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Testing interplanetary transfer of bacteria between Earth and Mars as a result of natural impact phenomena and human spaceflight activities

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Abstract

Interplanetary transport of microbes between Earth and Mars can be envisioned to occur either naturally as a consequence of impacts (lithopanspermia) or as a result of human and robotic spaceflight. In either case, the considerations for modeling successful transfer of microbial life are similar. The probability of microbes surviving either natural or human-mediated transfer is a function of: the initial population size and composition (i.e., the bioload); survival of launch, transit through space, entry and deposition; and ability to survive and proliferate on the recipient planet. Modeling this process for testing lithopanspermia and for mitigation of forward and back contamination for planetary protection purposes calls for accurate simulation of all aspects of transfer.

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1. Introduction

This short review is concerned with the interplanetary transfer of microorganisms, which is important from two broad and interlinked perspectives. First, there has been a recent upsurge of interest in the natural interplanetary exchange of microbes within rocks as the result of natural impact processes, variously dubbed “panspermia”, “lithopanspermia”, or “transpermia” [1–4]. Second, throughout the history of space exploration there has been much discussion, research and effort devoted to “Planetary Protection”, i.e., protecting Earth and other planetary environments from accidental or intentional microbial cross-contamination resulting from human spaceflight activities [5–8]. As we shall see below, these two fields of investigation share common

philosophical and practical linkages, consideration of which may serve to enhance our understanding of both.

1.1. Natural interplanetary transfer of microbes

Even though the notion of panspermia has been around for centuries (for historical review see [8]), until relatively recently it was thought that microorganisms could not survive the physical forces imposed during planetary transfer, thus the planets were biologically isolated. However, within the past two decades the existence on Earth of meteorites which originated from Mars has been recognized [9–12]. These meteorites represent fragments of martian crust which were ejected into space by large impacts, traveled through space, and landed on Earth. Their existence has sparked a renewed interest in the possibility of lithopanspermia, especially in testing the hypothesis that microbial passengers within meteors could survive the journey [1–4] (see below).

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1.2. Interplanetary transfer of microbes by human spaceflight

Humans are beginning to leave Earth and to expand our presence onto other planets and moons within the solar system, first using robotic probes (fly-bys, orbiters, landers, and rovers), followed by human exploration and colonization missions. Because microorganisms are ubiquitous in Earth's environment, humans leaving Earth to explore space carry along a large host of terrestrial microorganisms as accidental contaminants of spacecraft surfaces, and also as integral and beneficial components of biological life support systems and the astronauts themselves. The stresses placed upon microorganisms during transfer by human and robotic spaceflight are much gentler than those imposed by natural impacts; hence the probability of surviving human-mediated transfer is much greater. Because at least two immediate exploration targets, Mars and Europa, may harbor evidence of past or present life, one of the major challenges facing mission planners and explorers alike is protection of these pristine environments from "forward contamination" by terrestrial microorganisms, as well as "back contamination" of Earth environments by potential extraterrestrial microbes. Planetary protection is based on a UN Treaty and is regulated by guidelines established by the Committee on Space Research (COSPAR). Specifically, an international treaty has been established to prevent the introduction of microbes from the Earth to another celestial body or vice versa, whether this occurs intentionally or unintentionally (UN Doc. A/6621, December 17, 1966 and follow-on agreement UN Gen. Ass. Resol. A/34/68, December 5, 1979). Based on this treaty, a concept of contamination control has been elaborated by COSPAR under consideration of specific classes of mission/target combinations and recommended to be followed by each space-faring organization (e.g., see [13]).

2. Results and discussion

2.1. Probability theory for microbial survival

In considering interplanetary transfer of microbes by either natural or artificial processes, many of the same fundamental concepts of microbiology apply. First, from the field of microbial ecology we know that microbes are found in environmental samples as diverse populations of cells representing several distinct species, and each species exhibits its own characteristic survival properties. Second, from the field of sterilization/disinfection we know that the inactivation of

microbial populations by any particular physical stress is stochastic; hence we must immediately start our analysis by thinking in terms of the *probability* that a proportion of cells in a population will survive a given treatment. Therefore, in order to construct meaningful models of interplanetary microbial transfer we must obtain, as accurately as possible, several key pieces of information from each of four major categories: (i) What is the size, composition, and survivability of the initial microbial population? (ii) What are the types, magnitudes, and durations of the biocidal factors to which the populations will be exposed during launch, cruise phase, atmospheric entry, and deposition on the target planetary bodies? (iii) What fraction of the population would likely survive each biocidal factor? (iv) What is the likelihood that cells surviving transfer could proliferate in their new environment? Based on these premises, semi-empirical estimations of the probability of microbial interplanetary transfer by natural processes [2,3] or spaceflight [6] have been attempted.

