Discover Space

Brief Communication

Partial protection of *Bacillus subtilis* spores on simulated Martian media enhances survival against UV radiation

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Abstract
Mars is known to have once sustained an environment that may have been habitable. This idea has often been combined with theories of panspermia, to suggest that any life on Mars may have had a common origin with life on Earth. These ideas typically involve meteorites produced as impact ejecta as vectors for the transport of life between planets. However, such meteorites would be subjected to the harsh conditions of space, most notably, Solar UV radiation. Some Gram-positive bacteria generate endospores, which provide protection from extreme conditions and are common models for understanding bacterial survival in space. In this study, the spore forming bacteria *Bacillus subtilis* and the non-spore former *Staphylococcus aureus* were used to investigate the survival of bacteria exposed to UVB and UVC radiation on simulated Martian regolith. Spore survival was limited upon excessive doses of UVB and UVC radiation, although this was minimised when spores were covered with lysed bacteria which provided partial protection. These results build upon previous studies and suggest that lysed bacteria from the same bacterial species might offer spores on Martian meteorites a partial shield from UV radiation and enhance their viability in panspermia models.

1 Introduction

The transport of simple microbes from Martian meteorites to Earth (a process known as Panspermia), offers a theory for the origins of life independent of abiogenesis on Earth [1–3]. Similarities between Earth and an ancient Mars, Martian permafrost, and topographical imagery from various Mars missions suggest that Mars once had the capacity to sustain life [4–7]. These features lend credibility to the suggestion that ejecta from Mars may have transported simple microbes to Earth via Martian meteorites.

Investigations into the robustness of simple microbes are of interest for investigating the possibility of panspermia, but also ensuring life on Earth does not contaminate other celestial bodies as a by-product of space exploration [8–12]. Bacteria referred to as 'spore formers' undergo a process called endosporulation which creates a thick, protective wall around the cell's DNA, providing protection from exposure to harsh environmental conditions such as detergents, temperature, desiccation, and exposure to UV radiation [13, 14]. Due to this, the spore forming bacterium *Bacillus subtilis* is an ideal model for understanding the survival of microbes in space. This has seen *B. subtilis* spores subjected to UV exposure in the vacuum of space [8], and used as a model for various space UV radiation simulations [8–12]. Furthermore, simulations that assess the survival of *B. subtilis* spores under the pressure of departure via Martian ejecta, including their

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arrival on ejecta to Earth suggest that these spores could survive these extreme events [15–17]. These studies suggest that if Mars had once harboured life, then Earth and Mars may share a common ancestor.

This study aimed to address the survival of *B. subtilis* spores partially shielded by lysed bacteria of the same species as a potential barrier from UV radiation in space. Using simulated Martian regolith media (media with similar chemical composition to the surface of Mars) and exposure to UVB and UVC radiation, our results show that *B. subtilis* spores can withstand greater fluence of UV radiation if partially shielded. This adds important data to this field and complements previous studies that have investigated the feasibility of meteorites harbouring life [16, 17]. Although spores on Martian meteorites are likely to experience substantial doses of UV radiation, the partial bacterial barrier as outlined in this study could limit the consequences of such exposure, hence enhancing the possibility of panspermia from Mars to Earth.

2 Materials and methods

2.1 Cultivation of bacterial strains and extraction of *B. subtilis* spores

Strains used in this study included *S. aureus* ATCC25923 and *B. subtilis* FUHAC10 (obtained from the Federation University culture collection). Both strains were grown overnight in nutrient broth (NB) (ThermoFisher) with vigorous shaking at 37 °C then maintained on nutrient agar (NA) plates. *B. subtilis* spores were extracted using similar methods as outlined by Tauscher et al. [12]. In short, *B. subtilis* was incubated overnight by shaking at 37 °C in Schaeffer Sporulation Medium (SSM) [18]. Culture was then centrifuged at 10,000 g for 10 min at room temperature for harvesting and purified using the wash method described by Nicholson [19]. Following this, cells (and associated spores) were heat shocked at 80 °C for 10 min to destroy vegetative cells, and remaining spores were transferred to ddH20 for storage. Spores were assessed by phase-contrast microscopy for purity and viability was determined by incubating extracts on NA plates grown overnight at 37 °C. For Martian regolith medium, the chemical composition of Martian regolith as described by Seiferlin et al. [23] was used (Table 1), with 1.5% nutrient agar added to make solid media (ThermoFisher).

