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Assessment of the Forward Contamination Risk of Mars by Clean Room Isolates from Space-Craft Assembly Facilities through Aeolian Transport - a Model Study

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Abstract The increasing number of missions to Mars also increases the risk of forward contamination. Consequently there is a need for effective protocols to ensure efficient protection of the Martian environment against terrestrial microbiota. Despite the fact of constructing sophisticated clean rooms for spacecraft assembly a 100 % avoidance of contamination appears to be impossible. Recent surveys of these facilities have identified a significant number of microbes belonging to a variety of taxonomic groups that survive the harsh conditions of clean rooms. These microbes may have a strong contamination potential, which needs to be investigate to apply efficient decontamination treatments. In this study we propose a series of tests to evaluate the potential of clean room contaminants to survive the different steps involved in forward contamination. We used *Staphylococcus xylosus* as model organism to illustrate the different types of stress that potential contaminants will be subjected to on their way from the spacecraft onto the surface of Mars. *Staphylococcus xylosus* is associated with human skin and commonly found in clean rooms and could therefore contaminate the spacecraft as a result of human activity during the assembling process. The path the cell will take from the surface of the spacecraft onto the surface of Mars was split into steps representing different stresses that include desiccation, freezing, aeolian transport in a Martian-like atmosphere at Martian atmospheric pressure, and UV radiation climate. We

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assessed the surviving fraction of the cellular population after each step by determining the integrated metabolic activity of the survivor population by measuring their oxygen consumption rate. The largest fraction of the starting culture (around 70 %) was killed during desiccation, while freezing, Martian vacuum and short-term UV radiation only had a minor additional effect on the survivability of *Staphylococcus xylosus*. The study also included a simulation of atmospheric transport on Martian dust, which did not significantly alter the metabolic potential of the cells. The high survival potential of skin microbes, which are not among the most robust isolates, clearly underlines the necessity for efficient decontamination protocols and of adequate planetary protection measures. Thus we propose a series of tests to be included into the description of isolates from spacecraft assembly clean rooms in order to assess the forward contamination potential of the specific isolate and to categorize the risk level according to the organisms survival potential. We are aware that the tests that we propose do not exhaust the types of challenges that the microbes would meet on their way and therefore the series of tests is open to being extended.

Keywords Space exploration · Microorganism · Planetary protection · Mars

Introduction

Planetary protection comprises protecting planet Earth and Earth life against the intrusion of extraterrestrial life forms (back contamination), as well as the contamination of other planets and moons by terrestrial organisms, first and foremost microorganisms (forward contamination). The latter has a high priority for space agencies when preparing the exploration of extraterrestrial bodies, and rules and regulations regarding planetary protection have been formulated and implemented by the Committee on Space Research (COSPAR) since 1964 (DeVincenzi et al. 1998). Already in 1967, in the United Nations Outer Space Treaty, spacefaring nations had reached an agreement on regulating space contamination ("Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies" 1967).

In particularly our Moon and Mars have been in the focus of space exploration. E.g. since the 1970ies 7 spacecrafts have successfully landed on Mars and several arrived on Mars but failed. NASA and ESA plan to launch several landing missions in the next decade, culminating with a manned mission probably in the late 2020ies or the early 30ies (Davila et al. 2010; McKay et al. 2011). These activities, that apart from proving the principle of colonizing new territory outside Earth, have the search for extraterrestrial life as the overarching goal, emphasize the necessity for planetary protection.

Before microorganisms can contaminate the surface of Mars, there are several harmful conditions they will encounter, both during space travel and on Mars itself. In space, cells will be exposed to the highly desiccating conditions of space vacuum, extreme low temperatures, microgravity, and high levels of solar UV radiation. Space vacuum immediately causes dehydration and severe damage to cell components such as the cell membrane (Wolfe 1987; Chapman 1994; Santivarangkna et al. 2007). Temperatures in interplanetary space vary greatly depending on whether or not an object receives direct sunlight. The temperature at the surface of the moon, for instance, varies from around 110 K at night to around 390 K during the day (Vasavada et al. 1999). Although Mars has a thin atmosphere, it does not offer significant insulation, thus producing temperature variations from 150 K to 298 K (Horneck 2000). This

poses another challenge to microorganisms as freezing can damage cells in several ways. In hydrated cells, the effects of freezing are comparable to dehydration effects. Mazur et al. (1972) describe two processes that can explain freezing injuries depending on the freezing rate. At low freezing rates cells lose water fast enough in order to maintain osmotic equilibrium as the extracellular water freezes, which creates a dehydrated state. When freezing at high rates, cells will not be able to lose water fast enough, intracellular ice will form and damage to cell components will occur.

