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SOIL BIOLOGY

Sustainability of Extreme Microbial Ecosystems to the Comprehensive Impact of Physical Factors of the Martian Regolith

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Abstract—As the key parameters of the Martian regolith, we have studied the combined effect of gamma radiation (1 and 10 kGy), low temperature (−50°C), and low pressure (1 Torr) on the microbial communities of extreme ecotopes of Earth to estimate the duration of cryopreservation of hypothetical Martian ecosystems in a viable state. The obtained data suggest that cryopreservation of viable microorganisms in the surface layer of the regolith is possible for at least 130000 years; at a depth of 30 cm, for 170000 years; at a depth of 2 m (the depth which the ExoMars 2020 mission must reach), for 330000 years; and at a depth of 5 m, for 2 million years.

Keywords: astrobiology, Mars, gamma radiation, microorganisms, sustainability
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INTRODUCTION

Studies of extreme Earth habitats make it possible to assess the ultimate possibilities for the existence of earthly life forms, the only ones known to us. These studies fundamentally change the perception of the viability of organisms and play a leading role in the development of astrobiology, making it possible to assess the probability that other planets and their moons are inhabited, to develop technologies and tools for detecting life, and to plan space missions [26]. An important task is to study the adaptive capabilities of microorganisms outside terrestrial variations of the influencing factors, in conditions close to the specific target objects of astrobiology. Therefore, in cosmic and laboratory experiments simulating extraterrestrial and cosmic physicochemical effects, various organisms are exposed in a near-earth orbit [28].

Most of the studies focus on simulating conditions on Mars as one of the most promising objects in the search for life. In particular, the ability of bacterial growth at low temperatures under Martian atmospheric conditions [20, 29] and salinity typical of the Martian regolith [21] has been demonstrated. It was found that terrestrial nonextremophilic bacteria can develop in aqueous films formed during sublimation of ground ice through the regolith [23].

It is assumed that the hypothetical Mars biosphere can remain in a cryopreserved state, like the terrestrial microbial communities in permafrost. The duration of its conservation is limited by the effect of ionizing radiation [22].

It is necessary to study the radiation tolerance of microorganisms in conditions closest to the parameters of the Martian regolith to estimate the duration of conservation of the Mars biosphere with constant accumulation of radiation damage. It is important to reproduce the set of physical factors, in accordance with the possibility of modifying radiation effects [6], and simulate interactions between microorganisms in

Table 1. Chemical characteristics of samples

Index	SN	M-1/91	A-6/99-6
pH	8.11	7.51	8.21
NO_2^-, mg/kg	Traces	Traces	Traces
NO_3^-, mg/kg	1.03	0.89	0.78
NH_4^+, mg/kg	4.12	3.34	2.56
Cl^-, mg/kg	58.15	49.84	62.30
CO_3^{2-}, mg/kg	330.41	129.29	172.39
Na^+, mg/kg	512.70	87.57	915.15
Mn^{2+}, mg/kg	1348.20	3.23	331.35
Mg^{2+}, mg/kg	136.46	170.40	10.47
K^+, mg/kg	877.50	40.47	106.98
$Fe^{2+} + Fe^{3+}$, mg/kg	4.35	29.55	34.22
C_{org}, %	3.79	0.32	0.01

hypothetical Mars ecosystems and a heterogeneous mineral environment [3, 12].

The aim of this work is to study the combined effect of gamma radiation (1 and 10 kGy), low temperature (–50°C), and low pressure (1 Torr) on microbial communities in extreme Earth habitats as a simulation of the key parameters of the Martian regolith.

MATERIALS AND METHODS

Samples of ancient frozen sedimentary rocks of the Arctic and Antarctica, as well as serozem from the Negev Desert (Israel) were used for this study. Similar extreme habitats of the Earth are considered as Mars analogs [11].

