

# Growth of microorganisms in Martian-like shallow subsurface conditions: laboratory modelling

A.K. Pavlov<sup>1</sup>, V.N. Shelegedin<sup>2</sup>, M.A. Vdovina<sup>1</sup> and A.A. Pavlov<sup>3</sup>

<sup>1</sup>Laboratory of Mass Spectrometry, Ioffe Physico-Technical Institute of Russian Academy of Sciences, St. Petersburg, Russia

e-mail: Alexander.Pavlov@nasa.gov

<sup>2</sup>Department of Biophysics, St. Petersburg Polytechnical State University, St. Petersburg, Russia

<sup>3</sup>NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA

**Abstract:** Low atmospheric pressures on Mars and the lack of substantial amounts of liquid water were suggested to be among the major limiting factors for the potential Martian biosphere. However, large amounts of ice were detected in the relatively shallow subsurface layers of Mars by the Odyssey Mission and when ice sublimates the water vapour can diffuse through the porous surface layer of the soil. Here we studied the possibility for the active growth of microorganisms in such a vapour diffusion layer. Our results showed the possibility of metabolism and the reproduction of non-extremophile terrestrial microorganisms (*Vibrio* sp.) under very low (0.01–0.1 mbar) atmospheric pressures in a Martian-like shallow subsurface regolith.

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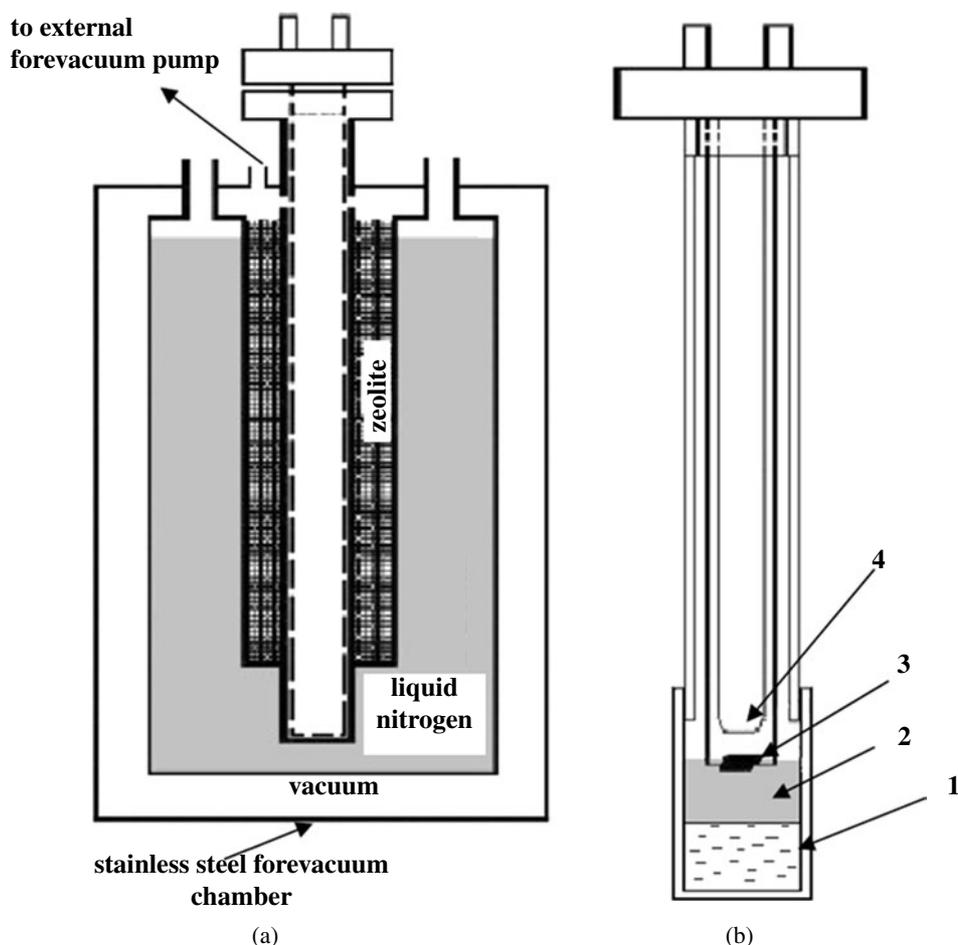
## Introduction