Another important challenge is radiation. In the solar system, the most damaging type of radiation is solar UV radiation. With respect to Mars, it had been identified as the single most harmful factor to the survival of microorganisms (Cockell et al. 2005; Newcombe et al. 2005; Schuerger et al. 2003, 2006). However, soil layers of only 1 mm in depth provide sufficient protection of cells against the short-term exposure of solar UV (Cockell et al. 2005; Mancinelli and Klovstad 2000). Hansen et al. (2009) have shown that less than 2 cm of Martian analogue soil was enough to protect a microbial community from the effects of solar UVC radiation equivalent to 80 sols. Microorganisms that contaminate Mars will first get into contact with dust that is covering the surface and the dust could potentially offer a means of transport if cells are getting attached to it. This would enable microorganisms to disseminate over long distances during global dust storms (Pollack et al. 1979; Tomasko et al. 1999). In addition, dust that would get in contact with the surface of a spacecraft could mobilize cells and transport them to the ground.

On the other hand, suspended Martian dust is likely to display triboelectric charging, which could damage the cells. Triboelectric charging or contact electrification is a charging process that occurs when two materials are separated after contacting one another. The process leads to electrical charging of the materials with the same absolute charge but opposite sign. The collision of particles leads to the formation of an electrical dipole, and when the dipoles potential exceeds the breakdown voltage of the surrounding atmosphere, a discharge will occur. Triboelectric charging and associated discharges have been observed in dust storms (Kamra 1972), dust devils (Farrell et al. 2004) and volcanic plumes (Gilbert et al. 1991) on Earth. The dry atmosphere of Mars has a lower conductivity than Earth's atmosphere and should maintain charges more easily. In addition, the low pressure reduces the atmospheres breakdown potential, which increases the frequency of discharge events compared to Earth. As suspended dust is a major constituent of the Martian atmosphere and dust devils and storms are common phenomena (Edgett and Malin 2000), dust charging and discharging is likely to happen on Mars (Farrell et al. 1999). Indeed, in laboratory simulations, the charging and discharging of Mars analogue dust has been observed under simulated Martian conditions in several studies (Fábian et al. 2001; Gross et al. 2001; Krauss et al. 2003; Merrison et al. 2004). The consequences on bacterial survival have not been addressed yet.

During the study that we present here, some critical steps of forward contamination of Mars were simulated including dehydration, exposure to vacuum and freeze/thawing, suspension in a Martian atmosphere and UV radiation. For this purpose the Gram-positive bacterium *Staphylococcus xylosus* that was used as model organisms in order to exemplify the effect of the different steps, was allowed to grow biofilms on quartz dust and subsequently vacuum-dried and frozen to simulate space vacuum and subzero temperatures, and finally injected into a wind tunnel where it was suspended in a simulated Martian atmosphere and irradiated by UVC.

Quartz was chosen as a carrier material as it is relatively common in Martian soil (Bandfield et al. 2000) and did not interfere chemically with our activity measurements that were used to

evaluate the activity after each treatment. *S. xylosus* is an appropriate model organism for the evaluation of our approach as 1. it is commonly found on the skin of humans and could therefore be a likely candidate to contaminate space crafts, 2. is known to form biofilms and therefore attaches well to surfaces and 3. as a potential contaminant that originates from humans it is less stress resistant than contaminants that originate from other sources including soil and consequently a more sensitive survivability indicator.

In addition our experiment tests the effect of transport of bacteria on dust in a simulated Martian atmosphere and thus also includes the influence of triboelectric charging on short timescale on bacterial survival.

