Identification and Characterization of Isolates Collected from the MSL at Kennedy Space Center

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Abstract

Planetary protection is governed by the UN under the Outer Space Treaty and the Committee on Space Research (COSPAR). One of the goals of Planetary Protection includes the practice of protecting planetary bodies from forward contamination by Earth life. Mars is considered a likely place to look for extraterrestrial life, given its proximity to Earth, the presence of carbon and other essential elements, and the presence of water in some form. Although our knowledge is growing, it is still unclear whether organisms from Earth, traveling on Mars-bound spacecraft, could survive and grow in a Martian atmosphere. The objective of our study is to determine if organisms on spacecraft pose a forward contamination risk.

Microbial isolates were collected from the Mars Science Laboratory (Curiosity) at Kennedy Space Center, prior to launch and tested under several extreme growth conditions. Conditions tested include high NaCl and pH, low temperatures, and anaerobic growth using various electron acceptors and carbon sources. Additionally, organisms were tested for their ability to persist after exposure to desiccation. It was determined that the majority of isolates belong to the *Bacillus* genus, although many of the organisms identified belong to non-spore forming genera. Most of the isolates can grow in media containing high concentrations of NaCl and in basic media. The majority of isolates were able to withstand desiccation for 2 weeks. Interestingly, many isolates identified as belonging to the same genus and species showed different levels of survival under the same conditions.

The data being generated from these and other studies provides much needed basic information regarding the types and survivability of microorganisms entering space as a consequence of space exploration missions. The knowledge gained from these studies will provide a better understanding of the actual risk of forward contamination, and may lead to improved spacecraft manufacturing and sterilization methods for future missions.

Introduction

Mars is a likely candidate in the search for extraterrestrial life, both past and present, since it contains the basic life such as carbon, potential energy sources, and water in some form. Conditions on Mars are thought to be analogous to those of early Earth. Studies on the ability of organisms to survive extreme conditions such as those found on Mars is one approach to investigating the potential for life beyond Earth, and to understand how life may have evolved on Earth.

Planetary protection of Mars is governed by NASA and international policy, since microorganisms transported on the surface of spacecraft to Mars could hinder the search for past or present life on Mars. This policy restricts the spacecraft's exposed surface areas, mated surface areas, and total encapsulated volume to a biobuden level of less than or equal to 5 X 10^5 spores and landed hardware to 3 x 10^4 spores (NASA NPR 8020.12D). During the build up of a spacecraft, such as MSL, a microbial sampling campaign is untaken to assess this bioburden actively throughout the mission build up and testing phases. The organisms that are enumerated from these campaigns are typically isolated and persevered for future study. There have been extensive studies to collect and understand the microbes that are associated with spacecraft surfaces such as that of the cleanroom facilities or ground support equipment (LaDuc et. al) however more studies looking at the microbes directly associated with the spacecraft are needed. Although studies have shown that many of the microbes originating from cleanrooms and support equipment are resistant to extreme conditions similar to those found on Mars, studies characterizing the ability of these organisms to utilize energy sources found on Mars are lacking. Understanding the biochemical potential and environmental tolerances of these organisms would directly provide insight into whether these organisms could survive under Mars conditions thereby enhancing the scientific knowledge base for predictive risk assessments for future missions.

The goals of our project are to identify organisms isolated from the surfaces of the Mars Science Laboratory (MSL) while at Kennedy Space Center (KSC) and investigate the potential of these organisms to withstand extreme conditions and utilize energy sources similar to those found on Mars. The information collected from this study should improve the knowledge base for predictive risk assessments for the survival of organisms to Mars and provide information as to whether organisms residing on the MSL are likely to survive Mars-like conditions.