The Martian surface is a harsh place to survive and grow for any known microorganism. It is sterilized by high ultraviolet (UV) fluxes, low temperatures, atmospheric superoxidants and ionizing radiation. Nevertheless, there are some known types of terrestrial bacteria, such as *Deinococcus radiodurans* and sporulating microorganisms, which can resist all of these harmful factors (Nicholson *et al.* 2000; Pavlov *et al.* 2006). Although a shallow subsurface layer (below ~1–2 mm of soil) is well protected from the most harmful factor – UV radiation (Hansen *et al.* 2009) – even there the hypothetical Martian biosphere would experience severe challenges, because liquid water is unstable under extremely low Martian atmospheric pressures – 3–7 mbar (Ingersoll 1970; Chevrier & Altheide 2008). Furthermore, the recent study by Schuerger & Nicholson (2006) suggested that the low pressure itself is a significant stress for biota and inhibits bacterial growth. Here we explored the possibility of terrestrial non-extremophile microorganisms reproducing in a Martian-like shallow subsurface layer under very low pressures. In our experiments microorganisms were given some amounts of nutrients and glucose (see Materials and methods), which may or may not be appropriate for Mars (see Discussion), therefore our current results should not be viewed as an exact replication of the Martian environment.

The spectacular Odyssey measurements of gamma-ray radiation and scattered epithermal neutrons (Boynton *et al.*

2002; Feldman *et al.* 2002; Mitrofanov *et al.* 2002) suggested the presence of a shallow (less than 1 m depth) ground ice layer covered by dry regolith at high latitudes on Mars. The presence of subsurface ice at a few centimetre depths was confirmed by the Phoenix Mission (<http://phoenix.lpl.arizona.edu/>; Smith *et al.* 2009). Two equatorial regions also show increased hydrogen abundance at shallow depths and Jakosky *et al.* (2005) pointed out that a high concentration of ground ice at low latitudes is possible as a result of long-term climate variations. However, the currently favoured explanation for the low-latitude subsurface hydrogen on Mars is hydrated minerals rather than water ice (Feldman *et al.* 2004). In general, the ground ice table depths vary from a few centimetres to 1 m (Boynton *et al.* 2002; Feldman *et al.* 2002; Mitrofanov *et al.* 2002). The observed water vapour pressure in the Martian atmosphere undergoes significant local oscillations in time, due to the processes of sublimation of the ground ice and polar ice caps and re-condensation of the water vapour (Mellon *et al.* 2004; Möhlmann 2004; Schorghofer 2007).

Modelling studies by Haberle *et al.* (2001) and the Mars Global Surveyor observations of thermal emission (<http://tes.asu.edu/>) show peak surface temperatures as high as 280–290 K, even at latitudes of 60–70 degrees in the southern hemisphere during the summer season. Diurnal temperature variations can be more than ~100 K. Peak surface temperatures above 273 K should result in the intense sublimation of the shallow subsurface ice during Martian daytime.



**Fig. 1.** Experimental setup. (a) Schematic view of the forevacuum chamber. (b) The internal Teflon tube with the experimental sample placed into a Teflon cup. It includes: 1 – a piece of ice at the bottom; 2 – the sand layer containing a biological sample; 3 – a thermocouple for the control of temperature at the sand’s surface; 4 – an electric heating element. Note that neither the Teflon cup with the sample nor the Teflon tube are in direct contact with the cooled surfaces.

Vapour diffusion through the upper Martian regolith with adsorption on the surfaces of grains and the production of thin films of liquid water in the space between the grains was previously considered by Schorghofer & Aharonson (2005), Möhlmann (2004), Bryson *et al.* (2008) and Chevrier *et al.* (2008). We hypothesize that such a ‘wet’ layer in the Martian shallow subsurface can be a suitable habitat for the potential biosphere, even though the present-day Martian atmospheric pressure is low.

In order to test our hypothesis, we simulated Martian-like pressure/temperature (P-T) conditions in a vacuum chamber. We placed a sample of ice covered with sand into the vacuum chamber and simulated multiple day–night temperature cycles observed on Mars. As expected, the subsurface ice intensely sublimated under very low atmospheric pressure. We placed non-extremophile bacteria into the layer of sand right above the ice so that the only source of moisture in the sand would be the water vapour sublimated from the ice layer underneath. We found that the ordinary non-extremophile bacteria were able to reproduce in such extreme Martian-like conditions.

## Materials and methods

### Chamber setup

Our experimental setup is shown in Fig. 1. The forevacuum chamber has a cryogenic pump inside for effective capturing of the water vapour and other gases (including CO<sub>2</sub>). The connection to an external forevacuum pump is also provided. The atmospheric pressure inside the chamber was maintained at 0.01–0.1 mbar. The surface of the studied sample is periodically heated by thermal radiation from an electric heater (see Fig. 1). We used an electric heater made of thin nichrome wire, which in our experiments generated ~0.2 W (adjustable). The temperature of the sand surface layer was measured by the internal thermoelectric couple during the whole experimental run.