Material and Methods

Model Organism *Staphylococcus xylosus* was kindly provided by Viduthalai Regina, Department of Bioscience, Aarhus University, Dk. *S. xylosus* was selected for this model study because it is common on the skin of humans and therefore is a likely bacterium to contaminate spacecrafts as they are known to attach well to surfaces. The strain is not pathogenic to humans or animals, which is a prerequisite to being a model organism. In addition, strains of the genus *Staphylococcus* are common among clean room isolates as reported in Moissl-Eichinger et al. (2012).

Preparation of the Test Material Quartz dust (<2 μm in diameter) was obtained from Alfa Aesar (UK). The dust was cleaned and sterilized by washing 250 g of quartz dust in 1 L 1 M HCL overnight by vigorous shaking at room temperature. The quartz suspension was transferred to sterile Falcon tubes and centrifuged at 5000 rpm for 10 min. The pellet was washed in sterile milliQ water. The procedure was repeated 6 times to remove the very fine material and traces of the acid. A starting culture of *S. xylosus* was prepared by inoculating a drop (approximately 50 μl) of an outgrown culture into 5 ml of 1 % (*w*/*v*) sterile TSB medium and overnight incubation at 37 °C. To grow cells that attached to the quartz particles 60 g of sterile quartz dust was added to 400 ml of sterile 1 % (*w*/*v*) TSB medium. 500 μl of the *S. xylosus* pre-culture were added and the suspension was incubated at 37 °C for 20 h on a shaker. The quartz-bacterium mix was centrifuged at 5000 rpm for 10 min and washed 3 times in 1× PBS. After each washing step the supernatant was discarded. The attachment of the cells to the quartz particles was checked by microscopic inspection after staining with LIVE/DEAD *Bac*Light Bacterial Viability Kit (Invitrogen, ThermoFisher Scientific, Waltham, USA) according to the manufacturer's instructions. The Live/Dead staining was used to evaluate whether the cells survived the washing steps. In general, more than 90 % of the cells stained green, which is interpreted as physically intact and therefore living cells.

Preparation the differently Treated Cultures

Vacuum Drying Washed *S. xylosus*/quartz cultures were transferred to sterile Falcon tubes and dried in a vacuum chamber overnight to remove most of the free water.

Freezing Vacuum-dried cultures were frozen in liquid nitrogen for 10 s, and thawed at room temperature.

Suspension of the Dust into the Wind Tunnel The wind tunnel facility (Merrison et al. 2002) (Fig. 1 Supplementary material) consists of a cylindrical central wind tunnel that is 40 cm in diameter and 1.5 m long and enclosed in an environmental chamber that is 1 m in diameter and 3 m long. A fan draws gas from the central wind tunnel and returns it into an outer cylinder for recirculation. The environmental chamber allows the wind tunnel to be evacuated to a few mbar and the composition of the gas to be controlled. Wind speeds of 1–10 m/s can be achieved. The biofilm coated quartz dust was injected through a nozzle that is situated on top of the chamber. For injection small amounts of dust (usually around 0.5 g) were filled into a closed air-filled tube, which is then opened to the vacuum of the tunnel. The turbulence created by the suction at the injection nozzle is sufficient to suspend the dust into the wind tunnel. Suspension usually lasts for about 20 min. The wind tunnel was evacuated to 4 mbar, filled with CO_2 to 60 mbar, and evacuated again down to 15 mbar to create a simulated Martian atmosphere. A wind speed of 4 m/s was used, and a total of 40 injections of dry *S. xylosus* coated quartz dust were made over 1 h for a total injected mass of ~20 g, with each injection using CO_2 in the injection tube. A control sample was placed into the wind tunnel in such a way that it was exposed to the vacuum and CO_2 atmosphere, but was not exposed to the wind or UV radiation. Each injection raised the pressure by ~0.4 mbar, and in between injections the chamber was evacuated to 15 mbar. The total pressure range during the experiments was 14–16 mbar. After the last injection it took the dust 20 min to settle. Than the wind tunnel was opened to air and the pressure rose to ambient pressure within half an hour, after which the samples were taken out. The total experiment, from evacuating the wind tunnel to sample collection took about 4 h. During the third experiment, a 200 W xenon-mercury (Hamatsu Photonics) lamp was used at 200 W. The lamp was placed in front of the quartz window to irradiate the dust with UV light. The intensity of UV light in the wind tunnel was measured with an AVASPEC 2048 UV/VIS spectrometer (Avantes, The Netherlands) at the opposite side of the wind tunnel by removing the glass window.