Materials and Methods

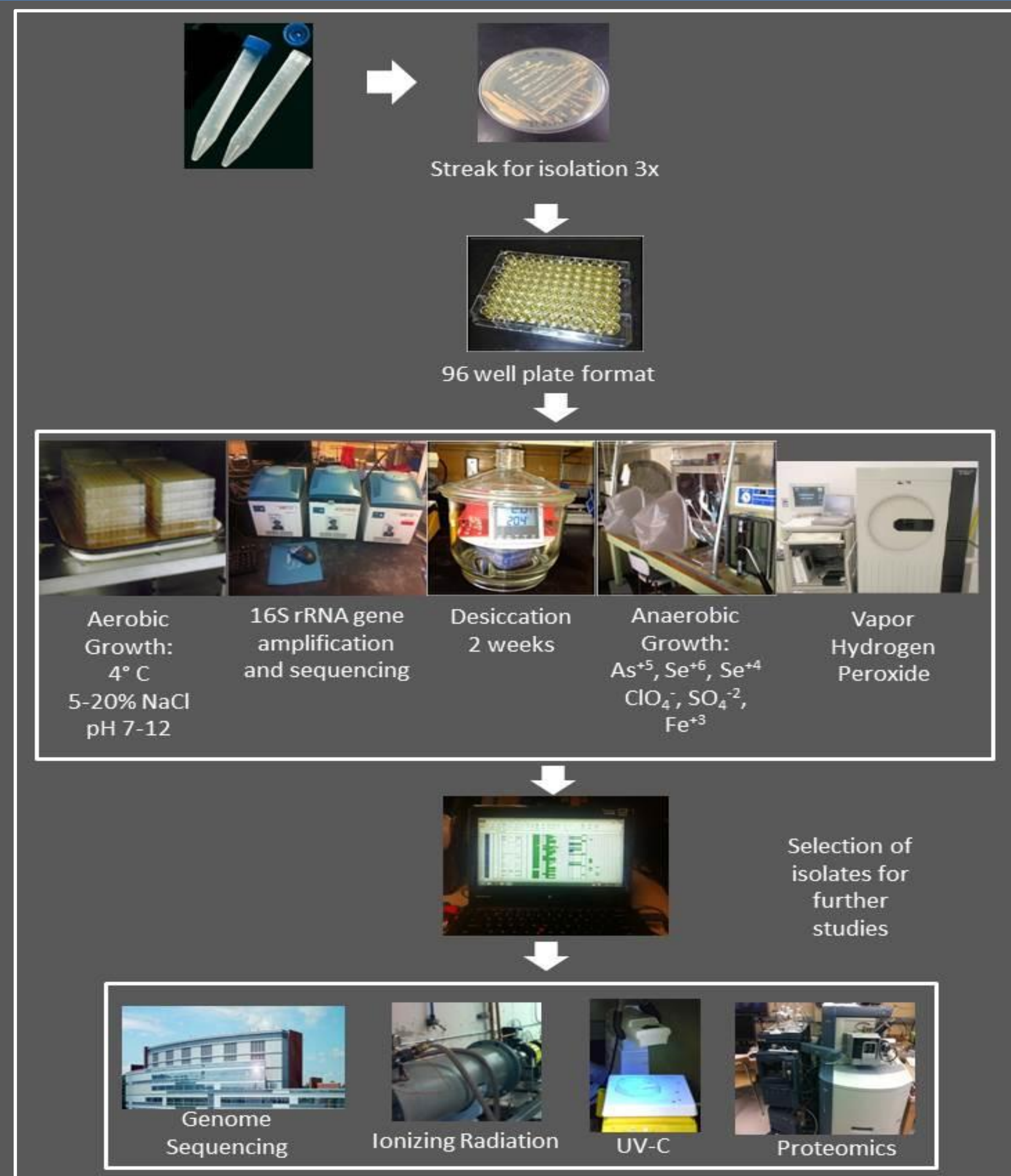

Figure 1: Process flow.

Table 1: Table of electron acceptor and donor sources used in anaerobic media (Modified ATCC #2106).

Media	Electron acceptor	Electron donor
1	Perchlorate (10 mM)	Acetate (20 mM)
2	Perchlorate (10 mM)	Lactate (20 mM)
3	Arsenate (10 mM)	Acetate (10mM)
4	Arsenate (10 mM)	Lactate (20 mM)
5	Selenite (5 mM)	Acetate (20 mM)
6	Selenite (5 mM)	Lactate (20 mM)
7	Selenate (10 mM)	Acetate (10mM)
8	Selenate (10 mM)	Lactate (20 mM)
9	Sulfate (50 mM)	Acetate (20 mM)
10	Sulfate (50 mM)	Formate (20 mM)
11	Fe (III) (80 mM)	Acetate (20 mM)
12	Fe (III) (80 mM)	Lactate (20 mM)

Figure 2: Launch of the Atlas V rocket carrying NASA's Mars Science Laboratory (MSL) in its payload. Courtesy: Scott Andrews/Canon. Photo Credit: NASA PHOTO NO: KSC-2011-8030

