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Results on the survival of cryptobiotic cyanobacteria samples after exposure to Mars-like environmental conditions

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Abstract: Tests on cyanobacteria communities embedded in cryptobiotic crusts collected in hot and cold deserts on Earth were performed under Mars-like conditions. The simulations were realized as a survey, to find the best samples for future research. During the tests organisms have to resist Mars-like conditions such as atmospheric composition, pressure, variable humidity (saturated and dry conditions) and partly strong UV irradiation. Organisms were tested within their original habitat inside the crust. Nearly half of the cryptobiotic samples from various sites showed survival of a substantial part of their coexisting organisms. The survival in general depended more on the nature of the original habitat and type of the sample than on the different conditions they were exposed to. The best survival was observed in samples from United Arab Emirates (Jebel Ali, 25 km SW of Dubai town) and from Western Australia (near the South edge of Lake Barley), by taxa: *Tolypothrix byssoidea, Gloeocapsopsis pleurocapsoides, Nostoc microscopicum, Leptolyngbya* or *Symploca* sp. At both places in salty desert areas members of the Chenopodiaceae family dominated among the higher plants and in the cryptobiotic crust cyanobacterial taxa *Tolypothrix* was dominant. These organisms were all living in salty locations with dry conditions most of the year. Among them *Tolypothrix, Gloeocapsopsis* and *Symploca* sp. were tested in Mars simulation chambers for the first time. The results suggest that extremophiles should be tested with taken into account the context of their original microenvironment, and also the importance to analyse communities of microbes beside single organisms.

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Key words: extremophiles, cyanobacteria, cryptobiotic crust, Mars, simulation.

Introduction

An important step towards the understanding if Earth-like life is able to survive on Mars and what kind of steps should be taken to realize planetary protection is the realization of analysis on the survival of microbes under Mars-like conditions. In this work, we present the results of our Mars simulation tests performed in the DLR Mars Simulation Facility (DLR-MSF), Berlin. The aim of this study is to analyse the survival after being exposed to different conditions we might encounter on Mars, as there are various parameters such as very low temperatures, gas composition, low gas pressure, low amounts of relative humidity and exposure to UV irradiation that might have important effects on many organisms. The parameters were adjusted artificially and were computer controlled and monitored by the use of a set of sensors inside the simulation chamber.

We realized survey-like analysis on the survival of different cyanobacteria. We are interested in the analysis of microbes inside cryptobiotic crusts, where several taxa of cyanobacteria are present in between mineral grains and weathering products, providing a small but complex ideal environment for survival in extreme conditions. Besides the simulation results, we also put emphasis on the possible benefits of realizing Mars-like simulation chamber tests with microbes embedded in their natural microenvironment (cryptobiotic crust) and to highlight the Mars relevant issues of these crusts. Using the experiences an outlook on an optimized realization of future chamber tests will be given.

Cryptobiotic crusts as microhabitats

The survival of cyanobacteria inside cryptobiotic crust samples was analysed. A cryptobiotic crust is often also called as microbiotic soil crust (Eldridge & Greene 1994), 'biological soil crust' (Belnap *et al.* 2001) or 'cryptogamic crust' (Strandling *et al.* 2002). We use the term of 'cryptobiotic crust' for the 0.05–5 mm thin layer on isolated or somewhere in shadowed rock surfaces on Earth, composed of weathered minerals and cryptogamic organisms (bacteria, algae, small sized fungi, lichens and small-sized bryophytes) embedded in a mucilagineous sheath (envelope) of cyanobacteria, algae and

related microfauna. The grains and organisms are agglutinated together. The major part of these organisms use sunlight for photosynthesis, and are in metabolic connection with the atmosphere, from where they gain water and carbon dioxide and keep it inside the crust as long as possible. After rewetting they resumes photosynthetic activity very fast (Campbell 1979; Potts & Friedmann 1981).

Some cryptoendolithic communities living under harsh conditions, such as in the McMudro Dry Valleys of Antarctica (Friedmann 1986; de la Torre *et al.* 2003) were already proposed as possible semi-analogue for possible Martian living organisms. Such crusts can be described only as semi-analogues, because certain environmental conditions (temperature, humidity and UV radiation) are different on Earth than on Mars, and these Mars-like stress conditions are never present together at the same location on terrestrial analogues.

The most important components of cryptobiotic crust that play a central role in these communities are cyanobacteria. They may serve as semi-analogues for hypothetic Martian organisms, because most of the year they are in a dormant state thats allows them to resist very dry periods, a capacity that might be of high relevance on Mars (Pocs *et al.* 2003, 2004). Cyanobacteria are also used because they photosynthesize after reactivation in the presence of water. This activity can be measured easily and can be one indicia of survival after being exposed to Mars-like conditions. The cyanobacteria take even part in the C and N circulations, produce Mycrosporin-like components for UV screening, tolerate salt concentration up to 200 g l^{-1} (Oren & Seckbach 2001) and they produce mucilaginous sheath what allows their preservation during the dry season (Dor & Danin 2001). Cyanobacteria are adapted to a wide range of temperatures, and at many locations the same taxa are present in hot and cold deserts (e.g. the *Microcoleus* species). They accumulate trehalose as a water replacement mechanism to maintain the functional integrity of membranes during anhydrobiosis (Wynn-Williams 2000).