Calculation of the Average UV Intensity in the Wind Tunnel The average intensity measured at 240 nm wavelength is about 60 $\mu W/cm^2/nm$. The wind tunnel has a diameter of 40 cm and a length of 1.5 m giving a total volume of 0.1885 m^3. The UV beam was about 15 cm in diameter, so the volume of the beam was 0.0071 m^3. As the dust spends half of its time in the wind tunnel recirculating through the outer cylinder, the fraction of time the dust spends in the beam is 0.0188 for an average UV intensity on the dust (240 nm) of 0.011 $W/m^2/nm$. The average intensity of UV light at 240 nm on the surface of Mars is 0.006 $W/m^2/nm$ (Hansen et al. 2009). The average time a dust particle would remain in suspension in the UV beam was 200 s, so the dust experienced 367 s of Martian UV equivalent.

Collecting the Wind Tunnel Samples Dust injected into the wind tunnel was collected using vertically positioned charged metal plates and a horizontally positioned glass plate (Fig. 2 Supplementary material). The glass plate of 20 × 20 cm was placed on the bottom of the wind tunnel to collect the bigger or uncharged particles that settled without attaching to the charged plates. A construction of 6 charged plates, 3 positively charged and 3 negatively charged, were placed vertically in the wind tunnel with a voltage of 300 V between them to collect the negatively charged and positively charged quartz-bacteria particles, respectively. The charged plates were positioned in the middle of the wind tunnel with the glass plate in front (Fig. 3 Supplementary Material). The injection nozzle and the UV lamp were placed behind the collection setup, therefore, the injection itself did not deposit any dust onto the

charged plates or the glass plate, and once attached, the dust was protected from UV light. After each experiment, the plates were removed from the wind tunnel, the metal plates were unscrewed and the dust was collected. Unfortunately, the amount of dust that was collected on the charged plates and on the glass plate was not sufficient to carry out measurements in replicates as it was done with bacteria coated quartz dust prior to the injection into the wind tunnel or with the wind tunnel control. Thus these results are the least reliable and can therefore only provide trends.

Measuring Oxygen Consumption Rate Oxygen consumption of the quartz-attached cells was used as survival proxy. The "untreated" samples were prepared as follows: After the washing step the centrifuged but still wet material was transferred to an exetainer (Labco Limited, High Wycombe, England) and the oxygen consumption rate was determined as described below. Then the medium was removed, the material washed and dried and the dry weight of the material was used to calculate the oxygen consumption rate. In all other measurements dry material was weight after the respective treatments, and the dry material was suspended in TSB medium. Depending on the rate of O_2 consumption, 0.1 g or 0.2 g of quartz-bacteria dust was suspended in 1 % TSB in 5 ml or 10 ml exetainers and incubated at room temperature with mild shaking. Oxygen concentrations were measured using an oxygen microsensor with a guard electrode (Revsbech 1989). Before each experiment, the sensor was calibrated by a 2 point calibration using air-saturated 1 % TSB and 1 % TSB containing 2 M sodium ascorbate as an oxygen free reference. The oxygen concentrations were determined at regular time intervals and rates are expressed as μmol oxygen per min and g dry weight of *S. xylosus* coated quartz.

Data Analysis The statistical significance of differences in oxygen consumption rates between consecutive treatments was evaluated by pairwise comparison using an unpaired two-sample student *t* test. The degree of freedom was 3 for all treatments.

Results and Discussion

By simulating several stressful conditions that microorganisms would encounter as they are transported to Mars we can get deeper insight into the likelihood of an accidental but successful inoculation of the red planet by terrestrial microorganisms. In particular, we are interested in addressing whether bacterial cells could survive the transport on Martian dust and thus spread from the landing site to the surroundings. In order to produce contaminated dust, cultures of *S. xylosus* were grown in the presence quartz dust <2 μm. The successful colonization of the quartz particles can be seen in Fig. 1.