2.2 UVB and UVC exposures

Exposure of bacterial strains to UVB and UVC radiation was achieved by using an Opsytec BS-02 irradiation chamber and dosage was monitored by using an Opsytec UV-MAT dosage controller (Opsytec). Exposures were performed for each bacterial strain on each media at UVB and UVC in separate exposures using 20 small agar plates (35 mm) per exposure. Each experiment was repeated three times, and the average of each exposure was calculated. For exposures on NA agar plates, strains were inoculated overnight at 37 °C on NA agar for single colonies and exposed to radiation. Post exposure, plates were left to rest in standard laboratory conditions for one hour before 10 single colonies from each plate was transferred to fresh NA plates, grown overnight at 37 °C and checked the following morning for growth. Spores were counted at 5.1×10^8 mL^{-1} ($\pm 1.0 \times 10^8$) then assessed by inoculating NA plates with 50µL droplets of purified spores prior to exposure. The values in Figs. 1 and 2 represent plates that exhibited growth post exposure after each dose of radiation rather than on a cellular basis. For experiments where spores were covered with lysed bacteria, a slurry of irradiated *B. subtilis* culture which had been centrifuged at 10,000 g for 10 min at room temperature to remove excess liquid was

Table 1 Martian regolith medium based on chemical composition of JSC Mars-1 reported in [23]

Chemical	%
SiO_2	43.5
$Al2O_3$	23.3
TiO_2	3.8
$Fe2O_3$	15.6
MnO	0.3
CaO	6.2
MgO	3.4
K_2O	0.6
Na_2O	2.4
P_2O_5	0.9

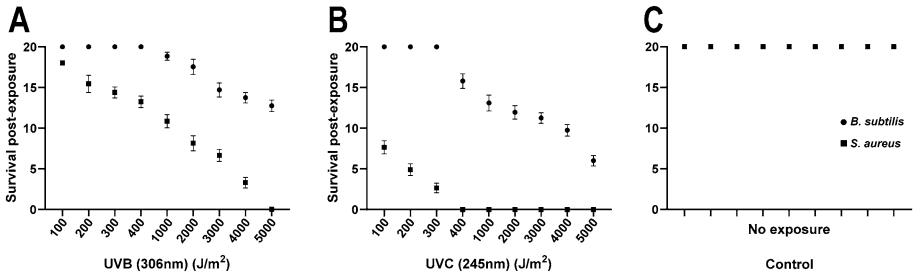

Fig. 1 Survival of *B. subtilis* and *S. aureus* upon UV exposure: **A**, **B** *B. subtilis* spores and *S. aureus* on normal growth media exposed to UVB and UVC radiation. **C** Control plates that were unexposed and at standard laboratory light conditions. X axis represents UV dose and no dose on control plates. Error bars = SDM. N = 30