Materials and methods

Next we describe the samples ('The samples used for the Mars simulation experiments' section) that were used for the tests and which were exposed to the conditions with varying parameters inside the simulation chamber. This section also contains the description of the photosynthesis analysing methods before and after the tests ('Pre-tests on the selected samples' section), as well as the chamber itself and the parameters of the Mars-like environmental conditions there ('Simulation chamber and realized tests' section).

The samples used for the Mars simulation experiments

The samples were collected from hot and cold deserts during the last 6 years and stored at a dry airing cupboard inside closed hermetic boxes at room temperature. The individual rock samples' diameters were between 3 and 8 cm and contained the nearly intact (unweathered) rock and the cryptobiotic crust in their shallow subsurface. The specimens were sliced into pieces with the entire size between 1 and 2.5 cm, and were placed in 3 cm diameter Petri-dishes with two samples in each dish. The samples were fixed by glue or double-sided tape before exposure to the simulated Mars-like conditions. The samples were numbered according to their locations where they were collected Table 1.

Pre-tests on the selected samples

Photosynthesis involves different pigments that absorb visible light of different wavelengths as chlorophyll absorbing the red and blue ranges, carotenoids absorbing the blue range, and phycobilins that are present in some species, absorbing from red to blue wavelengths. Before and after the chamber test the state of the organisms in the samples were checked by Olympus BX51 microscope with Nomarski DIC epifluorescent illumination in the Limnological Research Institute of the Hungarian Academy of Sciences (Tihany). The used excitation light was blue-violet (400–490 nm) and green (490–540 nm). In these bands the red fluorescence of active chlorophyll, active phycocyanin and phycoerithrin, and the bluish green fluorescence of fungal hyphae, and greenish yellow fluorescence of dead cells were observed. After excitement by the given light sources cyanobacteria cells showing red fluorescence have intact active photosynthetic apparatus. Green coloured material was composed of other substances or dead cells, dying cells are emitting a yellow coloured fluorescence (Fig. 1).

Simulation chamber and realized tests

The experiments were carried out at the MSF of the DLR Institute of Planetary Research in Berlin (Kochan *et al.* 1996, 2000; Mohlmann *et al.* 2006; Corinna *et al.* 2010; Panitz *et al.* 2010). The MSF is part of the Department of Experimental Planetary Physics and is used to perform laboratory experiments with controlled time-profiles (e.g. simulated diurnal variations) of temperature down to about 198 K. Atmospheric pressure and composition (including humidity) can be modified to simulate conditions on Mars. In particular, the MSF can be set for thermophysical conditions typical of Martian mid- and low latitudes (Maturilli *et al.* 2012). The different parameters can be realized separately as well as in combined modus. The MSF's measurement categories and controllable parameters included are summarized in Table 2.

Description of experiment and equipment

The main part of the MSF is a climate chamber (CC) with inside dimensions of 80 cm height, 60 cm depth and 50 cm width. The experiments were performed in the 'experimental chamber' (EC: inside the climate chamber), which can be cooled separately from the CC. The EC is of stainless steel, forming a cylinder with a volume of 10.3 litres with an inner diameter of 20.1 cm and an inner height of 32.4 cm. There are connections through the top plate for gas flows and electrical contacts (50 pins each in two D-Sub connectors; 100 in total) inside the top plate to connect to internal sensors or devices). The sensors can be placed 1 cm above the samples.

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Table 1. *The analysed cryptobiotic samples. (* The sample consists of the lichen thallus of Collema, in which the fungus mycelia embed the symbiont cyanobacterial filaments of a* Nostoc *species, which is responsible for the photosynthetic activity, CBC is for cryptobiotic crust.)*