2.2. Studies using model organisms

The major weakness of the above mathematical survival models lies in the scarcity of hard experimental data for several of the terms of the probability calculations. To remedy this situation, recent efforts in our labs and by others have been directed towards defining bacterial survival after application of robustly-simulated environmental stresses, using model organisms. Because bacterial spores are notoriously resistant to inactivation and are frequently-encountered inhabitants of both natural and man-made materials, spores of the model laboratory bacterium *Bacillus subtilis* have been studied extensively in this regard [1,4,7]. Other less-well studied model organisms include various spore-forming bacteria from the interior of rocks [14,24], those found contaminating spacecraft or their assembly facilities [15–18], the radiation-resistant bacterium *Deionococcus radiodurans* [19–21], halophilic archaea [22], and even lichens [23].

2.3. Initial bioloads

The initial population is often referred to as the "bioload", broadly defined as the total viable microorganisms in a given unit of material (e.g., gram of rock, square centimeter of spacecraft surface). The bioload is an ideal number practically impossible to obtain precisely, and which therefore must be estimated using a number of sampling methods with extrapolation. The following example illustrates some of the

difficulties involved in estimating initial bioloads of objects. We sampled the interior of near-subsurface granite from the Sonoran desert (Arizona, USA) and obtained $\sim 10^4$ culturable bacteria per gram of rock, of which $\sim 5 \times 10^2$ ($\sim 5\%$) were spores [24]. But what is the true concentration of potential passengers inside this rock? Immediately we encounter problems. It is known that only a fraction of bacteria (the usual number cited ranges from $<0.1\%$ to 1%) from environmental samples such as subterranean rock are culturable [25], but it is difficult to determine that fraction with precision. Nevertheless, using the above assumption we arrive at an estimate of $\sim 10^6$ – 10^9 total cells and $\sim 5 \times 10^4$ – 5×10^6 spores per gram of this particular granite sample. Mathematical impact models can be used to obtain estimates of the total number of impacts striking a biologically-inhabited zone per unit time, and to compute numbers and mass distribution of ejected fragments predicted to result from an impact of a given size, speed, and angle [2,3]. From these pieces of information the initial microbial population potentially launched into space can be estimated, but admittedly with a large margin of error. In a similar fashion, the initial bioloads of spacecraft before launch can be estimated after sampling various areas, with many of the same caveats [6,26]. Under present NASA planetary protection guidelines, robotic landers not carrying life-detection packages destined to land on Mars must contain fewer than 3×10^5 viable endospores per vehicle [5]. However, the total microbial bioloads on spacecraft, including spore-formers, non-spore formers, and non-culturable species, likely will be one to two orders of magnitude higher than this level based on models for the launched bioloads of unmanned robotic landers [26] and recent work with detecting non-culturable microorganisms on spacecraft surfaces [17,27]. Addition of astronauts to a spaceflight results in a dramatic increase in the microbial bioload, both within the spacecraft and indeed as a component of the astronauts themselves. Although the true number is not known with certainty, it is estimated that the human body contains about 10^{14} cells, of which 50–90% are non-human microorganisms!

2.4. Surviving launch from the donor planet

The only natural event energetic enough to eject pieces of planetary crust into space is a high-speed (10–20 km/s) impact by an asteroid or comet [1,3]. The physics of the ejection process have been extensively modeled [1,3]. Briefly, in the vicinity of the impact site lies a spallation zone in which near-surface rocks are

ejected at relatively low shock pressure and heating but at high speed. Experiments replicating shock and heating alone [28], acceleration alone [21], or all forces applied together [20,29,30] indicate that a fraction of spore and vegetative cell populations can survive the forces of an impact-driven launch. Of course, human and robotic spacecraft are launched under much gentler conditions than an asteroid impact, thus it would be expected that essentially all microbial passengers present on and within spacecraft would survive launch.