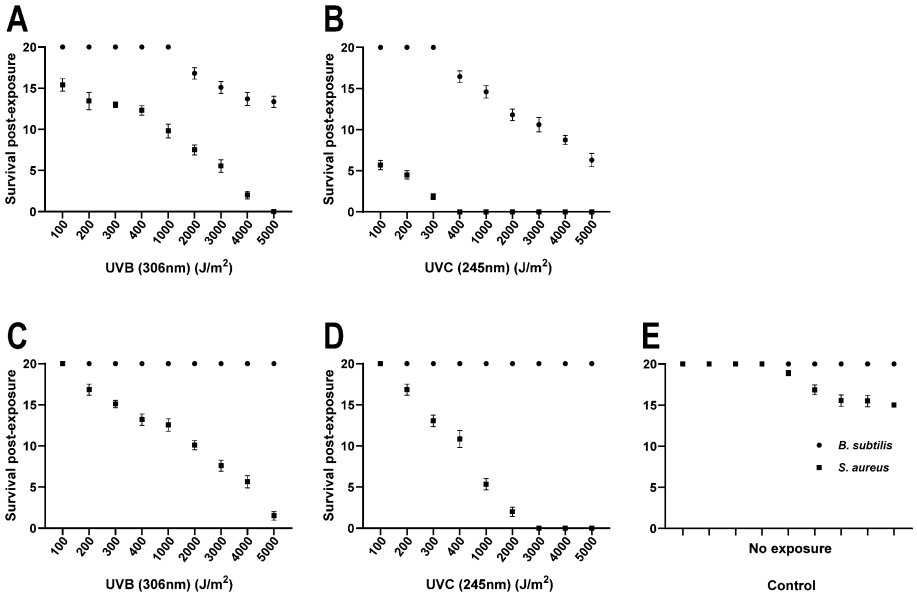

Fig. 2 Spore survival in the presence of vegetative bacterial cells on simulated Martian media: **A**, **B** *B. subtilis* spores and *S. aureus* on a Martian simulated media exposed to UVB and UVC. **C**, **D** *B. subtilis* spores and *S. aureus* on a Martian simulated media protected with 300 µl of pre-lysed bacteria then exposed to UVB and UVC. **E** Control plates unexposed on Martian regolith media. X axis represents UV dose and no dose on control plates. Error bars = SDM. N = 30

placed on top of the spore droplets to provide a partial shield from UVB and UVC radiation. The slurry was observed via microscopy to contain a mix of intact cells and cell debris. To ensure that the irradiated culture was non-viable, samples were lawn plated on NA agar to check for growth before proceeding. Control plates received the same treatment as the irradiated samples but were not exposed to radiation.

2.3 Statistical analysis and software

Construction of graphs and statistical analysis was performed using the Prism 5 software package (Graphpad). Figures were processed using Adobe Photoshop and Adobe Illustrator was used for annotation (Adobe).

3 Results

3.1 Endospore viability upon exposure to UVB and UVC radiation

The Earth's atmosphere blocks harmful UVC and most UVB radiation emitted by the Sun, although no such barrier exists in space [20]. To determine the germicidal influence that UV radiation has on the survival of spore and non-spore forming bacteria, endospores from *B. subtilis* were extracted and exposed to UVB and UVC radiation (Fig. 1A, B). This was performed alongside the non-spore former, *S. aureus,* which served as a control for the effectiveness of endospores in the presence of UV radiation. Due to the high germicidal properties of UVC, adding UVB exposure, which has milder germicidal activity [21] would ideally show trends in endospore decline that might not be as readily apparent with UVC

exposure alone. Unsurprisingly, UVC radiation had greater germicidal properties on both bacterial strains when compared to UVB exposure (Fig. 1A, B). Whilst this was expected due to the higher germicidal properties of UVC radiation, these results of concurrent UVB and UVC exposure show a similar trend to that observed in previous studies [8, 9, 11, 12, 21]. This suggests that this approach is ideal for assessing the influence of UVB and UVC on bacteria which could be used to simulate Solar UV exposure in space.

3.2 Martian regolith as a medium for endospore survival:

Previous studies have assessed the suitability of different bacteria in simulated space UV radiation conditions, although many of these have used conventional liquid or agar growth mediums, or spacecraft simulated agar as the bacterial environment [12, 22]. To address the conditions that bacteria on Martian ejecta might experience, a simulated media was generated based on the JSC Mars-1 Martian regolith research media (Table 1) [23]. Neither bacterial species inoculated on this medium were able to reproduce, suggesting that the Martian regolith medium is not ideal for sustaining growth of either of these bacteria. This could be due to the elevated levels of SiO_2 and Al_2O_3, which are present at toxic levels for normal bacterial survival [24, 25]. Simulated Martian regolith has shown to have no effect on *B. subtilis* spores [26], and control plates of spores placed on Martian regolith media that were exposed to normal laboratory light all germinated when moved to normal growth medium (Fig. 2E). Slight toxicity was observed in control plates of *S. aureus* over longer durations, showing that Martian regolith is not favourable for sustaining non spore-forming bacteria (Fig. 2E). Collectively, this suggests that although bacteria cannot thrive on Martian regolith media, *B. subtilis* spores can withstand conditions that are otherwise toxic for a non-spore forming bacterial species.