Sample no.	Location	Taxa	Description
11–17 (05128/III)	Bihar Mts., Romania	*G. pleurocapsoides, Gloeocapsa alpina, Gloeocapsopsis dvorakii*	On dry, half shady marble cliff near the entrance of Bear Cave (Pestera Urşului) at 500 m altitude
11–17 (07080/III)	Slovakia, Slovenský Raj. Stratenská Dolina 2 km ESE of Stratená	*Chroococcus lithophilus* (dominant), *G. pleurocapsoides*	S facing, dry limestone rocks at 890 m alt. 'Tintenstriche' (temporary watercourse)
21–27 (01069)	Australia, Northern Territories. W Macdonnel Ranges	*T. byssoidea* (dominant), *G. pleurocapsoides*, *N. microscopicum* and *N. minutissimum* in the upper layer, in the −0.1 to 0.4 mm deep lower layer *Schizothrix* aff. *kialingensis* without UV screening pigment	Open *Chenopodiaceae* semidesert in temporarily wet depression, 46 km WSW from Alice Springs, at 630 m alt.
21–27 (09001/I)	United Arab Emirates, Jebel Ali, 25 km SW of Dubai town	Scattered *Chroococcales* intermixed in the sandy soil, *Collema* sp. *.	Temporarily waterlogged depression in a coastal saltpan with desert vegetation dominated by *Chenopodiaceae* at 5 m alt.
31–37 (04197/I)	Western Australia. Dried out W branch of the salt Lake Barley along Youani Road, at 409 m alt.	Top layer: *T. byssoidea*. Subsurface layer to – 1 mm: *Leptolyngbya* or *Symploca* sp. + mycelia of fungi	
31–37 (04195/I)	Western Australia. near the S edge of Lake Barley, 410 m alt.	Top layer: *T. byssoidea* (dominant), *Microcoleus paludosus.* Subsurface layer 0–1 mm: *Crinalium epipsammum, Symplocastrum friesii* (dominant), *Microcoleus vaginatus.* Bottom layer 1–3 mm: *Symplocastrum penicillatum. Lyngbyella* sp.	dry (from October to April) salt lake bottom, with well developed trilayered CBC
41–44 (04195/I)	Western Australia. 410 m alt.	Top layer: *T. byssoidea* (dominant), *M. paludosus.* Subsurface layer to −1 mm: *C. epipsammum, S. friesii, M. vaginatus*. Bottom layer −1 to 2 mm: *Lyngbiella* sp., *S. penicillatum*	Dry (from October to April) salt lake bottom with well-developed trilayered CBC
41–44 (09001/B)	United Arab Emirates, Jebel Ali, 25 km SW of Dubai town	Lichen on soil, *Collema* sp. *	Coastal salty desert (sabkha) vegetation, dominated by *Chenopodiaceae*

A 'gas-mixing system' (GMS), which includes controls for humidity and a PC-based data and control unit completes the system. The GMS was developed to simulate planetary atmospheric conditions with respect to varying compositions and amounts of gases. The equipment is computer controlled to actively selected dew points [− 75 °C (198 K) to +10 °C (283 K) at 101 325 Pa], gas mixing rates and flow rates. The experimental setup allows the mixing of up to five gases. Six mass flow controllers (MFC) regulate the flow of the different gases and the moistened carrier gas (e.g. air) into the GMS. The gases are mixed in a pipe system, whereas the moist gas is produced before in thermally controlled scrubber bottles. Air or any other gas can be used as a carrier. Air, provided by a compressor is dried in two steps to a frost point of −74 °C (199 K) using a permeation dryer. The other gases are bottled. Humidity correlates to a water vapour pressure range of about 7–0.00073 Pa at 600 Pa ambient pressure, which corresponds to the expected H_2O vapour pressure on Mars. MFC's with a flow rate of 1.5–75 litres h^{-1} at standard ambient temperature and pressure regulate the maximum generated gas flow of 155 litres h^{-1}. Two membrane vacuum pumps regulate pressure (from 200 to 101 325 Pa). All experimental parameters and data are PC-controlled and logged (using LabView).

Configuration inside the experimental chamber

Sensors were also arranged inside the chamber at the sample holder, two PT100 temperature sensors and one capacitive humidity sensor. The humidity sensor and a PT100 were attached close to the biological samples (1–2 cm). The second PT100 was affixed in the middle of the chamber.

Prepared samples were wetted before and after the tests (0.2 ml) at 25 °C. The simulations were run under seven different condition types (tests performed separately and in combined modus) that are listed below and also in the Table 3. During the simulation the Xe-lamp irradiated the samples with the Mars-like sol spectra ($\geqslant$ 200 nm) as it appears on the surface of Mars and the PAR-LED/UV-B and some scattered light of the Mars-Xe-Lamp realized the simulation of Mars-like niche conditions (deVera *et al*. 2002).

The parameters of the tests were chosen according to produce the parameters that approach Mars-like conditions in order to identify which parameters could be limiting factors. The systematic approach of the used parameters is the following:

- the pressure of the atmosphere was stepwise changed from Earth- to Mars-like conditions in every cases (avoiding stress reactions of the organisms, as described previously);

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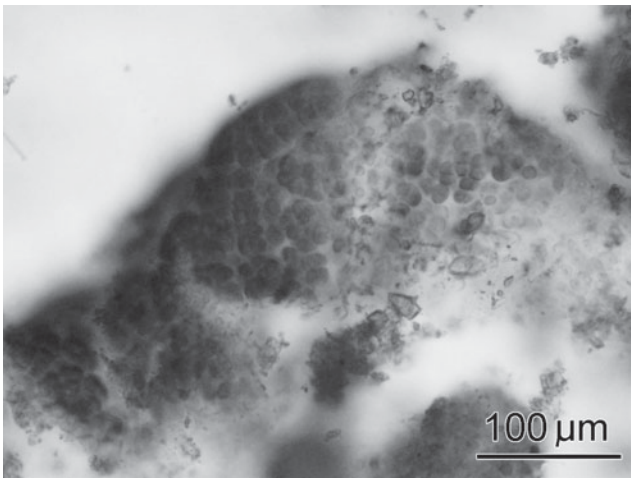

Fig. 1. Pre-test image of *Gloeocapsa* sp. from a location close to Dubai. The cells are excited by UV light. Coccoid cyanobacteria cells showing red (here dark) fluorescence have intact active photosynthetic apparatus. (Besides this, on the original photo in reality green coloured material is composed of other substances or dead cells, dying cells are emitting a yellow coloured fluorescence).

