp, [6]NASA Ames Research Center, Moffett Field, CA 94035-1000, USA, e-mail: Lynn.J.Rothschild@nasa.gov

Transporting materials from the Earth to Mars is very expensive as cargo capacity is limited and the launch cost is high because of the large energy requirement and infrastructure needed to overcome Earth's gravity. The high cost for a long-term settlement program by providing all materials from the Earth are impractical for living permanently off of the Earth. One of the ways to reduce the cost of supplies and increase the probability of mission success is the use of the native resources on Mars, known as *in situ* resource utilization (ISRU). Biotechnology offers a solution to the mass problem described above since a small initial mass of a single cell can replicate and grow using the resources around it. Recent advances in genetic engineering and synthetic biology have shown that humans are rapidly increasing the range of products capable of being produced. However, the Martian surface is highly arid and contains chloride salts; in addition, 0.4-0.6 (w/v) % (w/v is omitted below) perchlorates were found [1-3]. Thus, commonly used model organisms are not suitable for growth on Martian soil since they usually cannot grow in the presence of high concentration of salts, and perchlorate is toxic for most organisms. Further, there is not enough knowledge about perchlorate-resistant microorganisms and effects of perchlorate on microorganisms. Therefore, garnering this knowledge is important and beneficial for not only use on Mars but also to fully understand the ecological properties of halophilic and halotolerant microorganisms in general.

We screened perchlorate-resistant halophilic and halotolerant microorganisms from Big Soda Lake, Nevada, USA, and characterized them [4]. Perchlorate-resistant halophilic and halotolerant bacteria, BSL1, BSL2, BSL3 and BSL4 were isolated from Big Soda Lake. From the analysis of a rRNA-based phylogenetic tree, BSL1 shared the same root with *Bacillus licheniformis* (identity: 99.2%), BSL2 with *Bacillus pseudofirmus* (identity: 99.1%), BSL3 with *Halomonas salifodinae* (identity: 99.1%) and BSL4 with *Alkalibacillus filiformis* (identity: 99.1%).

The results of the salt resistance assay showed that BSL1 grew in the presence of 0 to 10% NaCl, while showing the highest OD_{600} at 24 h in the absence of NaCl suggesting BSL1 as a halotolerant bacterium. In contrast, BSL2, BSL3 and BSL4 grew in the presence of 2.5 to 10%, 2.5 to 17.5% and 7.5 to 15% NaCl, respectively, and all pointing that BSL2-4 are halophilic bacterium. Also, BSL3 showed the fastest growth in the isolates, reaching the highest OD_{600} in the presence of 7.5 or 10% NaCl.

The results of the perchlorate resistance assay showed that, BSL1, BSL3 and BSL4 can grow in the presence of variety of perchlorate salts at least 0.5%, concentrations which exist on Mars. The results of the perchlorate resistance assay in the presence of two-component perchlorate salts (calcium, magnesium and/or sodium perchlorate salts) has shown that BSL1-4 can grow at least in the presence of 0.5% mixed magnesium, sodium and/or calcium perchlorate, and the order of perchlorate resistance was BSL3 > BSL1 ≈ BSL4 > BSL2 based on the growth curves comparisons.

Therefore, some of the isolates could have potential to grow on Martian salts and perchlorates, and the search for life under these conditions is not only useful for ISRU on Mars, but also for use on perchlorate deposit on Earth.

References:

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