Antarctic frozen sedimentary rock was sampled (A-6/99-6) from a depth of 1.3–1.5 m from a 99-6 well drilled in the flat region of Beacon Valley (77°50′ S, 160°36′ E) [14]. According to various estimates, the age of rocks ranges from 50–300 ka to 8.1 Ma [14]. Sedimentary rock is coarse-grained sand with inclusions of pebbles cemented by ice into a massive cryogenic structure. The maximum negative temperature recorded in the studied frozen rocks is –18.5°C.

Arctic frozen sedimentary rock (M-1/91) was sampled from the 1/91 well [2] located in the Lower Kolyma Lowland region (Yakutia) between the Bolshaya Chukochya and the Malaya Konkovaya rivers in the sublatitudinal Oler uplift. The depth of the sampling was 34.0 m (the oldest layers of the Oler formation, which have not melted for 1.8–2 Ma). The temperature in the well was –7 to –12°C [13]. The procedure of permafrost rock sampling and sample transportation to the laboratory was described previously [14].

Serozem (SN) was sampled in the Negev Desert in the Avdat area (30°47′ N; 34°46′ E) from a depth of 5–10 cm (A) [5]. Annual precipitation here is about 100 mm, with zero from June to October. Soil-forming rocks are loesslike loams.

Chemical characteristics of the samples are presented in Table 1.

Before irradiation, soil samples were weighed, moistened with sterile water, incubated in a thermostat at a temperature of +28°C for 10 days in order to activate the microbial community, and air-dried for 1 day at the same temperature. The microbial communities in frozen rocks were activated in the same way, but without addition of water (moistening of the samples was due to melting ice). For irradiation, the samples were placed in the previously described climatic chamber [23], which can maintain a pressure of 1 Torr and a temperature of –50°C throughout the process. The irradiation was carried out on a K-120000 gamma irradiation facility with ^{60}Co sources in the following doses: 1 kGy for serozem and 10 kGy for all other samples. The radiation intensity was 3 kGy/h. Activated unirradiated samples served as the control. Samples were stored at –18°C until analyses.

The number of cultured heterotrophic bacteria was determined by a plating technique using dense glucose–peptone–yeast (GPD) nutrient medium (composition: peptone 2 g/L, glucose 1, yeast extract 1, casein hydrolyzate 1, $CaCO_3$ 1, agar-agar 20 g/L), as described in [4]. The platings were cultivated at +28°C. The total number of prokaryotes in the samples was determined by epifluorescence microscopy (EFM) with acridine orange, according to [4]. The potential metabolic activity and functional diversity of microbial complexes were assessed by multisubstrate testing [1, 16]. Mineral salts and a set of 47 test substrates were introduced in two replicates into the wells of the plate for enzyme-linked immunosorbent assays. Then suspensions of samples and an indicator of substrate consumption (triphenyltetrazolium bromide) were added. The plates were incubated in a thermostat at +28°C for 72 h; the optical density of the solutions in the wells was measured photometrically at a wavelength of 510 nm. The array of functional biodiversity coefficients reflecting the conditions of the microbe community was calculated from the obtained data using Eco-Log© software. [1]. Data were statistically processed using software STATISTICA 8.0, Microsoft Office Excel 2007, and Eco-Log©.

RESULTS AND DISCUSSION

After irradiation at 10 kGy, the number of cultivated bacteria in the serozem sample decreased by two orders of magnitude; in the samples of Antarctic and Arctic permafrost, it decreased by 60 and 325 times, respectively (Table 2). Meanwhile, the number of colony forming units (CFU) remained at a high level and varied from tens of thousands to millions of CFU per gram of sample. Earlier [17, 27], it was shown that

Table 2. Influence of model conditions of Martian regolith on number of prokaryotic cells