- the used atmospheric composition was Mars and also Earth like to determine the effect of changing CO_2 concentration;
- the relative humidity was used to simulate H_2O saturated conditions (both simulating surface frost and inside ice filled porous material);
- the UV radiation simulated surface conditions, the LED produced UV/VIS/PAR light simulating niche conditions with a certain amount of UV to shallow subsurface scattered or screened light.

Survival analysis

The procedure of survival analysis was performed in the following order: (1) general analysis of survival capacity before simulation experiments; (2) level of response after the simulations; (3) analysis to check potential differences in the results with respect to the applied different test parameters. After the simulation tests the samples were stored at isolated closed boxes and dry places at room temperature. Tests were realized by two methods after 2–6 months of the simulation, when samples were wetted half an hour before the epifluorescence test and for 24 h before the PAM tests using 200 microeinstein from 1 mm distance. Both tests were realized for all samples, and survival was stated where both tests showed it – actually in all survived cases both tests were positive.

Results

In this section, we are first giving an overview about the survival of some samples to the simulated conditions. Secondly, a general overview about the survival of the entire communities is listed according to the applied simulated conditions. Thirdly, we show the survival of the dominant taxa in the different characterized communities, by taken into account the different habitat locations.

Table 2. *Conditions during simulation experiments. The parameters were used separately and in combination in the experimental chamber (EC). The measurement categories which can be monitored by the use of sensors are: trace humidity in gases, water activity, material- and soil-moisture, pressure, temperature, photosynthetic activity, volume flow of gases, current, resistance, voltage, gas analysis*

Parameter	Range
Relative humidity	0–100%
Pressure	600 Pa
Temperature	− 50 °C + 20 °C (diurnal cycle with stepwise increase/decrease)
Gas mixture	95% CO_2, 4%N_2, 0.97% air, 0.03% H_2O
Controlled time profiles	humidity, gas mixture, temperature, pressure, LED-illumination (Mars-like niche irradiation), UV-irradiation (Mars-like surface irradiation)
Irradiation with xenon lamp via fibre (e.g. inside the EC)	spectral range from 0.25 to 2.2 µm with 0–0.4 W m^{-2} × nm on a 13 mm diameter spot – manually controlled

Among the 50 tests realized with different samples and conditions, in 29 cases the survival of samples was confirmed by both of the two methods used after the simulation tests. The diagrams in Fig. 2 are representing some examples of the photosynthetic activity of samples that survived the tests. Dark grey colour marks the control sample measurements, whereas bright grey marks the samples witnessed the chamber conditions. The vertical axis [effective quantum yield (EQY)] shows the level of response, while the tests were realized at 200 actinic light (µE m^{-2} s^{-1}) of photosynthetically active radiation (PAR). The examples, left and middle show samples where most of the organisms survived the simulated Mars-like conditions. Here the control samples presented roughly the same level of response than those that witnessed the chambers. The third example of the right shows, in contrast to the other, two of the organisms which survived the applied conditions in the simulation chamber reached higher activity levels when compared with the control measurements. Such behaviour was observed in approximately 14% of all tested samples.

General overview on the survival of all communities

Table 4 gives an overview of the survival according to the conditions in the chamber. Here we indicate these conditions separately, although their detailed analysis would require more targeted tests to see the effect of individual parameters. That is the aim for the future, here the aim was only to 'survey' the possibilities and select the best candidates for future analysis. The results indicated in Table 4 suggest that there was probably no one absolute limiting factor that would have prohibited the survival of all of the organisms in the analysed samples.

As a general summary it could be stated that no one of the used conditions: atmospheric pressure (Earth/Mars like), relative humidity (dry/saturated) and presence of UV radiation was absolute limiting factor. Certain organisms inside the

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Table 3. *Conditions of the seven different types of test run. The pressures were decreased in all experiments in stepwise approximately of about 60 mbar by every 30 min to avoid stress-reactions where positive measurements might not be possible and a long recovery might be possible after the simulation experiment (*CO_2 *atmosphere means mostly* CO_2*, together with the minor components described in Table 2.)*