### Preparation of experimental and control samples

To simulate the upper Martian permafrost layer, we used a tube with water ice at the bottom (~1 g) covered by ~1–1.5 cm of sand (Fig. 1). Although sand was not specially selected (small-sized grains from the Baltic seashore), the

water vapour diffusion rates through this sand layer were very similar to the diffusion rates reported for Martian analogues (see Discussion: *Soil selection*). The sand was sterilized and dried out by heating to temperatures above 200 °C for at least 30 minutes before the addition of nutrients.

To provide nutrition for the bacteria we added the inorganic M9 Standard Minimal Medium (with a dry weight salt fraction of  $\sim 5 \times 10^{-3}$  g/g) and glucose (with a weight fraction of  $\sim 10^{-5}$ – $10^{-4}$  g/g) to the dry sand mixture. This level of nutrients was chosen to avoid contradiction with the Viking results (see Discussion: *Nutrients*). The nutrients and sand were well mixed with an electric shaker.

For each experimental cycle we added 50 µl of water suspension containing cells of *Vibrio* sp. to the sand, with the total number of cells being  $10^5$ – $10^6$ . Prior to any experiments, the sample mixture of sand with bacteria and nutrients was dried outside the vacuum chamber at 40 °C for 1 hour to eliminate any water from the water suspension (named the ‘desiccation step’). Then, we separate the mixture into four identical samples with an approximately equal number of viable cells. All four samples were put in a refrigerator and remained frozen at a temperature  $-15$  °C at 1 atm. After 1 hour in freezing conditions, one sample (named the ‘experimental sample’) was taken out of the refrigerator and put on the surface of ice (Fig. 1). Within a few minutes the experimental sample with ice was put in the vacuum chamber and then cooled by liquid nitrogen, so that the ice did not melt. Then, the chamber was pumped down to pressure 0.01–0.1 mbar within 1 hour. Three control samples were maintained in the frozen conditions at  $-15$  °C in the refrigerator under regular pressure until the end of the experimental run. The main purpose of having three control samples for each experimental sample was to make sure that the mixture of bacteria with soil was uniform after the ‘desiccation step’ in the sample preparation routine.

#### *Biological sample characteristics*

To study the possibility of the metabolism in the Martian-like soil we chose the wild strain of *Vibrio* sp. This strain was originally isolated by one of the co-authors (Shelegedin) in 1998 from the Baltic seashore and is now available from the culture collection in the St. Petersburg Institute of Nuclear Research, Radiobiology Department. The characteristics of this strain were published in Babenko *et al.* (1998).

These bacteria are mesophiles and facultative anaerobes. The *Vibrio* sp. strain has the following optimal conditions for growth:  $5 < \text{pH} < 9$ , salinity  $< 3\%$  and the optimal temperature for growth is 37 °C. Under this temperature, the time period needed for doubling the cell’s population is equal to 0.5 hour under unlimited nutrient supply (Babenko *et al.* 1998). For this study, we also measured the increase of doubling time at low temperatures and we obtained the following results: at 15 °C–3.5 hours, 10 °C–4.5 hours, 6 °C–40 hours, 3 °C–96 hours. The doubling time for lower temperatures was too long for valid measurements.

Although *Vibrio* sp. does not ‘like’ to live in extreme environments (non-extremophile), it is able to survive

desiccation and freezing. *Vibrio* sp. can grow in the inorganic M9 Minimal Medium ( $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{NaCl}$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{MgSO}_4$ ,  $\text{CaCl}_2$ ) mixed with glucose.

#### *Experimental run*

We performed nine experimental runs in total and each experimental run lasted for three days. Therefore, each run included three Martian ‘diurnal’ cycles of heating and freezing. The diurnal cycles were simulated by turning on and off the heat source – thin nichrome wire – which is located in the chamber directly above the soil (Fig. 1). During daytime, the peak temperatures of the Martian surface can reach 280–300 K (Fig. 2) and after sunset (nighttime) the temperature decreases to  $\sim 200$  K or even lower for high latitudes. It was estimated that the diurnal temperature variations on Mars propagate down to the depths of a few centimetres (Mellon *et al.* 2004; Möhlmann 2005). Our experimental set-up allowed us to reproduce these basic features of the Martian surface environment.

In our chamber we heated the top of the sand layer for 5 hours during each diurnal cycle and the observed surface temperatures are shown on Fig. 3. The time for cooling down after the heater was turned off was approximately 1 hour. The power output of the heater is adjustable and allowed us to have the peak soil temperatures at either 280 K (three experimental runs) or 300 K (three experimental runs) (Fig. 3). Peak temperatures were typically reached after  $\sim 1$  hour of heating. The inner cryogenic pump was filled with liquid nitrogen during the whole run and therefore the ice at the bottom of the chamber was cooled all the time. While the heater was switched off (analogue of Martian nighttime), the sample had a steady-state temperature of  $\sim 200$  K. Three experimental runs were performed when the heater was turned off the entire time.