The route of forward contamination was arbitrarily divided into 4 stages: vacuum drying, freezing, suspension on dust particles at Martian pressure and short-term exposure to UVC radiation. The following parameters were not simulated: space vacuum, microgravity, and temperature, solar wind and cosmic rays. In order to evaluate these parameters we would need to include the international space station. This is feasible but would require long-term planning. We are convinced that the steps that could be evaluated in the terrestrial laboratory provide a valuable first proxy of a bacterial strains' forward contamination risk potential. The wind tunnel facility at Aarhus University is part of the Europlanet 2020 Research Infrastructure and can be accessed through the Europlanet application platform (http://www.europlanet-2020-ri.eu/) to carry out further in depth experiments.

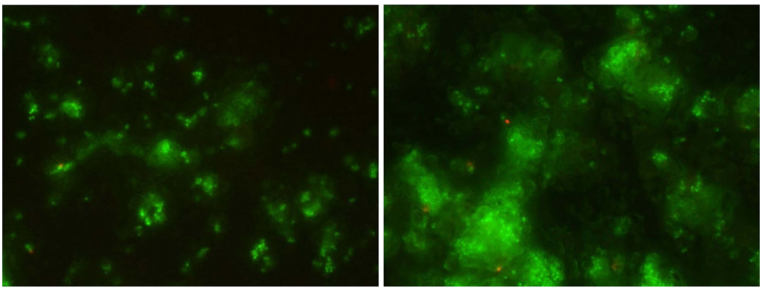

Fig. 1 The micrographs show two examples of cells of *Staphylococcus xylosus* after growth in the presence of quartz dust. The cells were stained with the Baclight Life/Dead kit. Cells with the green color are assumed to be alive while cells that stain with the red dye are assumed to be dead. Reflections of light by the quartz dust are strongest in the right picture. Text: S xylosus attached to quartz particles. The cells were stained with LIVE/DEAD *Bac*Light Bacterial Viability Kit (Invitrogen). *Green color* indicates live cells while the *red color* indicates dead cells. Reflection of the light by quartz particles can be seen especially well in the right picture, showing that the cells form aggregates with the quartz paticles

We present the outcome of a model study and propose that clean room isolates of spacecraft assembly workshops should be subjected to a similar series of tests, including the wind tunnel facility in order to standardize the risk assessment protocol. Usually stress effects on survival are quantified by viability tests determining the number of viable cells as colony forming units on nutrient agar plates. This procedure is difficult to automatize. We therefore suggest measuring oxygen consumption as a very sensitive activity proxy that integrates the activity of all aerobic microorganisms. Aerobic organisms are the most likely contaminants from clean rooms as indicated by the study of Moissl-Eichinger et al. (2012). The average oxygen consumption rates after each treatment are given in Table 1. Untreated biofilm covered quartz suspended in 1 % (*w*/*v*) TSB medium served as controls. In all studies vacuum drying induced

Table 1 Oxygen consumption rate[a] as function of sample treatment

	Untreated	Vacuum-dried	Frozen	WT-control	WT positive plate	WT negative plate	WT glass plate
Exp 1	0.69 ± 0.17	0.31 ± 0.05	ND	0.11 ± 0.02	0.05	0.07	0.09
Exp 2	1.0 ± 0.12	0.25 ± 0.03	0.17 ± 0.04	0.17 ± 0.02	0.1	0.16	0.14
Exp 3 (+UV)	0.5 ± 0.06	0.25 ± 0.03	0.21 ± 0.02	0.12 ± 0.03	0.05	0.04	0.05

The results of all experiments presented as oxygen consumption rates are summarized in this table. Detailed information about the treatments is given in the material and methods section. All rates are based on 4 replicates with the exception of the samples that were obtained after the bacterial coated quartz was suspended in the wind tunnel (WT) and collect on positively and negatively charged plates as well as on an uncharged plate. In this case there was not obtained enough material to carry out replicate measurements . Because of lack of material only one experiment was carried out with a sample from each plate. In experiment 2 a freezing step is included and in experiment 3 the bacteria coated dust was irradiated by UVC light while it was suspended in the wind tunnel atmosphere prior to collection on the plates. In experiment 1 the oxygen consumption rates where significantly different on a 5 % significance level between all treatments. In experiment 2 the oxygen consumption rates where significantly different on a 5 % level between the untreated control and all other treatments and the vacuum-dried treatment and all the other treatments. In experiment 3 no significant difference was observed between the vacuum-dried and the freeze-thaw sample while all the other treatments were significantly different on a 5 % level