No. of test	Gas composition	Relative humidity (%)	Pressure (p) (Pa)	Radiation	Exposure time	Samples
1	Earth-like (380 ppm CO_2)	75–100	Decrease from Earth-like $p = 101300$ Pa to Mars-like $p = 600$ Pa	LED (UVB/ VIS/PAR)	1 day	05128 II (11), 01069 (21), 09001 II (21), 04197 I (31), 04195 II (31), 04195 I (41), 09001 B (41)
2	CO_2	75–100	Decrease from Earth-like $p = 101300$ Pa to Mars-like $p = 600$ Pa	LED (UVB/ VIS/PAR)	1 day	05128 II (12), 07080 III (12), 01069 I (22), 09001 I (22), 04197 I (32), 04195 III (32), 01195 I (42), 09001 B (42)
3	Earth-like (380 ppm CO_2)	0	Decrease from Earth-like $p = 101300$ Pa to Mars-like $p = 600$ Pa	LED (UVB/ VIS/PAR)	1 day	05128 II (13), 01069 (23), 04197 I (33), 07080 II (13), 09001 I (23), 04195 III (33)
4	CO_2	0	Decrease from Earth-like $p = 101300$ Pa to Mars-like $p = 600$ Pa	LED (UVB/ VIS/PAR)	1 day	05128 II (14), 01069 I (24), 04197 I (34), 07080 III (14), 09001 I (24), 04195 III (36)
5	CO_2	75–100	Decrease from Earth-like $p = 101300$ Pa to Mars-like $p = 600$ Pa	UV (sol $\lambda \geqslant 200$ nm)	1 day	05128 II (16), 01069 (26), 04197 I (36), 04195 I (43), 07080 III (15), 09001 I (25), 04195 III (35), 09001 B (43)
6	CO_2	0	Decrease from Earth-like $p = 101300$ Pa to Mars-like $p = 600$ Pa	UV (sol $\lambda \geqslant 200$ nm)	1 day	05128 II (16), 01069 (26), 04197 I (36), 07090 III (16), 09001 I (26), 04195 III (36)
7	CO_2	0–100 (daily cycle)	Mars-like pressure $p = 600$ Pa	LED (UVB/ VIS/PAR)	4 day	05128 II (17), 01069 I (27), 04179 I (37), 01495 I (44), 07080 III (17), 09001 I (27), 04195 III (37), 09001 B (44)

crusts survived any of the conditions and all of the used combinations.

Survival of various communities

As we are interested in the survival of the different communities of organisms that are embedded in the crust and mineral matrix, we list below the results of survival according to the samples and their original locations. Table 5 lists those samples where both epifluorescence and PAM 101-103 have indicated the survival of the microorganisms in the crust. With this 'survey method' only the general survival can be determined (the survival at least of the dominant taxa in that community). The aim of this kind of analysis was to select and identify the best locations where samples can be collected for future tests, where under Mars-like conditions additional test runs can further be started with extending the exposure times and with the use of isolated cultures gained from the collected different sample communities.

The above presented list (Table 5) contains samples from three locations: 09001 (survival at seven test types), 01069 (survival at six test types), 04197 (survival at 11 test types). The test types do not differ substantially from each other. The observations suggest the survival in general depends on the origin and the type of sample, e.g. the different mineral composition within the crust as well as the differing composition of microorganism associations analysed here which might be triggered differently by the conditions they witnessed.

There was no survival in the case of samples from 05128/III (Bihar Mts., Romania), 07080/III (Slovakia, Slovenský Raj) locations. The main difference between 'no-survival' and the above mentioned three 'high survival' locations are that the samples from these 'good locations' were characterized in all of these cases by as dry salty desert regions, with low humidity and good salt tolerance. It is known that the adaptation for salty environment on Earth (Todd & Irwin (2000) helps to preserve cells and to maintain their survival capacity (Krumbein *et al.* 2004; Stan-Lotter *et al.* 2004; Baxter *et al.* 2007). This would be one of the main important factors which might allow resisting to all of the main environmental factors we might encounter on Mars (as there are: low temperatures, low gas pressure and dryness).

Survival of various organisms

Below in Table 6 we list the survival grouped according to dominant taxa in the samples and not only according to the samples of different origin. One sample was usually dominated by one taxa, but there were several cases (such as 21u, 31l, 41l, 22u and 42u), where the same sample was composed of two taxa in bigger amount.

To conclude we have to differentiate as follows:

- The results showed that differences exist in the survival behaviour of analysed organisms and communities. We had to differentiate the results taking care of the analysed

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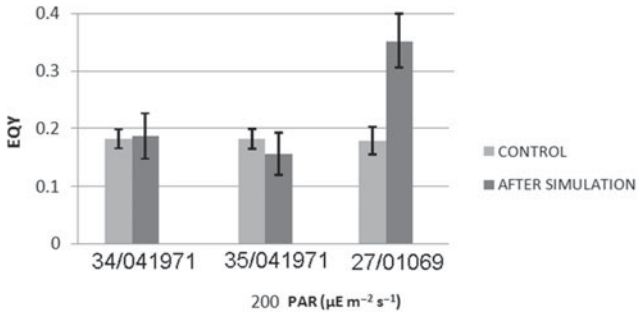

Fig. 2. Characteristic example diagrams for survival, comparing the control samples (light grey) and those that were exposed to the simulation conditions (dark grey) measured with four different levels of actinic light. The vertical axis shows EQY. In the left and middle cases roughly the same response by the treated and the control samples is measured, whereas in the right example presents an interesting case where the response of the organisms was better after the simulation test if compared with the control. The used samples: (a) and (b) were *Tolypothrix byssoidea, Leptolyngbya* or *Symploca* sp. from a dried out salt section of Lake Barley in Western Australia; whereas (c) *T. byssoidea* (dominant), *Gloeocapsopsis pleurocapsoides, Nostoc microscopicum*, from Australia, Northern Territories, W Macdonnel Ranges. The used test conditions were the followings – left: under Mars CO_2 atmosphere + Mars pressure with low humidity rh ~0 and LED-light; middle: under Mars CO_2 atmosphere + Mars pressure + UV and high humidity rh ~1 and LED-light; right: under CO_2 atmosphere + Mars pressure, temperature down to −50 °C + LED light, for 4 days with diurnal temperature cycles 16 h light, 8 h night.

categories as there were various type of tests (exposure conditions) and different observed composition of the groups of taxa, which were exposed (different tolerance).

