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SURVEYING THE SURVIVAL OF CYANOBACTERIA IN CRYPTOBIOTIC CRUST UNDER MARTIAN CONDITIONS

Dulai S. (1), deVera J.-P. (2), Kereszturi A. (3,4), Koncz L. (2), Lorek A. (2), Marschall M. (1,2), Möhlmann, D. (2), Pocs T. (1,3)
(1) Department of Plant Physiology and Botany, Eszterhazy Karoly College, Eger, Hungary, (2) DLR, Berlin, Germany, (3) New Europe School for Theoretical Biology and Ecology,
(4) Konkoly Astronomical Institute, Research Centre for Astronomy and Earth Sciences, HAS, (E-mail: kereszturi.akos@csfk.mta.hu)

Introduction

This work is to present results on the simulation tests realized at DLR Mars Simulation Laboratory (DLR-MSL) in Berlin; under a Hungarian German cooperation. The first report of these tests can be red in [1], here more details are summarized. The aim was to analyse the survival of cyanobacteria after being exposed to different conditions on Mars, like very low temperatures, gas composition, low gas pres-sure, low amounts of relative humidity and exposure to UV irradiation. 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 of survival of different cyanobacteria to select the best candidates for more detailed future simulation.

Figure 1. The samples after preparation in petridishes

Methods

The samples were collected from hot and cold deserts [2] during the last 6 years and stored at a dry hermetic boxes at room temperature (Figure 1.). List of sampling locations are in Table 1. Epifluorescence before the simulation (Figure 2.) test was investigated by an Olympus BX51 microscope with Nomarski DIC (epifluorescent illumination) for checking if the organisms are living inside the mineral matrix. The experiments were car-ried out at the Mars Simulation Laboratory at DLR Institute of Planetary Research that is able to control time-profiles of temperature down to about 198 K [2]. Atmospheric pressure and composition (including hu-midity) and can be set for thermo-physical conditions typical of Martian mid- and low latitudes. The parame-ters used for the 7 test run types are listed in Table 2. below.

Table 2. Realized test types

test no	gas com-po-sition	rel. hum. (%)	pressure (p) (Pa)	radiation	expo-sure time
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
2	CO_2	75-100	Decrease from Earth-like p=101300 Pa to Mars-like p=600 Pa	LED (UVB/VIS /PAR)	1 day
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
4	CO_2	0	Decrease from Earth-like p=101300 Pa to Mars-like p=600 Pa	LED (UVB/VIS /PAR)	1 day
5	CO_2	75 - 100	Decrease from Earth-like p=101300 Pa to Mars-like p=600 Pa	UV (sol $\lambda \geq 200$ nm)	1 day
6	CO_2	0	Decrease from Earth-like p=101300 Pa to Mars-like p=600 Pa	UV (sol $\lambda \geq 200$ nm)	1 day
7	CO_2	0 - 100 (daily cycle)	Mars-like pressure p=600 Pa	LED (UVB/VIS /PAR)	4 day

Results

Because we are interested in the survival of the different communities of organisms which are embedded in the crust and mineral matrix, we analyzed these organisms inside their rock crust. With this "sur-vey method" (analysis of communities from several locations) only the general survival can be determined (the survival at least of the dominant taxa in the com-munity), to select and identify the best locations where samples can be collected for future tests.

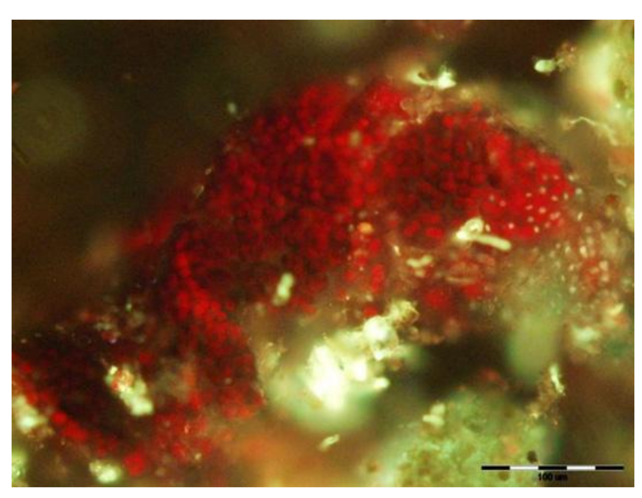

Figure 2. Pre-test image of Gloeocapsa sp from close to Dubai in UV excitation light

Samples that showed the best survival are from:

•United Arab Emirates (survival 7 test types),
•Australia, Northern Territories. W Macdonnel Ranges (survival at 6 test types),
•Western Australia. Dried out W branch of of the salt Lake Barley along Youani Road, at 409 m alt (survival at 11 test types).