Since the qualities of Martian regolith, coupled with high doses of UVB and UVC radiation, would limit bacterial survival, we hypothesised that spores might be able to withstand these extreme conditions if partially protected from UV radiation. Indeed, lysed bacteria of the same bacterial species were added as they may serve as a shield from UV exposure. To assess this, *B. subtilis* spores were extracted, placed on Martian regolith medium and covered with a 300 µl slurry of pre-lysed bacteria prior to exposure to UVC and UVB radiation (Fig. 2). After exposure, spores (and bacteria for *S. aureus*) were transferred to normal growth medium to assess viability. Interestingly, spores that were subjected to doses of UVB and UVC radiation germinated after being transferred from Martian regolith media to normal growth media (Fig. 2C, D). In contrast to this, unprotected spores on Martian regolith media that were exposed to the same UV radiation failed to germinate at doses as low as 400 J/m^2 UVC and 1000 J/m^2 UVB (Fig. 2A, B). The UV barrier provided by the lysed bacteria did provide a short term means of protecting the non-spore former *S. aureus* from UVC radiation. This mild protection for non-spore forming bacterium is in a similar trend as reported for the non-spore forming halophilic archaea *Halorubrum chaoviator* and cyanobacteria *Synechococcus nägelli*, which have both shown low rates of survival after two years exposure to the vacuum of space on the EXPOSE-R facility on the International Space Station [27]. Although *B. subtilis* spores were more robust post-exposure than *S. aureus* vegetative cells, these data indicate that spores that form within sufficient vegetative growth of *B. subtilis* are viable after exposure to normally lethal doses of UV radiation due to UV shielding by other bacterial cells.

4 Discussion

Successful transfer of bacteria from meteorites of Martian origin to a primordial Earth may be feasible, though there are several variables that would impact cell viability. For example, any bacteria capable of inter-planetary transfer would likely be spore formers that phenotypically match the conditions of an early Archean Earth. Several studies have investigated the effect of UV radiation on *B. subtilis* in space [8–12, 26], while some investigations have employed extremophilic bacteria such as *Deinococcus radiodurans* [28, 29]. However, *D. radiodurans* has an extremely efficient DNA damage repair system, as well as other metabolic advantages suggesting it is not a simple base microbe (Zahradka et al., 2006). Therefore, bacterial panspermia approaches that utilise simple spore forming microbes are more amendable models for such studies.

Candidate bacteria would require protection from UV radiation during the voyage from Mars to Earth to avoid the inevitable decay of spores under the prolonged UV radiation exposure. This study addresses this hurdle and shows that *B. subtilis* endospores increase their protection to UV radiation when shielded by lysed bacteria of the same species. Survival rates of *B. subtilis* spores in simulated space UV radiation studies have been previously reported [8, 10, 12, 26]. Our findings also support those reported for *B. subtilis* spores as reported from the EXPOSE-E mission, where spores exposed for

nearly six years in space vacuum showed a low survival rate [8]. This is further complemented by the survival of non-spore forming *Halorubrum chaoviator* and *Synechococcus nägelli* exposure on the EXPOSE-R facility on the International Space Station, where low rates of survival were recorded after two years of exposure to the vacuum of space when partially shielded from UV exposure [27]. These reports coupled with the findings presented in this study suggest that bacteria, particularly spore formers, could survive extended periods on meteorites if partially protected from UV radiation.

While this study does offer new insights that add to the theory of panspermia via Martian meteorites, the time required for this voyage placing constraints on bacterial survival is potentially a valid criticism. However, spores from the bacterium *Thermoactinomyces sacchari* as old as 9000 years have been shown to be viable [30]. Moreover, it is not currently understood how long spore forming bacteria can remain in this suspended state. While this does not compare to the extensive time scales that a meteorite could spend in transit from Mars to Earth [31], it does show that spore forming bacteria can last extensive periods in conditions that are less than favourable. However, DNA damage has been observed in *B. subtilis* spores upon UV exposure [32] which needs to be considered in panspermia models. An additional limitation of these approaches is that the surface of Mars likely had different chemical composition when it had conditions viable for life [33]. We also note that the exposure an asteroid receives from the Sun varies with heliocentric distance. Therefore, converting the UV doses in this study into 'time-in-space' doses would require knowledge of the orbit of a given piece of debris, which will be covered in detail in a future study. Nonetheless, this work builds on the evidence obtained in previous studies [8–12], suggesting that spore forming bacteria could potentially survive long durations on Martian meteorites with partial protection from UV radiation.

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Data availability Access to data of all replicated experiments is available via personal communication to the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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