2.5. Surviving space transit

Once boosted into space, microbes on or within rocks or spacecraft would be subjected to an entirely different set of environmental stresses, namely extreme vacuum, desiccation, solar and cosmic radiation, microgravity, and both extreme hot and cold temperatures. Of these factors, solar UV is the most immediately lethal, but is relatively easily shielded [7]. Spaceflight experiments have shown that with minimal shielding a number of types of bacteria, archaea, and viruses can survive at least short-term (weeks to months) exposure to space in low Earth orbit; most notable among these are *B. subtilis* spores, which survived nearly 6 years in space on the Long Duration Exposure Facility (LDEF) [1,31]. However, in the space environment microorganisms would be unable to maintain metabolic and cellular repair activities, leading to inexorable degradation of their biological molecules and stochastic inactivation [1,3,4,32]. Under these conditions, transit time in space becomes an important factor. Despite the fact that fast meteorite transits (a few months to a few years) between Earth and Mars are theoretically possible [33], the actual collection of martian meteorites spent from ~ 0.6 to 15 million years in space traveling from Mars to Earth [11,12]. Models using thermal and radiation inactivation kinetics suggests that these extremely long transit times limit the probability of viable microbial transfer [34,35]. In contrast, spacecraft fly along trajectories carefully calculated to transfer between Earth and Mars in 6–8 months, dramatically increasing the probability of microbial survival to space transit. In addition, human spaceflight would provide a protected habitat for microbes on the journey inside the spacecraft and within the astronauts themselves. However, recent work has demonstrated that high vacuum encountered during the cruise phase to Mars can impart some biocidal effects on spacecraft contaminants [16,36–38]. For example, the viability of endospores of *B. subtilis* exposed to interplanetary pressures (3×10^{-12} mb) can be reduced approximately 1 order of magnitude

during the 6–8 months cruise phase to Mars [6]. Although the data is less plentiful for non-spore forming species [36,39,40], populations of non-spore forming species are reduced to an even greater extent than spore formers during long-term exposures to interplanetary vacuum; perhaps by as much as 1–3 orders of magnitude of lost viability during the cruise phase to Mars.

2.6. Surviving atmospheric entry and deposition

Although the physics of high-speed meteorite and spacecraft entry through the atmosphere is quite well understood [41,42], the survival of microbes during this last phase of transfer has received the least amount of experimental attention. Frictional heating melts the surface of the meteorite and aerodynamic forces ablate the liquid rock, carrying away the heat with it. Because of the short transit time through the atmosphere (a few tens of seconds), apart from a thin (<1 mm) fusion crust at the surface, the interiors of meteorites are not heated significantly [1,41,43]. We have recently demonstrated that substantial numbers (up to 13%) of minimally-shielded *B. subtilis* spores deposited on the surface of artificial granite meteorites were able to survive ballistic space flight on sounding rockets, including two high-speed atmospheric passages of 1–2 km/s during ascent and descent [30,41], suggesting that microbes buried deeper within rocks would probably survive the entry phase in high numbers. Impact with the surface at the end of the

flight would occur at terminal velocity, roughly 50 m/s for Earth and 300 m/s for Mars; the forces generated during final impact would be quite modest compared to those prevailing at launch, and would further serve to intimately mix the meteorite material with the martian or Earth regolith [1]. In contrast to entry of natural meteorites, robotic (and future human) flights employ various protective devices (heat shields, deceleration rockets, parachutes, air bags) in order to accomplish “soft landings” on Mars, thus maximizing microbial survival to planetary entry and landing. The only losses in the viable bioloads during reentry on spacecraft would occur on those areas of the vehicles directly exposed to the heat of reentry. In the case of spacecraft, this would comprise the thin outer layers of the heat shield that are ablated away during atmospheric descent. However, these losses of viable bioloads are minor compared to the overall microbial populations present within the protected regions of the spacecraft.

2.7. Surviving and proliferating on Mars

For microbial cross-contamination of planets to be ecologically relevant, upon arrival the interplanetary travelers must be able to both survive and proliferate in their new environment. Certain types of microorganisms exist on Earth, called extremophiles, which exhibit an astounding capability for growth and survival under extremes of temperature, pH, salinity, pressure, etc.

Table 1
Comparison of surface conditions on Earth and Mars^a

Environmental parameter	Earth	Mars
Solar UV spectrum at surface	$\lambda \geq 290$ nm	$\lambda \geq 190$ nm
Solar constant	1368 W/m ²	590 W/m ²
UVC + UVB flux at surface	~ 2 W/m ² (UVB only)	0.37–8.6 W/m ^{2b}
Solar particle events (SPE)	None	Up to 0.1 Sv/h
Galactic cosmic radiation (GCR)	1–2 mSv/yr	0.1–0.2 Sv/yr
Mean atmospheric pressure	1013 mb (10 ⁵ Pa)	7.1 mb (710 Pa)
Atmospheric shielding	1000 g/cm ²	16 g/cm ²
Atmospheric composition	78.1% N ₂	95.3% CO ₂
	20.9% O ₂	2.7% N ₂
	0.0375% CO ₂	1.6% Ar
	Variable % H ₂ O	0.1% O ₂
		0.03% H ₂ O
Surface air temperature range	–89 to +58 °C	–125 to +20 °C
	Mean = +15 °C	Mean = –61 °C
Gravity	1 × g	0.38 × g
Day length	23 h 56 min 4.1 s	24 h 37 min 22.7 s
Year length	365 days	687 Earth days
Glow discharge	No	Likely
Globally distributed oxidizing soil	No	Likely

^aAdapted from Schuerger [6].