#### *Measurements of biomass increase*

It would be incorrect to simply compare the number of viable cells in the experimental sample after the experimental run with the number of viable cells originally injected into the soil mixture, because of the ‘desiccation step’ in our sample preparation procedure. We performed a separate test experiment and observed that the ‘desiccation step’ alone causes  $\sim 100$  times decrease in the viable cells numbers compared to the number of cells initially injected into the soil. Therefore, the only meaningful comparison of the change in viable cells should be done against the control samples, which passed through exactly the same sample preparation stresses. This also means that each experimental run (nine in total) should have its own set of control samples (27 in total) (see Table 1).

After each run, the experimental sample and the control samples were placed in separate cups with aminopeptide solution (peptone analogue). The sand and solution were mixed with a mixer and let stand at 37 °C. This causes large sand grains to settle down and bacteria to grow.

Another test experiment demonstrated that the number of viable cells in the dried and frozen control samples did not

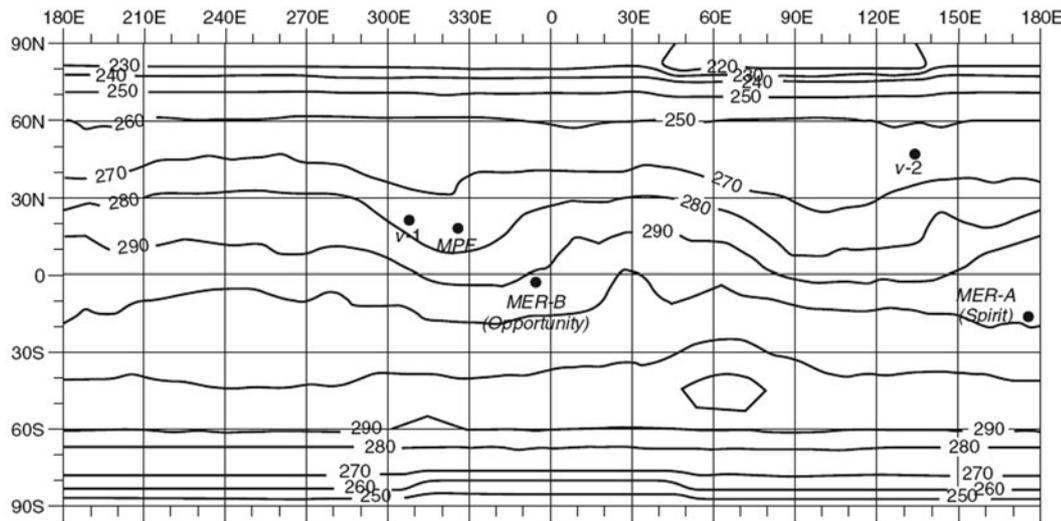


Fig. 2. Peak surface temperature on Mars (Haberle *et al.* 2001).

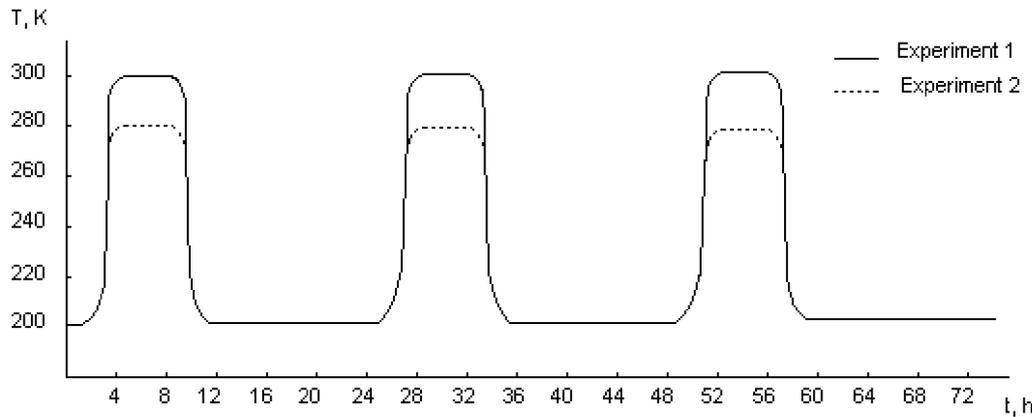


Fig. 3. Surface temperature cycles for Experiment 1 (runs 1–3) and Experiment 2 (runs 4–6).

Table 1. CFUs in the control samples for specific runs. In each experimental run the original soil–bacterial mixture was divided into four samples of equal weight. One sample was placed into the chamber for Martian simulation and the other three were used for control and kept in a refrigerator at a constant temperature ( $-15^{\circ}\text{C}$ ). Each experimental run (nine in total) had its own three controls – see the CFU 1, CFU 2, CFU 3 columns (27 in total).