Sample	Number of cultivated bacteria, CFU/g	Total number of prokaryotes (EFM), cells/g	Index *K*
SN, control	$2.5 \times 10^8 \pm 0.4 \times 10^8$	$5 \times 10^8 \pm 0.2 \times 10^8$	2.0
SN, 1 kGy	$1.5 \times 10^7 \pm 0.3 \times 10^7$	$9.4 \times 10^7 \pm 1.2 \times 10^7$	6.3
SN, 10 kGy	$2.2 \times 10^6 \pm 0.5 \times 10^6$	$9.8 \times 10^7 \pm 1.2 \times 10^7$	44.5
M-1/91, control	$1.5 \times 10^7 \pm 0.3 \times 10^7$	$1 \times 10^8 \pm 0.3 \times 10^8$	6.7
M-1/91, 10 kGy	$4.6 \times 10^4 \pm 0.6 \times 10^4$	$1 \times 10^8 \pm 0.3 \times 10^8$	2173.9
A-6/99-6, control	$7 \times 10^6 \pm 1.5 \times 10^6$	$5 \times 10^7 \pm 0.7 \times 10^7$	7.1
A-6/99-6, 10 kGy	$1.2 \times 10^5 \pm 0.3 \times 10^5$	$3.8 \times 10^7 \pm 0.8 \times 10^7$	316.7

gamma radiation at a dose of 10 kGy under normal conditions reduces the number of CFU bacteria in different soils by two to five orders of magnitude, which in general agrees with the results obtained for the SN and M-1/91 samples. The microbial community in Antarctic frozen rock demonstrated increased stability in comparison with the known data. In particular, in [19], irradiation of frozen rock from the same region (McMurdo Dry Valleys) at a dose of 6 kGy resulted in a decrease in the number of bacterial CFU by more than two orders of magnitude.

After irradiation at doses of 1 and 10 kGy, the total number of prokaryotes in the serozem sample decreased by five times; in the frozen rock samples it remained at the level of the control (Table 2). Comparison of the stability of microbial communities according to this indicator with the data of other researchers is difficult, since in the overwhelming majority of cases, they detected viability of microorganisms only by culture methods [17]. However, according to the results obtained during irradiation of the endolithic microbial complex in volcanic tuff, the total number of prokaryotes irradiated at doses 1–10 kGy decreased 1.5–2 times [24], which is close to our data. The reduction in the number of CFU with conservation of the total number of cells at the level of the control indicates the transition of some microbial populations to a nonculturable state [10]. A similar effect was observed earlier after radiation exposure [24]. Under favorable conditions, the ability of cells to cultivate can be restored [25]. It should be noted that with an increase in radiation dose, the coefficient *K* (the ratio of the total number of prokaryotes to the number of cultured cells) also increases. That is, most of the bacterial complex passes into an uncultivated state (Table 2). The sharp increase in coefficient *K* in irradiated frozen rocks should be noted. It may indicate increased availability of cellular stress-protecting mechanisms.

After irradiation at doses of 1 and 10 kGy, the serozem microbial community retained high potential metabolic activity and functional diversity (Table 3; Fig. 1). The value of index *d*, reflecting the stability of the microbial community, increased. Nevertheless, the value of this parameter did not exceed 1, which indicates the reversible nature of the damage (values of index $d > 1$ are characteristic of irreversibly disturbed systems) [16]. The specific metabolic work decreased 1.5 times; a sharp decrease in the consumption of amino acids, salts of organic acids, polymers, and alcohols was observed along with a slight increase in the consumption of pentoses and oligosaccharides. At the same time, the quantity (index *N*) and the variety of consumed substrates, the Shannon index (*H*) and the evenness (*E*) hardly changed in comparison with the control sample. The irradiated microbial community retained its original functional character. Earlier, the irradiation of soil samples at the same doses under normal conditions resulted in much greater inhibition of microbial communities: a decrease in the potential metabolic activity by six or more times and a reduction in the variety of consumed substrates by four or more times (up to the total absence of activity) [18, 24]. The possibility of restoring metabolic activity and functional diversity during moistening and subsequent incubation of irradiated samples was also demonstrated [18].