- Survival of taxa in several samples was observed in every test type, suggesting that there was no absolute limiting factor.
- The survival rates in most cases (both for taken into account the type of sample and the applied test conditions) were relatively high above 60% (one exception was observed for the test conditions in test type 3).
- The gas composition seems not to be a strong limiting factor, as there were no characteristically worse survival rate for the CO_2 composed atmospheres (tests 2, 4, 5, 6, 7) than for Earth-like atmospheres (tests 1, 3).
- The worse survival rate for many samples was observed at the test type 3. The reason for it is difficult to identify as there were many factors analysed and varied in our work, but the very dry conditions (humidity near to zero) might be a reason
- The worse tolerance in general was observed at *Chroococcus, Chroococcales* (25–41% of all tests together), whereas the best general tolerance was observed at *Nostoc* with 100% survival in both cases.

Discussion

In this section, we discuss the general characteristics of the survival after being exposed to the Mars-like conditions.

Table 4. *Survival rate according to the selected conditions in the chamber (gas composition was CO_2 in every cases, *marks cyclic changes between 0 and 1 in relative humidity)*

Conditions in the chamber (no. of test type)	Ratio of samples survived
Pressure: Earth-like (1, 3)	31% (4 of 13)
Pressure: Mars-like (2, 4, 5, 6, 7)	64% (21 of 33)
0 relative humidity (3, 4, 6, 7*)	50% (9 of 18)
1 relative humidity (1, 2, 5, 7*)	54% (13 of 24)
LED light (1, 2, 3, 4, 7)	56% (18 of 32)
UV radiation (5, 6)	57% (8 of 14)

Table 5. *List of samples where the epifluorescence and the PAM 101-103 analysis both demonstrated survival of substantial amount (~ 50%) of the organisms*

United Arab Emirates, Jebel Ali, 25 km SW of Dubai town	Western Australia, near the S edge of Lake Barley, 410 m alt.	United Arab Emirates, Jebel Ali, 25 km SW of Dubai town
21l – 09001/I	31l – 04195/III	41l – 09001/B
21u – 01069	32l – 04195/III	42l – 09001/B
22u – 01069	34l – 04195/III	43l – 09001B
23l – 09001/I	34u – 04192/I	44l – 09001/B
23u – 09001/I	35l – 04195/III	
24u – 01069	35u – 04197/I	Western Australia, near the S edge of Lake Barley, 410 m alt.
25u – 01069	36l – 04195/III	
26l – 09001	36u – 04197/I	
26u – 01069	37l – 04195/III	41u – 04195/I
27u– 01069	37u – 04197/I	44u – 04195/I

We are focusing first on the temperature, then the importance of the organisms' microenvironment and their analysis as a whole community instead of individual taxa. Finally, we summarize the differences between various samples which survived.

Survival and temperature issues

Among the 50 realized tests, nearly half of the analysed samples showed survival, although the survival was observed after being exposed to different simulated Mars-like conditions. The harshest conditions the organisms have to face were the simulation experiments that were as close as to Mars-like environments, excluding the lowest temperature range. In our tests, we did not go below −40 °C because of technical reasons. On Mars the absolute minimum is at the frost point of carbon-dioxide that depends on the local atmospheric pressure but is about −122 °C (Kahn 1985; Kieffer *et al.* 2000).

The most important limiting factors of the low temperature are: (i) the lower chemical activity (and metabolic rate if taking into account the active metabolism and not just survival) and (ii) the mechanical consequences of the water ice crystal formation and at even lower temperature the contraction of this ice. In our cases, the pure H_2O froze about 0 °C in bulk volume and at even lower as a thin interfacial layer (Zent *et al.* 1993; Mohlmann 2004, 2009, 2010).

