Current Trends in Natural Sciences Vol. 6, Issue 12, pp. 147-151, 2017

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

THE EXTREMELY HALOPHILIC MICROORGANISMS, A POSSIBLE MODEL FOR LIFE ON OTHER PLANETS

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Abstract
The group of halophilic Archaea was discovered in the beginning of XX th century. They are able to live in more than 2-3 M of sodium chloride concentration that can be found in hypersaline natural lakes, in alkaline saline lakes, in man-made hypersaline mats, in rock salt, in very salted foods, on salted fish, on salted hides, in stromatolites, in saline soils. Their adaptations consist in resistance to high ionic contents with internal accumulation of K ions in order to face high Na ion content from the near environment. They belong to the Halobacteriaceae family. Their adaptation and their resistance to UV radiation and their resistance in oligotrophic conditions in rock salt, apparently over geological times, increase the possibility to find similar microorganisms in the Martian subsurface and in meteorites, and to support the panspermia theory. Some of the research of a working group in this field of activity and their possible uses are shortly reviewed here.

Keywords: halophilic archaea, panspermnia, radiation resistance.

1. INTRODUCTION

Halophilic organisms. The organisms and especially halophilic microorganisms can be divided into halotolerants resisting a concentration of 7-10% NaCl (Oren et al., 1999), halophiles which resist 10-20% salt concentration and extreme halophiles resisting higher concentrations of salt up to saturation. These organisms are very diverse from viruses which are parasiting the halophilic Archaea as Myoviridae (Le Romancer et al., 2007). There are halophilic bacteria, some of them photosynthetic like *Halospirulina*, cyanobacteria like *Aphanotece halophytica* and *Oscillatoria* species (DasSarma and Arora, 2001). In these environments there are *Halorhodospira* (anaerobs) methanogens (*Methanohalophilus halophilus)* and *Salinibacter* (Anton et al, 2005). Algae like *Asteromonas gracilis* and *Dunaliella salina* (DasSarma and Arora, 2001), fungi such as *Wallemia ichthyophaga, Candida famata, Gymnascella marismortui* live also in hypersaline waters (Gunde-Cimerman et al., 2003). The protozoans *Tetramitus salina,* the cilliate *Fabrea salina* and arthropods *Artemia salina,* live in hypersaline waters too. Halophilic Archaea belong to the family *Halobacteriaceae*, there are 55 genera containing 159 species (year 2016), some of them being isolated from rock salt of geological ages (Fendrihan et al.,2006).

Habitat of halophiles archaea. Hypersaline waters are one of the well known environments, hypersaline soda waters (extreme halohiles and alkaliphiles).

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Salt lakes, like the Dead Sea, salt lakes from Australia, stromatolites, rock salt and man-made brines resulting from mining activity, fermented salted foods, salted fish and salted hides, are habitats for this kind of extremophilic Archaea. A special discovery was the isolation of some strains of extremely halophilic archaea like *Halococcus dombrowskii* and *Halobacterium noricense* from fluid inclusion from 200 million years old halite.

Resistance to environmental extremes. This kind of microorganisms are resistant to high concentration of salt and resistant to other environmental factor, such as UV radiation, dryness, lack of enough nutrients at values to which other microorganisms cannot resist. Such properties recommend them for studies related with extreme environments and space studies. The environments from other planets are similar to some extreme environments from Earth, and some meteorites of Martian origin contain halite. The experiments with haloarchaea embedded in salt crystals reproduced a natural process from ancient times in Earth evaporates, and they could be detected with Raman spectroscopy (Fendrihan et al., 2009).

Astrobiology and space studies. Astrobiology studies the origin of life on the known planets including Earth, the possibility of life on other planets, and the spread of life in the universe. That is why scientists have focused on these extremophile microorganisms - because similar microorganisms may exist in planets or in their subsoil - as Mars possesses halite ore as demonstrated by meteorites of Martian origin. At the same time, the question arises if life could not be spread by meteorites in space from one planet to another.

There are some previous studies on effects of irradiation on halophilic archaea (Baliga et al., 2004) and some scientists considered the protective role played by pigments like bacterioruberin from these halophiles (Shahmohammadi et al., 1998). Previous space studies were done with a *Haloarcula* strain in Biopan experiment (Mancinelli et al., 1998). Some Archaea such as *Halococcus dombrowskii* were discovered in halite of Permian age in the Alps (Stan-Lotter et al., 2002). We demonstrated the existence of the live cells in fluid inclusions in artificial halite with special staining (Leuko et al., 2004; Fendrihan and Stan-Lotter, 2004).

2. MATERIALS AND METHODS

The methods were used for the ADAPT experiment, a space and ground experiment of exposure of some microorganisms and lichens to space condition UV irradiation under atmosphere of CO_2 or vacuum. We report in this paper the preparation phase of some ground experiments. The cells of *Hcc. dombrowski* were embedded in artificial salt crystals, were exposed for different times to UV radiation and the effects on survival were estimated with the help of a special device which simulates the Martian irradiation (Kolb et al., 2005).