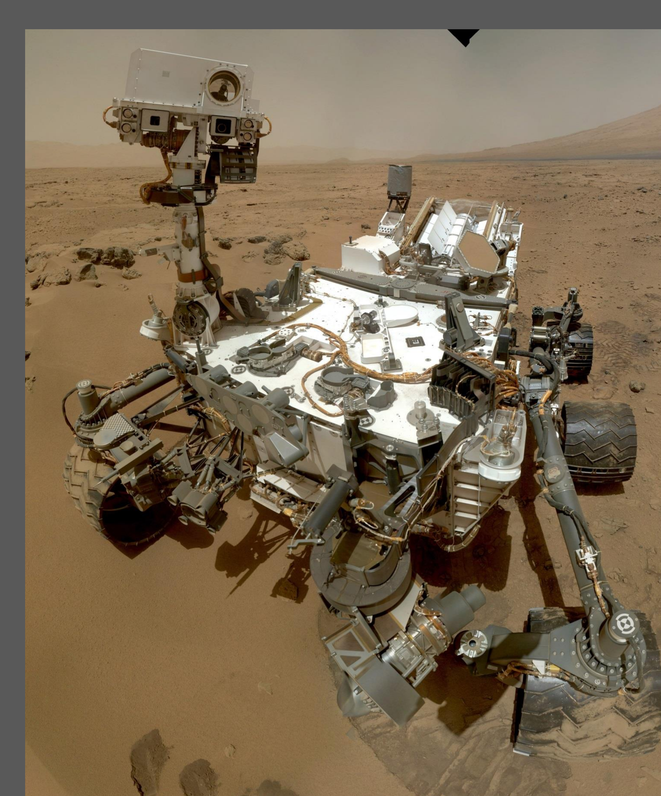

Figure 3: Curiosity self portrait. Image credit: NASA/JPL-Caltech/MSSS

Results

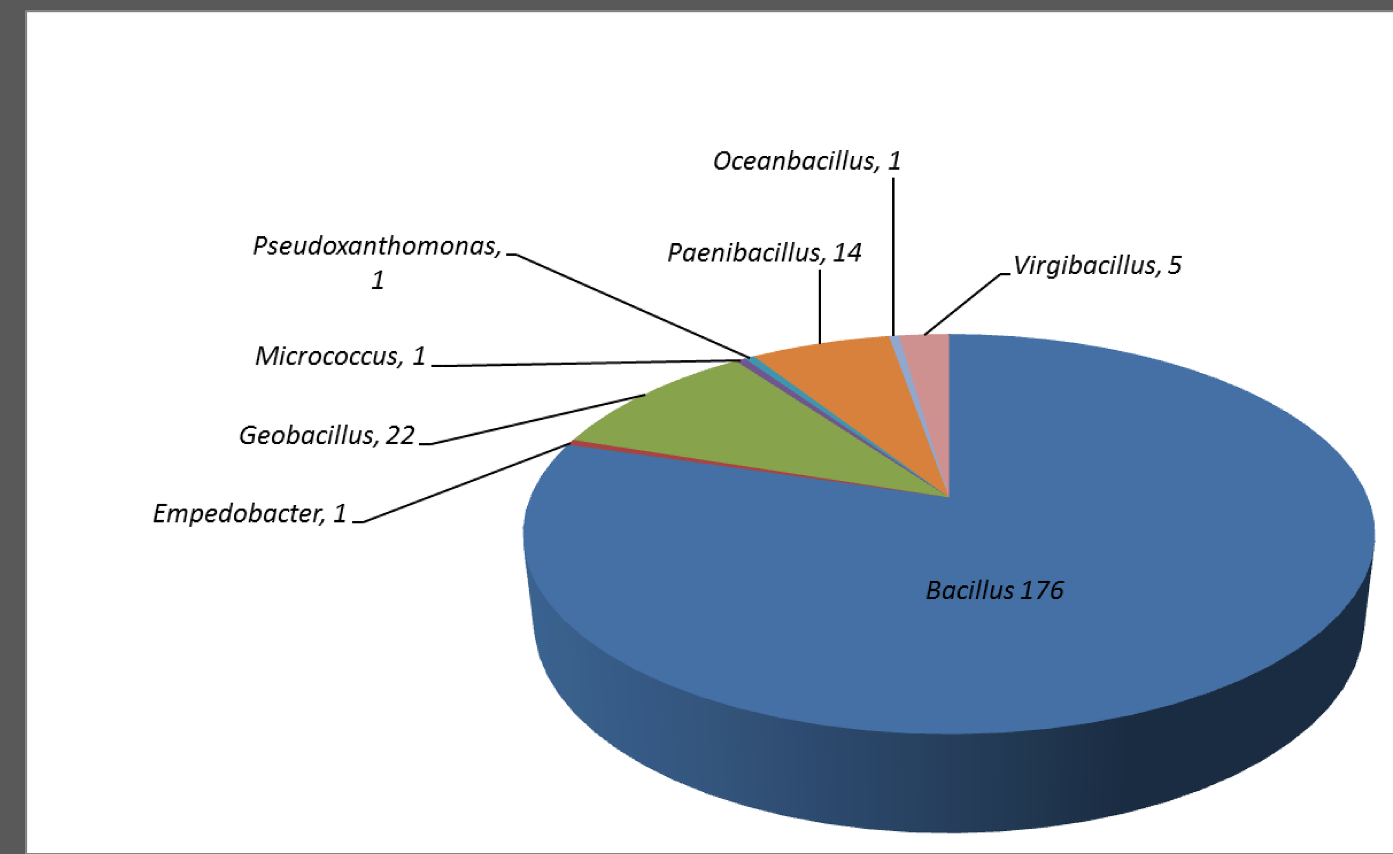

Figure 4: Identification of isolates by genus.

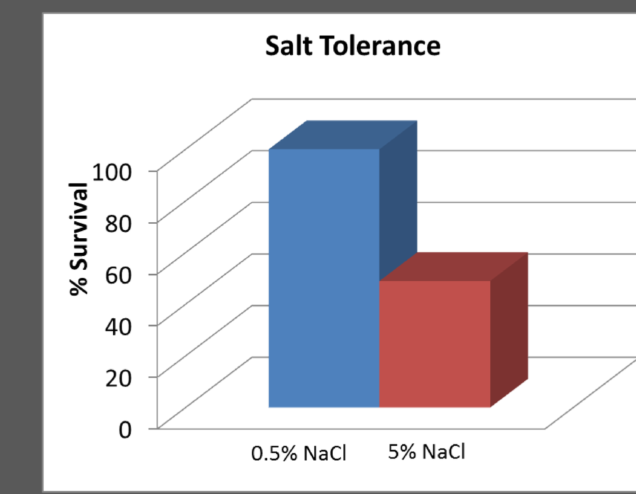