Run number	CFU 1	CFU 2	CFU 3	Mean CFU of control samples	Standard deviation of CFU in control samples
Run 1	205	197	208	203	6
Run 2	163	151	156	157	6
Run 3	222	228	235	228	7
Run 4	289	264	276	276	13
Run 5	256	229	274	253	23
Run 6	120	134	118	124	9
Run 7	174	161	147	161	14
Run 8	193	219	206	206	13
Run 9	211	257	231	233	23

change in the refrigerator at  $-15^{\circ}\text{C}$  within 72 hours. Therefore, if the experimental sample after the Martian simulation run in the chamber has a higher number of cells than the control samples in refrigerator, this would indicate the true biomass growth in the chamber.

#### Measurement of biomass growth by Colony-forming Units count

We took small portions of the aminopeptide solution with bacteria extracted from our experimental and control samples, put it in Petri dishes with agar jelly and counted the number of Colony-forming Units (CFUs). We used subsequent dilutions of the aminopeptide solution with bacteria to bring the CFU numbers from all experimental samples to roughly similar levels. The dilution factors are listed in Table 2 and allow calculation of the net biomass growth in the chamber experiments. For example, a 100-times increase in the CFU count in the experimental sample was reported when the suspension from the experimental sample had to be diluted 100 times (Table 2,  $D=100$ , runs 1–3) in order to get approximately the same number of CFUs as in the

Table 2. Increase of the biomass in the simulated Martian conditions.  $T(\text{peak})$  is the maximum surface temperature of the soil in a particular run during simulation of the Martian diurnal cycles in the chamber (see also Fig. 3).  $CFU_0$  is the number of CFUs in the experimental samples after dilution.  $D$  is the dilution factor of bacterial solution from the experimental samples after the Martian simulations runs. The last column represents the ratio of CFUs in the experimental samples (multiplied by the dilution factor) to average the control CFUs from Table 1. These ratios represent the net increase of the biomass in our experiments.

$T(\text{peak})$	$CFU_0$	$D$	$CFU_0 \times D / CFU_{\text{control}}$
Run 1. 300 K	210	100	103
Run 2. 300 K	135	100	87
Run 3. 300 K	263	100	115
Run 4. 280 K	233	5	4
Run 5. 280 K	260	5	5
Run 6. 280 K	152	5	6
Run 7. 200 K	159	1	1.0
Run 8. 200 K	187	1	0.9
Run 9. 200 K	236	1	1.0

Petri dishes with bacteria from the control samples (see Table 1).

#### Experimental protocol and bacterial identification

To avoid possible contamination problems, all experimental procedures were performed following the protocol from the ‘Principles of Microbe and Cell Cultivation’ by Pirt (1975). Furthermore, *Vibrio* sp. has a set of specific properties that allowed easy identification of those bacteria in the amino-peptide solution (resistance to ampicillin, ability to change pH from neutral to 9 at the last stage of growth, growth on chitin powder) after measurements. Note that any procedures of identification were held only after experimental runs and measurements. Any specific additions (including ampicillin and chitin powder) were made only for the recovered populations when all of the measurements were finished, so that these procedures could not affect the results.

## Results

Firstly, we obtained an experimental confirmation of the ice sublimation and diffusion of the water vapour through the sand layer in the vacuum chamber. In the beginning of the experimental cycle, our ice sample was covered by the dry layer of sand. At the end of the experimental cycle, the structure of the sample included ice remaining at the bottom, a thin dry layer of sand on the top and a large layer of ‘wet’ sand (vapour diffusion layer) in the middle, with an average water content up to  $\sim 30\%$  (Fig. 4) determined by the weight change of the soil layer before and after the experimental run. Note that part of the water in this wet sand layer was in the form of ice. However, as we show in the biological experiment, some amount of liquid water had to be present in the wet layer as well.