Table 3. Changes in parameters of functional diversity of serozem (SN) microbial community after exposure to combination of physical factors of Martian regolith

Parameter of functional state of microbial community	Control	1 kGy	10 kGy
Rank distribution coefficient of substrate consumption spectrum, *d*	0.624	0.855	0.873
Number of consumed substrates, *N*	36	35	34
Specific metabolic work, *W*	1702	1118	1089
Evenness, *E*	0.97	0.98	0.99
Shannon index, *H*	5.0	5.0	5.0

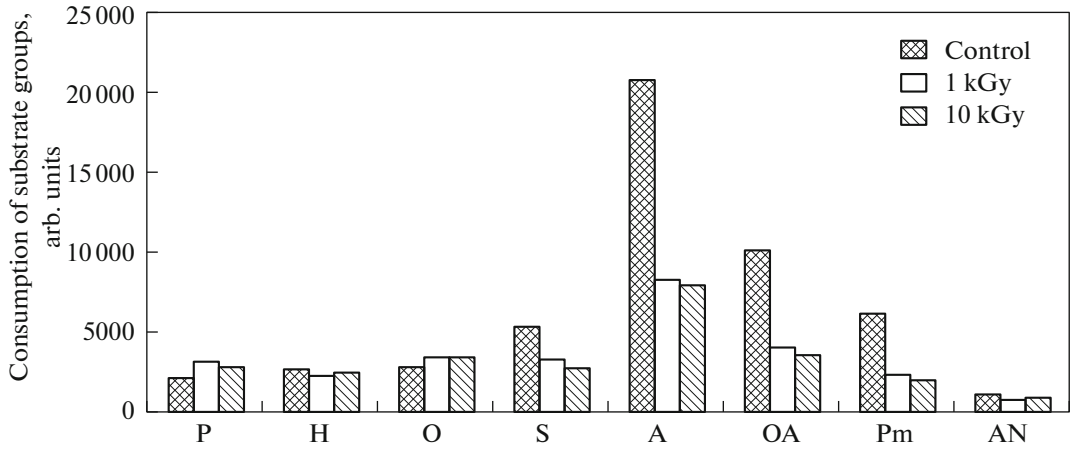

Fig. 1. Influence of model conditions of Martian regolith on consumption of different groups of substrates by serozem microbial community: P, pentose; H, hexose; O, oligosaccharides; S, alcohols; A, amino acids; OA, salts of organic acids; Pm, polymers; AN, amides, nucleosides.

CONCLUSIONS

The investigated microbial communities demonstrated high tolerance to comprehensive effects of physical factors of the surface layer of the Martian regolith. Irradiation in model conditions led to less damage to microbial complexes than the effect of the same doses of gamma radiation under normal conditions. This is probably due to a decrease in radiation damage at low temperatures [9, 19], as well as a decrease in the concentration of oxygen and water (the main sources of free radicals formed during irradiation) in the environment and in cells due to pressure decrease [6]. Microbial communities in frozen rocks showed slightly greater radiation tolerance in comparison to the prokaryotic complex of serozem soil, manifesting itself in retention of the total number of cells at the control level. This may be explained by the adaptation of microorganisms of extreme low-temperature ecotopes to cold stress and, consequently, tolerance to oxidative stress [7] arising from exposure to radiation.

Taking into account the known data on the intensity of ionizing radiation on the surface of Mars (0.076 Gy/yr [15]), our results allow us to assume the possibility of preserving viable microbial communities in a cryopreserved state in the surface layer of the regolith for at least 130 ka after loss of a significant part of the atmosphere. The minimum duration of conservation of living microorganisms with an increase in depth and decrease in radiation intensity [8, 9] is: at a depth of 30 cm, about 170 ka; at a depth of 2 m (depth of planned sampling of the ExoMars 2020 mission [30]), 330 ka; and at a depth of 5 m, 2 Ma.

It is obvious that the radiation doses applied by us are far from the limits of the stability of natural microbial systems of extreme habitats. Therefore, for more accurate predictions of the habitability of Martian regolith, it is necessary to search for sterilizing doses of radiation. It is important to study not only the effect of various types of ionizing radiation, but the entire set of factors and their synergistic action. As well, the need to detect microorganisms in situ in such experiments should be noted, taking into consideration the possibility of their transition to a nonculturable state.

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