Figure 5: Growth of isolates in elevated salt.

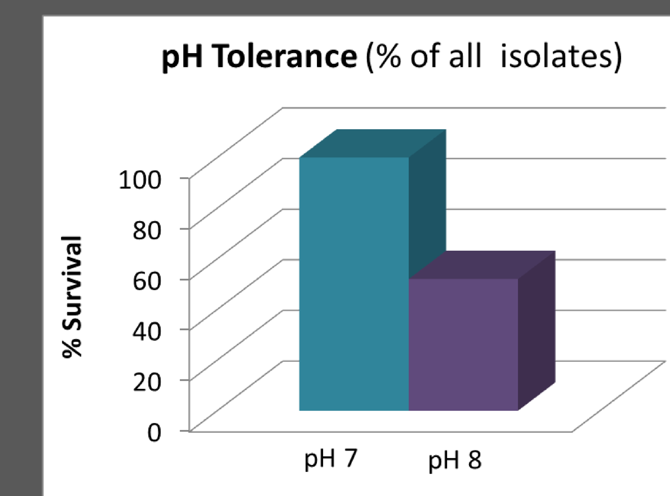

Figure 6: Growth of isolates in medium with elevated pH.

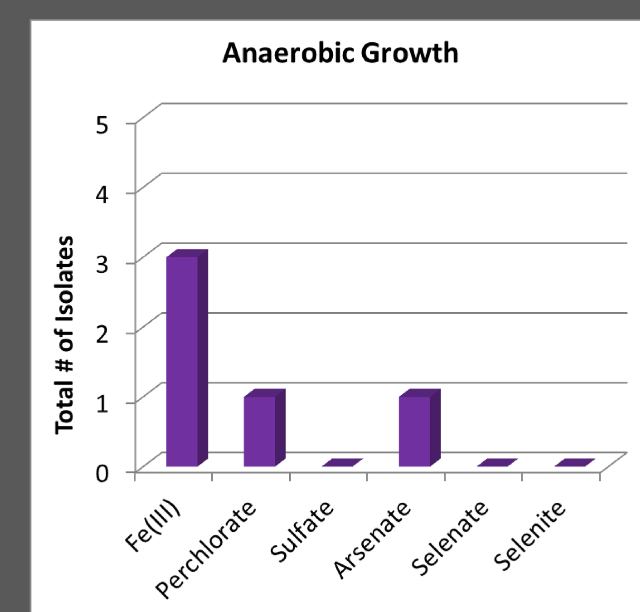

Figure 7: Number of isolates showing anaerobic growth.