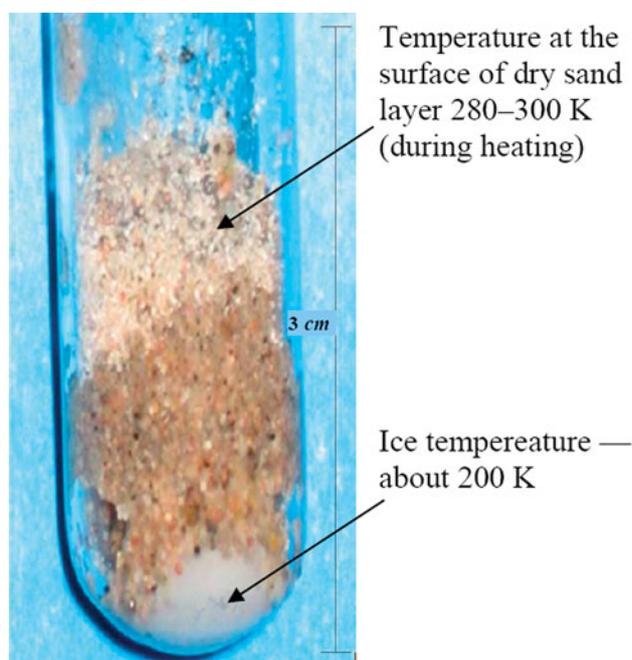


Fig. 4. Sample structure after experimental run: ice remnant on the bottom, thin dry layer of sand on the top and ‘wet layer’ (darker sand) in the middle with mean water content up to  $\sim 30\%$ . The ‘wet’ sand layer is actually solid – part of the water is in the form of ice, but some amount of liquid water films has to be present.

The comparison of the initial and final weight of the whole sample allowed us to estimate the average sublimation rate ( $S$ ) of ice in our chamber as  $\sim 0.01 \text{ g}\cdot\text{cm}^{-2} \text{ hour}^{-1}$ . This  $S$  value is consistent with the pure ice sublimation rate at the temperature of 190 K or the rate of adsorbed water evaporation at 285 K (Möhlmann 2004; Chevrier *et al.* 2008).

$S$  is strongly correlated with temperature. Therefore, the sublimation rate of ice could have been much higher in our experiments during relatively short periods of heating. However, the total contribution of these heating periods (5 hours for each one) to the average sublimation loss of ice was minor. We did a control run for which the heater was turned off at all times and it took only  $\sim 10\%$  longer to lose all ice from the sample. Therefore, the short heating periods in our experiments were not important to the overall evolution of the ice layer, which is consistent with the current diurnal variability on Mars.

Our average  $S$  value somewhat exceeds the mean annual sublimation rate of the Martian ground ice estimated by Möhlmann (2005), Mellon *et al.* (2004), etc. The main reason for such a difference was the lack of the vapour recondensation process in our experiment. The cryogenic pump captured and stored the water vapour during the entire experimental cycle. In contrast, recondensation of the water vapour takes place on Mars during the nighttime due to the significant decrease of the surface temperature.

We discovered that the non-extremophile bacteria *Vibrio* sp. were able to reproduce in the Martian-like P-T conditions. The biomass increased by up to 80–120 times compared to the

control samples after three Martian-like diurnal cycles with peak surface temperatures at  $\sim 300$  K (runs 1–3) (Table 2). The experimental run with less intensive heating (peak temperatures  $\sim 280$  K) still showed a 4–6 times increase of the biomass (see Tables 2, runs 4–6). The growth of the population took place only in the narrow vapour diffusion layer described above (Fig. 4), where the liquid water abundance (probably in the form of liquid films) and the temperature were sufficient to support the metabolic processes of *Vibrio* sp. Note that even though we did not measure the temperature in the ‘wet’ layer directly, we performed a separate sensitivity experiment where *Vibrio* sp. bacteria was held at 276 K outside the chamber in the incubator. Under this temperature and given the same nutrient concentrations, the population doubling time was  $\sim 4$  days. Therefore, the temperature in the ‘wet’ layer in our experiment should have exceeded 276 K, but on the other hand it must be lower than 283 K according to the temperature dependence of the doubling time mentioned above (see Materials and methods: *Biological sample characteristics*).

All experimental runs, with heating, confirmed the capability of *Vibrio* sp. bacteria to metabolize and reproduce under very low atmospheric pressure of 0.01–0.1 mbar and Martian-like temperature conditions. We found that short heating periods were crucial for the bacterial reproduction, because *Vibrio* sp. is non-extremophile bacteria. We carried out a separate experimental run in which the heating was turned off in the chamber and the temperature was thus maintained at 200 K (see Table 2, runs 7–9). The results of this experiment show no growth of bacteria within the 10% error compared to the control samples, although the sublimation of ice occurred due to low pressure maintained in the chamber.

## Discussion

### *Uncertainties and limitations*

Although the P-T conditions in our Martian simulation chamber were close to the current Martian conditions, we made a few assumptions that may or may not be correct for Mars and our experimental setup had a few limitations, which should be addressed in future studies:

(a) *Nutrients*. We performed our Martian simulations on *Vibrio* sp. and since it is a heterotrophic microorganism, it needs organic substrate (glucose) for growth. The Viking mission did not detect organic matter on Mars. Therefore, it was assumed that the concentration of organics in the upper layer of the Martian soil should have been less than  $10^{-9}$  g/g (Biemann *et al.* 1976) and the possibility for the Martian biosphere was put into question. However, recent research (Navarro-González *et al.* 2006) showed that the thermal volatilization-gas chromatography-mass spectrometer method used in the Viking mission was not able to detect any organic compounds in samples taken from Earth’s deserts at the levels of  $10^{-5}$ – $10^{-4}$  g/g and even higher