Cells of *Halococcus dombrowskii* strain H4 were cultivated in Erlenmeyer flasks until they reached an optical density of about 1 (measured with a Pharmacia photometer). Liquid cultures of washed cells of *Hcc. dombrowskii* were spread on quartz disks (7 mm diameter) and dried. Halobacterial cells accumulated in fluid inclusions of crystals as previously demonstrated (Fendrihan and Stan-Lotter, 2004). A normal culture had a reddish appearance (due to the containing pigments microorganisms). Other cultures without pigments were obtained by growing cells in a culture medium with added glycerol which is reducing the production of pigments). After growth cells were centrifuged in an Eppendorf mini-centrifuge at 5000 rpm (2236 gs) for 5 min with the rotor F 2402 of the Beckman centrifuge, obtaining a thick pellet. For experiments 8 un-pigmented and 8 pigmented samples of concentrated haloarchaea were prepared, being adjusted at OD (600nm), diluted 1.5 fold and mixed in about 1600 µl of TN buffer (233 g NaCl Sigma, 12.1 g Tris Base ICN Biochemicals, Millipore water was added at 1000 ml, pH adjusted with conc. HCl at 7.4 4 M NaCl,

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Tris solution). Aliquots of 40ul were deposed on sterilized quartz disk of 11 mm diameter and inserted in tissue culture plates with lid (Multiwell Primaria 24 well, Becton Dickinson Labware, USA). Samples were used for drying and subsequent UV irradiation with a special UV lamp at different irradiation times. Following irradiation, the samples were resuspended in 3.5 M TN buffer at 300 µl each. From these aliquots portions were used for cultivation on M2 solid culture media and determination of CFUs. Fifty µl were used for the LD test (LIVE DEAD BacLight Kit L-7012, Molecular Probes USA), 100 µl for immunoassay experiments for the detection of cyclobutane pyrimidine dimers (CPDs), using a Thymine Dimer Antibody (Gene Tex Inc, USA), and Goat Anti Mouse IgG antibodies (Invitrogen Molecular Probes USA), and 20 µl for cultivation on liquid media in test tubes. The photos were taken with a CCD camera of the fluorescence microscope, and with a laser confocal microscope allowing the quantification of the dead/live bacteria by using the photos.

3. RESULTS AND DISCUSSIONS

The results of the tests of survival using cultivation and flurorescence microscopy showed that *Hcc. dombrowskii,* embedded in salt crystals, survived practically to a dosis of 10 kJ/m^2. The role of pigments on protection by UV radiation is being investigated by inhibition of carotenoid synthesis of *Hcc. dombrowskii* with diphenylamine. For the detection of carotenoids in salt crystals, Raman spectrometry measurements were performed and the peaks of carotenoids are present (figure 1).

The results are shown in the following tables. From the photographs obtained with LIVE DEAD kits (fig. 2) the number of red (dead bacteria) and green (live bacteria) was calculated (Table 1).

At the same time we found that a low amount of cells (2 - 5 %) of *Hcc. dombrowskii,* which were exposed to space conditions and UV irradiation for 18 months, contained cyclobutane pyrimidine dimers (CPDs), as evidenced by immuno reactions, with no significant difference between pigmented and non-pigmented strains. The results suggested that some DNA damage has probably occurred during exposure (Groesbacher, 2010).

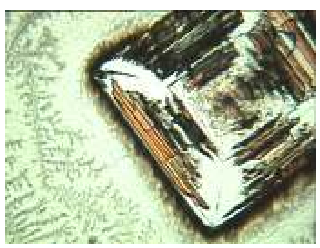

Figure 1. Haloarchaea embedded in salt crystals in fluid inclusion

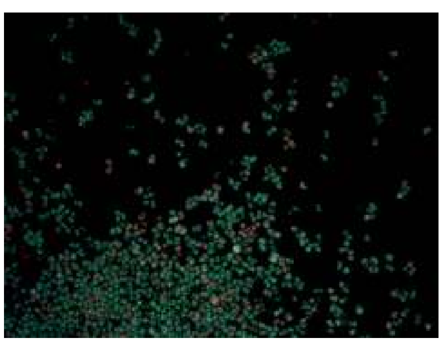

Figure 2. Halococcus dombrowski H4, culture, stained with LD kit fluorescence microscopy, x100. Green are living cells, red dead cells

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Table 1. Results of UV irradiation experiments

sample	green	red	sum	(%)
1.2, 1800 s	116	93	209	55.5
74,16 kJ	50	92	142	35
	38	24	62	61.2
2.2 1020s	36	15	51	75.4
42,02 kJ	24	14	38	63
	8	41	49	16.3
	20	36	56	35
	42	25	67	62.6
3.2, 765 s	30	24	54	55.5
31,52 kJ	58	25	83	69.8
	149	68	247	68.6
4.2, 510s	1134	736	1870	60,6
21 kJ	1023	576	1596	69.09
5.2, 255 s	349	167	516	67.6
10.5 kJ	320	162	582	66.2
	523	241	764	68.4
7.2, 3600s	21	47	68	30.88
148,52 kJ	161	82	343	66.2
	58	103	161	36.2
	68	68	129	50
	117	60	177	66.1
control	244	149	393	62
unexposed	281	154	435	64.5
	455	239	694	65.6
	461	231	692	66.6

4. CONCLUSIONS

The extraordinary resistance of halophilic archaea, to high dosis of radiation, including in space and ground laboratory experiments prove a power of adaptation which is practically useful for living in condition of other planets like Mars, included in subsurface halite. In the same time, this made panspermia theory to be more credible. Life on a planet can have double origin by evolution on its own, and second by elements which are comming from other planets.

5. ACKNOWLEDGEMENTS

First, I want to thank, to Professor Dr. Helga Stan Lotter, mentor and leader of the group studying the extreme halophilic Archaea, and to the colleagues prof. Maurizio Musso, Christian Radax, Andrea Legat, Marion Pfaffenhuemer, Claudia Gruber, Stefan Leuko, Gerhard Weidler, Anita Holzinger, and others who collaborated with me in projects and provided a positive and helpful attitude.

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