[a] μmol oxygen min^{-1} g^{-1} (dry weight)

the largest activity drop. The activity generally decreased after the subsequent treatments including freezing and thawing and injection in a simulated Martian atmosphere. However, non of these treatments lead to a statistically significant decrease with the exception of addition of UVC light upon suspension in the wind tunnel. In more detail, to test whether the differences were significant, Student's t Tests were performed to test for equal means between each treatment after testing for equal variances using F tests. Hence, statistically significant differences in average oxygen consumption rate were found after vacuum drying, and between the vacuum-dried and wind tunnel control samples. The difference between the vacuum-dried and wind tunnel control samples is somewhat unexpected as the wind tunnel control sample is exposed to mild wind tunnel vacuum but only for a few hours whereas the vacuum drying takes place overnight. We can currently not explain why cells would be more vulnerable to further drying in a desiccated state than in a hydrated state. The only difference between the two treatments is the CO_2 atmosphere the wind tunnel control sample is exposed to, but also this is not likely to have a significant influence. Similar results were obtained during pilot experiments using low-pressure ambient air in the wind tunnel (data not shown). The experiment was repeated, with the modification of including a freezing and thawing step. The oxygen consumption rates after each treatment are shown in Table 1. A statistically significant decrease was found after vacuum drying and after freezing and thawing. In this experiment, no significant difference was found between the wind tunnel control sample and the previous sample. Overall the same trend was seen as in the first experiment. The combined results of the two experiments clearly show that desiccation of the cells in vacuum had the largest effect on the bacterial activity as this was reduced by 60 % to 80 % compared to the control. Freezing seemed to have an additional but only minor effect. Short-term simulated atmospheric transport, on the other hand, had little to no influence on the activity of the cells. A third experiment was performed to investigate the influence of UV radiation in relation to all other treatments. The bacterial coated dust was subjected to UV radiation during suspension in the wind tunnel. The results are shown in Table 1. It is well known that UV radiation damages cells mainly by causing the formation of bipyrimidine lesions in the DNA. Horneck et al. (1995) have shown that this is also the case when microbes are exposed in space. In addition to pyrimidine photoproducts, DNA strand breaks and DNA-protein crosslinking were observed in cells of *Deinococcus radiodurans*, spores of *Bacillus subtilis* and conidia of *Aspergillus ochraceus* during exposure to solar UV in space (Dose et al. 1995). In general, DNA repair mechanisms are not able to cope with this rapid destruction of DNA by UV light. In space, repair is even more unlikely as cells are in a dormant state, which compromises their ability to repair DNA damage. Considering that the amount of UV radiation received buy the cells in this experiment is equivalent to only 6 min on Mars, it is not surprising that many cells survived the treatment. On Mars the situation is different. In contrast to quartz dust, used in our experiments, that is fully transparent for UV and therefore provides little protection, Martian dust is made of opaque minerals including basalts that would shield cells from radiation (Hagen et al. 1970). In several studies in Mars simulation facilities it had been shown that a thin layer of dust is sufficient to protect cells from UV induced damage (Hansen et al. 2009; Mancinelli and Klovstad 2000; Schuerger et al. 2003, 2006). In these studies the effect of aerosolization is not included, a process, which comes into play when cells are released from the spacecraft and get deposited on the surface. This process could be mediated by vibration during landing and/or by dust particles that impact the surface of the spacecraft as it could happen during a dust storm. While cells that fall of the spacecraft due to vibration would not have any protection against UV radiation, cells that are removed by dust particles would

receive shelter by the particle (Hagen et al. 1970) and would more easily get buried under a layer of surface material within a couple of minutes and thus escape from lethal UV radiation.