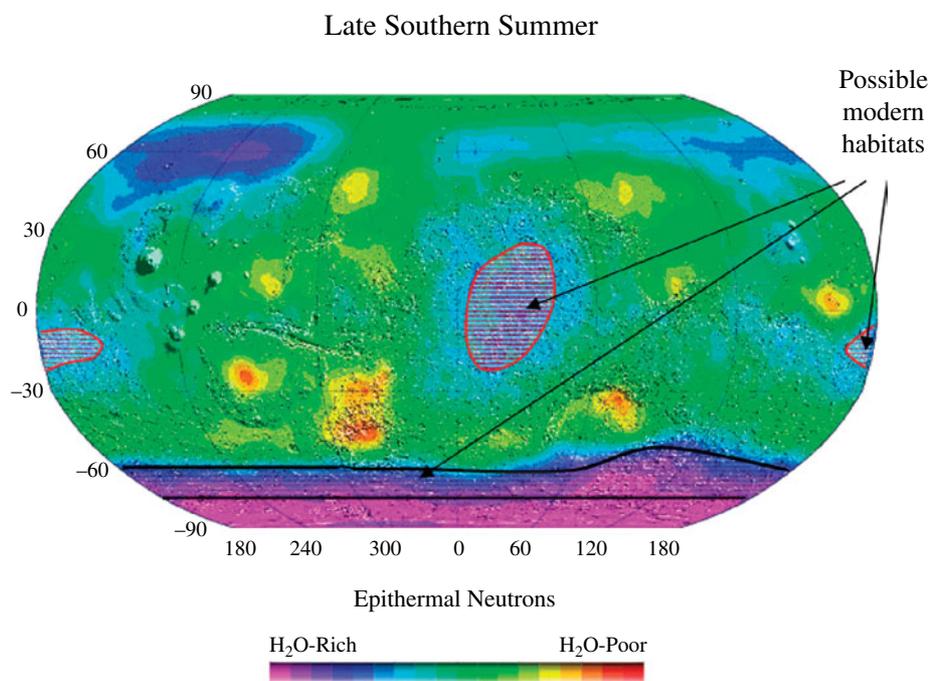
(although see Biemann (2007) for an alternative point of view). Hence the real abundance of organic matter in the Martian soil could have been much higher and the addition of  $10^{-5}$ – $10^{-4}$  g/g of organics to the sand in our experiments is not in contradiction to the Viking observations. Nevertheless, the level of Martian organic abundance in the shallow soil is an open question and we plan to study the reproduction of the autotrophic microorganisms in our chamber in future studies.

(b) *Chamber pressure*. We performed our experiments at 0.01–0.1 mbar of air pressure instead of 5–7 mbar of  $\text{CO}_2$ . The cryogenic pump in our setup removes  $\text{CO}_2$  very effectively and we could not maintain 5–7 mbar. However, we do not view lower pressures in the chamber as a disadvantage to our results or conclusions. At low pressures (up to  $\sim 1$  mbar), water molecules from sublimating ice predominantly collide with the walls of the soil grains rather than with other atmospheric gaseous molecules. Therefore, in this regime, termed Knudsen diffusion, the other gases do not affect the transport, and the diffusion flux of water vapour ( $J$ ) depends only on the gradient between the water vapour density inside the soil pores ( $\rho_1$ ) and in the inner chamber space ( $\rho_0$ ), and the depth of the soil layer ( $\Delta z$ ):  $J = -D \cdot (\rho_1 - \rho_0) / \Delta z$ . Because  $\rho_1$  is much higher than  $\rho_0$ , the mass flux depends mostly on  $\rho_1$  (Hudson *et al.* 2007) and the background gas composition should be irrelevant. If the gas pressures in the chamber were 5–7 mbar, then collisions of water vapour with  $\text{CO}_2$  might become important. However, additional collisions with  $\text{CO}_2$  would only slow down the diffusion of the water vapour. Hence, the conditions for supersaturation in the soil (and liquid films of water) could be achieved even under lower spike temperatures.

(c) *Soil selection*. We did not have access to the standard JSC Mars-1 analogue. However, for our experiments the most important soil characteristic is the soil vapour diffusion coefficient. The average coefficient of the vapour diffusion through the sand layer in our chamber was  $(1.55\text{--}1.65) \times 10^{-4} \text{ m}^2 \text{ s}^{-1}$ , which was estimated by measuring the total weight decrease of the initial soil sample with ice (due to ice sublimation) over the duration of one diurnal experimental cycle. This value is close to the diffusion coefficient  $1.74 \times 10^{-4} \text{ m}^2 \text{ s}^{-1}$  of the Martian regolith analogue JSC Mars-1 (Chevrier *et al.* 2007). Note that the typical theoretical estimate of the vapour diffusion coefficient is also  $\sim 10^{-4} \text{ m}^2 \text{ s}^{-1}$  (Smoluchowski 1968; Schorghofer & Aharonson 2005). Therefore, we would not expect a significant change of results if our sand layer were substituted with JSC Mars-1, although this has to be checked in future studies.