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Table 6. *Survival according to taxa groups (certain taxa might occur together with another one, but here they are separately indicated below). The survival marked by bold and shaded style at the two digit sample number. The conditions for each test type are available in Table 3*

Taxa name	Test type 1	Test type 2	Test type 3	Test type 4	Test type 5	Test type 6	Test type 7	Ratio of taxa survived
Gloeocapsopsis, Gloeocapsa	11u, **21u, 22u**	12l	13l, 13u, **23u**	14u, 14l, **24u**	15l, 15u, **25u**	16l, 16u, **26u**	**27u**	41% (7 of 17)
Chroococcus, Chroococcales	**21l**	12l, 22l	13l, **23l**	14l, 24l,	15l, 25l	16l, **26l**	27l	25% (3 of 12)
Tolypothrix	**21u,31l,** 31u, **41u**	**22u, 32l,** 32u, **42u**	**23u,** 33l, 33u, 43u	**24u, 34l, 44u**	**25u, 35l, 35u**	**26u, 36l, 36u**	**27u, 37l, 37u**	79% (19 of 24)
Nostoc	**21u,**	**22u**	**23u**	**24u**	**25u**	**26u**	**27u**	100% (7 of 7)
Microcoleus	**31l, 41u**	**32l, 42u**	33l, 43u	**34l, 44u**	**35l**	**36l**	**37l**	82% (9 of 11)
Crinalium	**31l, 41u**	**32l, 42u**	33l, 43u	**34l, 44u**	**35l**	**36l**	**37l**	82% (9 of 11)
Symplocastrum	**31l,** 31u, **41u**	**32l,** 32u, **42u**	33l, 33u, 43u	**34l, 44u**	**35l, 35u**	**36l, 36u**	**37l, 37u**	59% (10 of 17)
Ratio of samples survived	82% (14 of 17)	69% (11 of 16)	28% (5 of 18)	75% (12 of 16)	69% (9 of 13)	77% (10 of 13)	90% (9 of 10)	

Mechanical consequences and cell damage is produced around the freezing temperature that takes place most of the water ice between 0 and about −10 °C. At the minimal temperature the samples witnessed in the chamber of −40 °C nearly all of the water content is frozen, and the chamber experiments were suitable to test the mechanical cell damage. At temperatures even lower than −40 °C only small mechanical contraction might take place, and substantial changes are not expected.

Below 0 °C the ratio and mass of thin liquid water along the contact surface between H_2O ice and non ice materials water ice contracts decreases exponentially, having only few molecule thin liquid layer below −40 °C down to about −75 °C (Mohlmann 2004). While the existence of this thin liquid film plays an important limiting role in the metabolic activity at subzero temperatures, the aim of this study was the analysis of survival instead of checking the dependence of metabolic activity on presence or absence of liquid water.

Best taxa showing survival

Analysing the survival from statistical point of view, the best survival was observed at samples: United Arab Emirates (Jebel Ali, 25 km SW of Dubai town) and Western Australia (near the S edge of Lake Barley, 410 m alt.). These organisms survived most of the simulated conditions inside the chamber. The main reason for the high survival results is connected to the resistance of the different taxa, probably enhanced by the association form and composition of the microorganism community, and by the microscopic properties (small voids and hygroscopic polyssacharides) of the crust itself.

The dominant organisms inside the samples which have shown the best survival were: *Tolypothrix byssoidea, Gloeocapsopsis pleurocapsoides, Nostoc microscopicum* (United Arab Emirates), and *T. byssoidea, Leptolyngbya* or *Symploca* sp. (Western Australia). In the context of exposure to Mars-like environments, many of them were poorly analysed. In this work, the *Tolypothrix, Gloeocapsopsis* and *Symploca* sp. were tested in Mars simulation chambers for the first time. *Nostoc* sp. was already analysed in Martian regolith simulant and vacuum (Arai *et al.* 2008) and its other properties were tested, which are relevant to astrobiology (Wang *et al.* 2007, 2010; Jänchen *et al.* 2010). *Leptolyngbya* has not been tested within Mars analogue conditions, but was mentioned in possible extraterrestrial context (Foster & Mobberley 2010; Olsson-Francis & Cockell 2010) and its adaptation to extreme conditions on the Earth was analysed (Allen *et al.* 2003), while the *Tolypothrix* group was only mentioned previously as a Mars relevant organism because of its UV resistance (Cockell 2002).

Microscopic structure

The microscopic structure of the crust is important with its densely packed and hygroscopic components because it is reaching a decrease of water loss and is also able to screen UV radiation. To get insights into what are the specific issues in this aspect of the resistance at the analysed samples and their

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communities, the best survival should be compared and are presented as follows:

- In sample 09001, a xerotolerant lichen body of *Collema* sp. the filamentous cyanobacteria (*Nostoc* sp.) were well protected by the blackish brown pigment of lichen cortex and by their own scytonemin pigment against harmful UV and heat radiation (Sorrels *et al*. 2009).
- Both samples 01069 and 04197 from Australian desert areas had multilayered structure in which the dominant upper layer consisted of a weft (dense filamentar texture) formed by cyanobactera rich in UV screening, protecting, dark violet brown scytonemin in their mucilaginous sheath. These protect the cyanobacteria well from the harmful radiation if situated in a lower layer where no UV screening pigments were present. The structure of this cryptobiotic crust type was discussed in Pocs (2009: 158) in details, as 'Multilayered, epi-endoterranean type'. The top 0.2–0.4 mm thin layer was composed of purely cyanobacteria without lithic grains. Below this, 2–3 mm thick layer of cyanobacteria were present between the rock grains with 5–20 vol%.