^bVaries depending upon atmospheric dust load.

In addition, recent sampling of spacecraft destined for Mars and their assembly facilities has uncovered a number of spore-forming *Bacillus* species whose spores exhibit a high degree of resistance to harsh environments prevailing in space and on Mars [6,15,17,18,27]. However, “extreme” from the terrestrial perspective could be rather mild compared to the martian environment. The surface environments of Earth and Mars are compared in Table 1. As can be seen, the martian environment presents a number of obstacles which could be potentially lethal or at least inhibitory to Earth microbes, such as high solar UV flux, low atmospheric pressure, scarcity of liquid water, low or absent organic nutrients, extreme cold, and potentially highly-oxidizing soils (Table 1), to name but a few. Recent experiments have been directed toward testing the ability of a number of terrestrial bacterial species to survive and grow under robustly-simulated Mars environmental conditions. The results suggest that it is very difficult, if not impossible, for unshielded Earth bacteria, even hardy spores found on spacecraft surfaces, to survive for more than a few minutes, hours, or days of exposure to the full suite of Mars surface conditions including UV [6,16,44]. However, the possibility exists that Earth microorganisms could survive and proliferate in protected refugia that might provide somewhat more clement conditions than those prevailing on the martian surface. Current experiments in our labs are directed towards defining which martian environmental parameters limit bacterial growth, and understanding the molecular and cellular basis of these limitations. Preliminary experiments in our labs [45] with 13 species of spore-forming and non-spore forming bacteria suggest that many terrestrial microorganisms found on spacecraft surfaces may not be able to grow under the low pressure and low temperature conditions found on Mars, but much further work is needed in this area.

3. Conclusions

The ancient environments of Earth and Mars may have been more closely similar than they are today; indeed, evidence has been steadily accumulating that ancient Mars may have had a thicker atmosphere and a warmer and wetter past [46,47]. This early period in the evolution of the two planets and the origin of life on Earth coincided with the final stages of planetary accretion, the period of heavy bombardment, when there was active exchange of ejected surface rocks between the two planets. These observations have sparked the speculation that, if life originated on Earth or Mars, it could have spread to the other planet by natural

impact processes. This scenario predicts that Mars life, if it exists today, may share its ancestry with present-day Earth life, hence may be demonstrably related both genetically and biochemically. Indeed, currently planned life detection mission experiments rest on the basic assumption that present or past Mars life will leave traces recognizable by terrestrial biochemists and microbiologists [48]. However, since the time of their formation, Earth and Mars have embarked on divergent evolutionary pathways to the point that their present environments are dramatically different. It stands to reason that Earth and (putative) Mars life also evolved to adapt to the changing environmental conditions on their respective planets. Current results suggest that present-day terrestrial microbes may encounter severe difficulties surviving and proliferating in the present-day Mars surface environment; the logical extension of this reasoning is that Mars microorganisms might also have a difficult time prospering in today’s Earth environment.

After a journey of nearly 12 million years in space, one of the most famous martian meteorites, Nakhla, fell on June 28, 1911 at 9 A.M. in northern Egypt [11; www.meteorlab.com]. Thus, natural interplanetary exchange of rocks continues to this day, albeit at a rate much reduced from the period of heavy bombardment. In contrast, within the past four decades the number of human-directed flights from Earth to Mars has increased dramatically, and two-way traffic is planned for the near future with upcoming robotic sample return and human missions. Since 1962, nearly 40 robotic missions (flybys, orbiters, landers or rovers) have been launched with Mars as the destination. Many of these missions either missed Mars, crashed onto the martian surface, successfully landed, or landed and then drove over the martian terrain. Despite our best precautions, there is little doubt that a small but poorly constrained number of Earth microbes have already traveled to Mars. With the planned advent of human missions to Mars, the number of microbes taking this journey will likely jump by several orders of magnitude. Because of the undisputed impact of microbial life at every level, ranging from astronauts’ personal health to global ecological and geological processes, understanding the interplanetary transfer and fate of microbes is, and will continue to be, an essential part of human space exploration.

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