### *Comparison to previous studies*

We have discovered that the reproduction of the terrestrial non-extremophile microorganisms, *Vibrio* sp., is possible in the Martian-like shallow subsurface environment even if the gaseous pressure above the soil is only  $\sim 0.1$  mbar.



**Fig. 5.** Locations of the hypothetical habitats on Mars.

Our results are not in conflict with the results of Schuerger & Nicholson (2006) due to significant differences in the experimental setup. Schuerger & Nicholson simulated the effects of the combined stress of low pressures and somewhat low temperatures. They observed a failure of growth and germination of *Bacillus* spp. strains for atmospheric pressures below 25 mbar. They placed the unsealed Petri dishes with the bacterial culture into the chamber at low atmospheric pressure. The bacteria were planted onto the surface of agar media. Both the bacteria and the surface layer of agar should have been losing water at a high rate due to direct contact with the low atmospheric pressure. Therefore, the environment probably became too dry for the bacterial growth, at least in the top layer of their Petri dishes. In fact we observe this effect as well – the top ~0.5 cm of soil exposed to low pressures in our chamber looks completely dry (see Fig. 4) and not favourable for life. However, the soil at 1 cm depth clearly is not dry. In our experiment, water vapour from the ice sublimation had to diffuse through the layer of sand. Water vapour can adsorb onto sand grains during diffusion, forming thin layers of liquid films. Our experiments show that ordinary terrestrial non-extremophile bacteria were able to take advantage of those films. Therefore, we speculate that terrestrial bacteria could reproduce on Mars in the areas with shallow ground ice and high peak temperatures if there is an adequate nutrient supply.

#### Implications

Large diurnal temperature variations have been observed on Mars by all Martian missions. These variations cause intensive sublimation of the ground ice in daytime and condensation of water vapour at night. The map of the peak

surface temperatures is shown in Fig. 2. Note that areas with temperatures above 0 °C spread from the equator to 40° north latitude and to 75° south latitude. Summer temperatures at mid latitudes and polar regions of the southern hemisphere are much higher than in the northern hemisphere, because the southern hemisphere summer occurs when Mars is in its perihelion position.

Analysis of the reported ice table depths map (Boynton *et al.* 2002; Feldman *et al.* 2002; Mitrofanov *et al.* 2002) combined with data on peak surface temperatures (Fig. 2 from Haberle *et al.* 2001) and our experimental results on *Vibrio* sp. growth allow us to identify three areas on the surface of Mars with the best chances of harbouring the active biosphere (Fig. 5). The area between 60° and 75° in the southern hemisphere and two small equatorial regions (Fig. 5) have the shallow ground ice or subsurface liquid water (Bryson *et al.* 2008; Chevrier & Altheide 2008) and the proper temperature conditions for the *Vibrio* sp. biota to grow and reproduce at the present Martian conditions. Note that the annual average temperatures at these latitudes are low, but it only takes a few hours of peak heating for bacteria to grow. Our estimate of the potential habitat is quite conservative for two reasons. Firstly, it is possible (although we did not test it) that the films of liquid water might occur even without intense sublimation at higher atmospheric pressures (the northern hemisphere on Mars). Secondly, *Vibrio* sp. is not an extremophile and it is highly possible that the habitat for extremophiles would be much larger.

Long-term variations of the ground ice table depth due to oscillations of obliquity over a main period of 120 000 years have been discussed in a number of papers (Head *et al.* 2003; Chamberlain & Boynton, 2004). Curiously, the mean ground

ice depth was predicted to be only a few centimetres at all latitudes during the periods of high obliquity ( $>27^\circ$ ). Based on our experiments, it is possible that hypothetical Martian microorganisms could spread in large areas of Mars during those periods.

## Conclusion

Our experiments demonstrate the possibility for metabolic activity and the reproduction of terrestrial non-extremophile bacteria in the Martian-like shallow subsurface layer at very low atmospheric pressure of 0.01–0.1 mbar and cold average temperatures. The necessary conditions for metabolism and reproduction are the sublimation of ground ice through a thin layer of Martian soil and short episodes of warm temperatures in the vapour diffusion layer. At present such habitats could occur on Mars between  $60^\circ$  and  $75^\circ$  in the southern hemisphere and possibly in two equatorial regions.

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