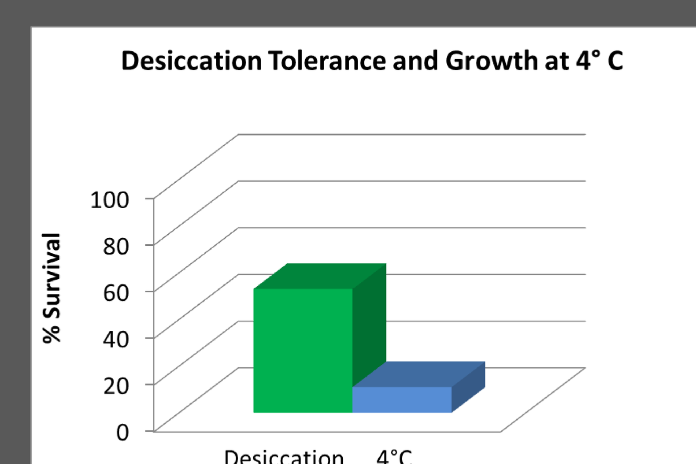

Figure 8: Number of isolates showing growth at low temperatures, and after exposure to desiccation and 5% H_2O_2.

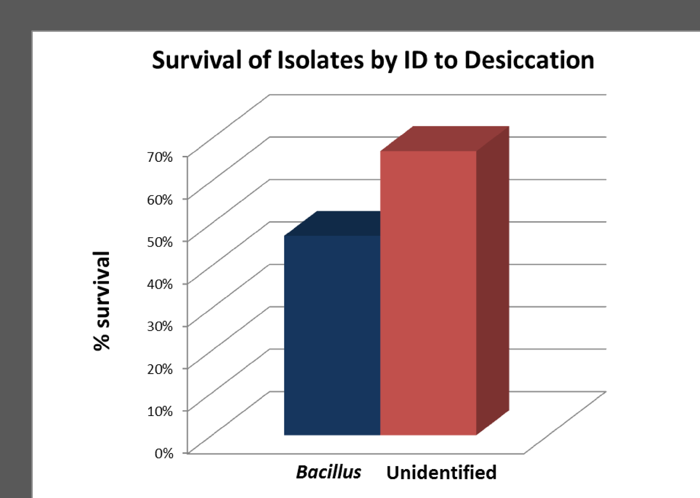

Figure 9: Isolates showing growth after a 2 week desiccation period by genus.

Conclusions

Bacterial isolates, collected from the MSL while at KSC, show a much higher proportion of *Bacillus* and related genera compared to isolates originating from the MSL while housed at JPL. This may be due to the difference in environment (i.e. brackish march environment vs. arid desert climate) or to differences in storage of isolates between the two facilities (i.e. glycerol stocks vs. slants). It should be noted that a higher percentage of the agar slants from KSC could not be cultured in comparison to the glycerol stocks obtained from JPL, so some selection for spore formers may have occurred which may explain why 30% of isolates collected from JPL are non-spore forming organisms while those collected from KSC appear to be almost exclusively spore forming organisms.

To date, approximately 1/3 of the isolates have been identified with another 2/3 still undergoing both growth studies and identification processes. However, preliminary results are indicating that the isolates aboard the MSL while at KSC are less hardy to extreme environmental conditions than those at JPL. Studies will need to be completed before a direct comparison between the 2 facilities can be made. Studies on vapor hydrogen peroxide and UV-C and ionizing radiation tolerance, to be performed shortly, should give more insight to the hardiness of these isolates and their ability to survive in a space environment. Proteomic experiments on the hardiest of isolates may provide insight into the ability of these organisms to survive extreme environments such as those found in space and on other planetary bodies.

Continuing identification of, and growth studies on, the remaining 757 isolates collected from the spacecraft while at KSC should help inform NASA on the probability of forward contamination of Mars or other planetary bodies. Ultimately, completion of these studies should point towards methods which can be utilized to improve cleaning and/or sterilization protocols in the future which would be needed for future life detection missions.

Selected References

1. Benardini, J.N., *M.T.* La Duc, *R.A.* Beaudet, *R.* Koukol . *(2014)* Implementing planetary protection measures on the Mars Science Laboratory. *Astrobiology*. 14(1):27-32
2. La Duc, M.T., M. Satomi, and K. Venkateswaran (2004) Bacillus odyssey sp. nov., a round spore forming bacillus isolated from the Mars Odyssey spacecraft. *Int J System Evol Microbiol* 54:195-201.
3. La Duc, M.T., A. Dekas, S. Osman, C. Moissl, D. Newcombe, and K. Venkateswaran (2007) Isolation and characterization of bacteria capable of tolerating the extreme conditions of clean room environments. *Appl Environ Microbiol* 73:2600-2611
4. NASA (2011) Planetary protection provisions for robotic extraterrestrial missions. NPR 8020.12. NASA, Washington, D.C.

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