General and common property of the organisms inside the samples that presented the best survival was, they are adapted to salty locations with dry condirions most of the year. General characteristic of the analysed samples was that they are composed of several taxa living in symbiosis. There was interdependence between the components in time and space. Even in unilayered crusts the photosynthesizing cyanobacteria, lichens and algal components supplied organic nutrients to the heterotrophic bacteria, fungi and for the microfauna. In addition, the cyanobacteria with their mucilaginous sheath cement and UV radiation protection with screening pigments was useful for other microbes. In multilayered crusts, the situation was even more complex, being microorganisms of different metabolism types in the strata above each other, the upper zone was occupied mostly by cyanobacteria, the lower by algae, the deepest by colourless sulphur bacteria or sulphate-reducing bacteria (Seckbach & Oren 2010).

The cryptobiotic communities and the physical, chemical properties of the crust where they were living in were strongly coupled, and might show simple but important interactions that are useful under extreme conditions. Although there is no proof or even strong hint on the possible life on Mars today (Cockell *et al*. 2012), if once it appeared there in the past, it had long time to adapt (facing to an environment getting more harsh as time passed by), and as a result of adaptation during long duration it could evolved towards a more complex state than commonly assumed (Gibson *et al*. 2009). The analysis of extreme tolerant communities instead of individual organisms (de la Vega *et al*. 2007) is twofold: (1) any possible Martian biota need not be composed of only one taxa (using the Earth-related definition), and biologists are often using models with several organism groups when they are exploring the origin of life (LUCA is also taken to be a poorly defined, interconnected group of organisms (Glansdorff *et al*. 2008; Theobald 2010)); (2) the analysis of analogue communities (instead of single organism groups) could point to such behaviour that are important in the exploration of astrobiological issue in general.

Conclusion

In this section, we summarize the conclusions after having analysed the results obtained by the Mars simulation tests that were showing the survival of extremophilic microbial communities within cryptobiotic crusts mainly composed by cyanobacteria species and realized in the DLR-MSF Berlin. We analysed organisms embedded in their original rock crusts. The crust might play a major role in the survival of the organisms. The nature of the protective rock crusts might also give a hint to what kind of microenvironments are favourable on Mars for possible extremophiles. This paper reports the first results of series of tests, and the selection of the best candidates for further analysis within the context of planetary analogue environments. We used a 'survey-like' method to study many samples under various conditions realized by the chamber. Those results that might be important for the scientific community are listed below.

- The survival rates in most cases (both for sample types and test types) were relatively high, above 60%. It means that these organisms would survive at least some hours in inactive phase on Mars, despite if strong UV radiation is present there.
- Every test types were survived at least several samples, suggesting there was no absolute limiting factor regarding the effect of the used Mars relevant temperature combined with Mars-like pressure and gas composition.
- Among the simulated conditions, more organisms survived the Mars analogue environment under Martian pressure than Earth atmospheric pressure.
- Despite simulating various combinations of environmental parameters, the survival in general depends stronger on the origin (location) and the type of taxa in the sample than the differences between the conditions they were exposed to. It suggests there are some communities that show high survival in general, under any combination of the used environmental parameters.
- Most of the samples that showed the best survival were salt-tolerant organisms from United Arab Emirates, Jebel Ali, 25 km SW of Dubai town and from Western Australia, near the S edge of Lake Barley, at 410 m altitude.
- The best survival was shown by taxa: *T. byssoidea, G. pleurocapsoides, N. microscopicum* (United Arab Emirates), and *T. byssoidea, Leptolyngbya* or *Symploca* sp. (Western Australia).
- *Tolypothrix, Gloeocapsopsis* and *Symploca* sp. were tested for the first time in a Mars simulation chambers.
- Based on the favourable conditions that the crust provides for the orgnaisms which were living inside these protective envelopes, it would be important to compare tests with organims embedded in their natural microenvironment and without such envelopes.
- The analysed communitites showed high survival in general, suggesting future research might put more emphases on testing community level behaviour of extreme organims besides analysing the individual taxa. Testing such communities is relevant for astrobiology first as their interaction

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provides important results to understand the process behind survival under harsh conditions, and second as their members are simple and ancient organisms, they are still relevant for extraterrestrial conditions and do not represent highly evolved systems.

Many of the tested organisms survived Mars-like conditions, where the best survival was presented by such organisms that have salt ability and tolerance at very dry locations. Salt tolerance might be important on Mars based on the liquids which could contain elevated concentration of salts (Tosca *et al*. 2008; Altheide *et al*. 2009; Mohlmann & Thomsen 2011), and salt-tolerant organisms found among the most durable extremofiles (Abrevaya *et al*. 2008, 2011). High survival was presented by *Tolypothrix, Gloeocapsopsis* and *Symploca* sp. such taxa that have not been deeply tested on a possible Mars-like organism yet. Regarding the other dominant strains in the samples, *T. byssoidea* and *N. microscopicum* have already been identified at the interesting Mars analogue location of Schirmacher Oasis in Antarctica (Hoover *et al*. 2008) and other terrains (Stojanovic *et al*. 2008; Schmidt *et al*. 2009), and were found to be a possible candidate for Mars analogue research using their adaptation to low temperature and elevated salinity.

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