Table 1. The analyzed cryptobiotic samples

sample no.	location	taxa	description
11-17 (05128/III)	Bihar Mts., Romania	*Goeocapsopsis pleurocapsoides, Gloeocapsa alpina, Gloeocapsopsis dvorakii*	On dry, half shady marble cliff near the entrance of Bear Cave (Pestera Ursşului) at 500 m altitude
11-17 (07080/III)	Slovakia, Slovenský Raj. Stratenská Dolina 2 km ESE of Stratená	Chroococcus lithophilus (dominant), Gloeocapsopsis pleurocapsoides	S facing, dry limestone rocks at 890 m alt. "Tintenstriche" (temporary watercourse)
21-27 (01069)	Australia, Northern Territories. W Macdonnel Ranges	*Tolypothrix byssoidea* (dominant), *Gloeocapsopsis pleurocapsoides, Nostoc microscopicum* and *N. minutissimum* in the upper layer, in the -0.1-0.4 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.	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 of the salt Lake Barley along Youani Road, at 409 m alt.	Top layer: *Tolypothrix byssoidea*. Subsurface layer to – 1mm: *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: *Tolypothrix 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: Tolypothrix byssoidea (dominant), Microcoleus paludosus. Subsurface layer to -1 mm: Crinalium epipsammum, Symplocastrum friesii, Microcoleus vaginatus. Bottom layer -1-2 mm: Lyngbiella sp., Symplocastrum 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	dominated by Chenopodiaceae. Lichen on soil	Coastal salty desert (sabkha) vegetation

The observations suggest the survival in general depends on the origin and the type of sample, e.g. the different mineral composition within the curst as well as the differing group 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 Bihar Mts., Romania and Slovakia, Slovenský Raj locations. The main difference between "no-survival" and the above mentioned "good survival" locations are that the good ones are from dry salty desert region, with low humidity, and good salt tolerance.

Conclusion

Analysing the taxa related survival, strong and obvious trends are difficult to identify. As our aim was a survey-like test the exact determination of limiting factors requires future more targeted analy-sis. Despite this general some statements can be drawn:

• survival of taxa in several samples was observed in every test types, suggesting there was no absolute limiting factor,
• the survival rates in most cases were relatively high, above 60% (excluding the test type 3),
• the gas composition alone seems to be not being a strong limiting factor, as there are 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 any sample were ob-served at the test type 3. The reason is difficult to identify as there are many factors analyzed and varied in our work, but the Earth-like atmospheric composition together with the very dry (near to ze-ro humidity) might be a reason – although only fu-ture tests could clarify it,
• the results suggest both difference exist in survival regarding the type of tests (conditions witnessed) and the group of taxa (different tolerance),
• the worse tolerance in general was observed at *Chroococcus, Chroococcales (25-41% of all tests together), while the best general tolerance was ob-served at Nostoc species with 100% survival in both cases) (the 100% means all of the analyzed samples showed substantial fraction of organisms that surveyed the chambers).*
• Table 3. gives an overview of the survival accord-ing to the conditions in the chamber. The results indicated in the table suggest that there was proba-bly no one absolute limiting factor that would have prohibited the survival of all of the organisms in the analyzed samples.

Table 3. Survival rate according to the selected conditions in the chamber (the gas was CO_2 in every cases)

conditions in the chamber	ratio of samples survived
pressure: Earth-like	31% (4 of 13)
pressure: Mars-like	64% (21 of 33)
0 relative humidity	50% (9 of 18)
1 relative humidity	54% (13 of 24)
LED light	56% (18 of 32)
UV radiation	57% (8 of 14)

No one of the used conditions: atmospheric pres-sure (Earth/Mars like), relative humidity (dry / satu-rated) and presence of UV radiation was absolute limit-ing factor. Any of the conditions and all of the used combination were survived by certain organisms inside the crusts.

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References [1] Dulai et al. 2012. EPSC2012-877. [2] Pocs T. 2009. Acta Botanica Hungarica 51, 147-178. [3] de Vera, J-P. et al. 2010. Astrobiology 10, 215-227.