Both vacuum drying and freezing in combination is often used to preserve bacterial cultures. Although these processes work well in preserving a small part of the initial bacterial culture for a long time, they still impose considerable stress on the culture and generally kill a large part of the cells. In both processes the cell membrane has been identified as the Achilles heel of the cell. Inactivation is mainly caused by dehydration of the cytoplasm. In a low-pressure environment, water will evaporate rapidly leaving the cells in a desiccated state. This is damaging to the cell membrane as membrane lipids undergo phase transition when water leaves and enters the cell, which causes the membrane to leak. During freezing extracellular water freezes to ice depleting the amount of water available for the cell, and depending on the cooling rate this can lead to dehydration damage or the formation of intracellular ice, which is damaging to cell components such as proteins and membrane lipids (Santivarangkna et al. 2008). The results of the experiments presented here confirm the damaging effect of low-pressure environments, as damage occurs both during vacuum drying and during exposure to a low-pressure CO_2 atmosphere in the wind tunnel. Freezing seems to have a minor effect, most likely because the cells were already in a dehydrated state when frozen. This greatly limits the effect of freezing, as it only causes damage when water is still present. On the other hand, the low-pressure environment in the wind tunnel did have an effect even though the cells were already dehydrated.

We were also investigating whether charging would have an effect on activity. Thus we hypothesized that the sample from the glass ground plate would have slightly higher oxygen consumption activity than the samples from the charged plates because the particles on the ground plate are generally less charged - no such difference was observed. However, in our experiments, most charging occurred upon injection rather than during suspension (Jonathan Merrison, personal communication) and although the particles electrified the same as during experiments where charging was induced by lift-off of the particles inside the wind tunnel rather than by injection, this does mean that not all particles in our experiment were charged to the same extent. The difference in distribution between the plates is mainly caused by the size of the particles rather than due to charge of the particles as larger particles are generally found on the ground plate. In a study by Mainelis et al. (2001) it was shown that both Gram-negative and Gram-positive bacteria have an overall net negative charge in atmospheric suspension. Mainelis and colleagues have shown that aerosolized *Pseudomonas fluorescens* and spores of *Bacillus subtilis* on average carry more negative charges than positive charges, with the exact charge distribution depending on the dispersion method. Some of the cells and spores carried several thousand elementary charges (Mainelis et al. 2001). When charges were induced upon *P. fluorescens* cells, the viability of the bacteria carrying between 4100 negative and 30 positive charges decreased with 40 % to 60 %, while the viability of bacteria carrying more than 2700 positive charges decreased by more than 98 % (Mainelis et al. 2002). In line with these results, one might expect the sample collected from the positively charged plates to have slightly higher oxygen consumption activity than the sample obtained from the negatively charged plates. This was not the case, most likely because the method used to measure activity is not sensitive enough to detect these differences, as the number of bacteria carrying a very high positive charge is low. The effect of charging on cell activity and survival would also depend on the length of the period the cells stay in atmospheric

suspension. In the current study, this period was only 20 min at a maximum and was probably too short to significantly affect the activity status of the cells. In future experiments the atmospheric suspension period should be increased to study the time dependency of the effect of charging on survival of the suspended microorganisms.

Conclusion

The combined results of the 3 pilot experiments provide important clues with regard to the influence of different challenges microorganisms encounter on their survival during forward contamination of Mars or other rocky bodies in our solar system. The desiccating effect of vacuum seems to have a large influence on survival, reducing the activity of the culture by 70 % compared to the control while subzero temperatures have a minor influence on dried cells, and will not contribute a lot to sterilization of spacecrafts. Little influence on cell activity could be attributed to simulated Martian atmospheric transport, indicating that cells once attached to Martian dust could spread from the landing sites. The only other factor that contributed to reduction in activity was UV radiation, which removed 50 % of the activity within minutes compared to the non-irradiated control sample. However, several experiments have shown that bacteria are easily protected from radiation by a thin layer of dust or extracellular cellular polymers. Consequently, microbes including clean room contaminants that survive on their way to Mars could contaminate the surface and would not be killed during atmospheric transport as long as they are not exposed to UV radiation for extended periods of time. These bacteria could interfere with life-detection assays and produce false positives or in the worst case outcompete aboriginal Martian biota. However, most of the clean room isolates are aerobic heterotrophs with limited chances of proliferation on the surface of Mars, as also would be the case for our test organism. Therefore they would mainly contaminate Mars by introducing genetic material and biomass, which could affect the indigenous Martian biota and interfere with search for Martian Life missions.

Overall, we suggest that the study of the physiological properties of clean room isolates of spacecraft assembly facilities should include tests of the kind that we carried out in this study to assess their potential of reaching Mars or any other planetary body in a viable state.

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