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Effects of Permafrost Microorganisms on the Quality and Duration of Life of Laboratory Animals

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Bacillus strain 3M isolated from samples of permafrost from Mamontova Mountain (Yakutiya) affected the quality and duration of life of "elderly" mice. Microorganisms were given i.p. to CBA mice aged 17 months. After treatment with microorganisms, animals showed increases in motor activity, muscle strength (the load-lifting test), the feeding instinct (the dosed starvation test), and cellular immunity in the in vivo delayed-type hypersensitivity reaction, along with improvements in psychoemotional status (the open field test). Mean duration of life increased by 9.19%, minimal duration of life by 41.93%, and maximum duration of life by 7.68%. The duration of "survival" (lifetime after treatment with microorganisms) increased even more significantly: the minimal by 141.9%, the mean by 28.0%, and the maximal by 20.4%. The mechanisms of the influence of strain 3M microorganisms on increases in the duration of life of laboratory mice to the upper limit for the species are suggested to occur via positive influences on quality of life.

KEY WORDS: permafrost microorganisms, quality and duration of life in laboratory mice.

Healthy humans depend to a significant extent on the biosphere, every layer of which is occupied by different species of microorganisms. Viable microorganisms have been found in samples of ice and soil obtained from different depths, all the way to the 4-km mark [2]. Some of them are millions of years old [10, 13, 14]. Microbiological material, including that with altered antigenic properties, can reach the surrounding environment and, thus, the biological community, when cryolithozones become degraded. It is impossible to assess their potential role for humans, as the mechanisms of their influences on ecosystems, including the ecosystems of humans and current organisms, have not been established.

Many examples of the interaction of extreme physical environmental conditions and living organisms indicate that living conditions induce properties adapting the organism to the conditions obtaining. During evolution, a whole series of adaptive reactions directed to preserving viability arose during exposure to the most unfavorable environmental conditions. These reactions are associated with changes in intracellular structures and the appearance of a whole series of specific protective reactions [5]. The influence of the set of severe conditions present in permafrost (low temperature, metabolusesc depletion, lack of light) on the biological potential of microorganisms is of particular interest. In this context, there is interest in studies of the influences of microorganisms found in permafrost on the adaptive systems of current mammals. Moreover, in circumstances where the study of aging is still a current fundamental question, investigation of the biological potential of cells able to survive thousands of years may be of interest in gerontology.

The aim of the present work was to study the influences of permafrost microorganisms, i.e., *Bacillus* strain 3M, on measures of the quality and duration of life in laboratory animals.

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METHODS

Studies were performed using *Bacillus* strain 3M isolated from samples of permafrost From Mamontova Mountain in central Yakutiya, where one of the most ancient exposures of permafrost in Eurasia is located. Samples were collected from permafrost which in all probability has not thawed in more than 3.5 million years [13]. The nucleotide sequence of the 16S rRNA has been deposited in the DDBJ/EMBL/GeneBank under accession No. AB178889, identification No. 20040510203204.24251. Studies of the acute toxicity of strain 3M were highly consistent with results of *B. cereus* probiotic *Bacillus* strain IP5832 from the medicinal agent Baktisubtil [6, 9]. At the same time, a number of differences in the biological potential of these strains have been demonstrated. Thus, freezing for 72 h decreased the toxicity of strain 3M as compared with strain IP5832; a series of experiments on dose-dependent effects showed that small doses of strain 3M, i.e., 5000 cells, stimulated cellular and humoral immunity [4], while this effect was not seen with strain IP5832.

The present study was performed using 54 “elderly” CBA males aged 519–525 days (17 months). A total of 24 animals received single i.p. doses of strain 3M microorganisms at a dose of 5000 cells in 100 μl of physiological saline. Before administration, bacilli were passed through a “freeze-thaw” cycle. In view of the differences between microorganisms from permafrost (strain 3M) and their contemporary analogs (strain IP5832), control animals (n = 30) received solvent, i.e., physiological saline.

Animals were observed until they died naturally, and physiological status was assessed once each month. All studies were performed in accordance with the “Regulations for Studies Using Experimental Animals” (Decree of the USSR Ministry of Health of 08.12.1977, No. 755) and the “European Convention for the Protection of Vertebrates” of 03.18.1986. The physical status of the animals was assessed in terms of muscle strength [8] and the level of feeding instinct activity was assessed using the 18-h dosed starvation test [8]. Motor activity and central nervous system activity were evaluated in the psychoneurological open field test [3]. The functional activity of cellular immunity was assessed in terms of delayed type hypersensitivity in vivo [15]. Minimal, mean, and maximal durations of life were measured in days, as were survival times after administration of strain 3M bacilli.

Results were analyzed statistically using Student’s test run in SPSS for Windows.

RESULTS

These experiments showed that parenteral administration of strain 3M *Bacillus* microorganisms from permafrost promoted significant increases in the duration of life of laboratory animals (Fig. 1). The minimal duration of life in mice of the control group was 589 days (survival from the start of observations was 70 days) and the maximal was 833 days (314 days from the start of observations). The minimal duration of life of mice from the control group was 836 days (317 days from the start of observations) and the maximal was 897 days (378 days from the start of observations).

Thus, the first animal from the experimental group died five days after the death of the last animal of the control group. The mean duration of life of the experimental animals was 846.3 ± 4.7 days, compared with 775.1 ± 18.1 days in controls (p < 0.01). The increase in the minimal duration of life in experimental life was by 41.93%, the increase in the mean was by 9.19%, and the increase in the maximal duration of life was by 7.68%. Duration of life after administration of strain 3M microorganisms (the survival time) increased even more significantly: the minimal by 141.9%, the mean by 28.0%, and the maximal by 20.4%.

Parenteral administration of strain 3M microorganisms was followed by a significant change in the quality of life of “elderly” animals relative to their contemporaries from the control group. The quality of life of laboratory animals was assessed in terms of the nature of metabolusesc processes, physical activity, and the state of the immune and nervous systems.

Metabolusesc processes were assessed in terms of changes in the animals’ body weight in natural living conditions and changes in the level of the feeding instinct in the “dosed starvation” test. In natural conditions, the body weight of the animals of the control group decreased over 10 months, from 29.0 ± 0.2 g (at age 17 months) to 24.5 ± 0.2 g (at age 27 months), compared with a decrease in experimental animals over the same period from 28.6 ± 0.3 to 26.6 ± 0.2 g, i.e., animals of the control group lost an average of 4.5 g (15.5%) during natural ageing, while animals of the control group lost only 2.0 g (6.9%) of the initial body weight.

At eight months after administration of bacilli (at age 25 months), the animals’ feeding instinct was assessed in the “dosed starvation” test and the activity of cellular immunity was assessed in terms of delayed-type hypersensitivity in vivo. Mice of the control group lost weight by 19.6% at 18 h of starvation, with a 66% recovery of weight 2 h after taking food. Animals of the experimental group lost 8.8% of body weight in starvation, with 96% recovery 2 h after eating. Immunological studies showed that the level of cellular immunity in control animals, assessed in terms of in vivo delayed-type hypersensitivity, was 12.4 ± 1.6%, while that in mice of the experimental group was two times greater, at 26.5 ± 3.1% (p < 0.01).

Motor activity and muscle strength provide important measures of quality of life (Table 1). The animals’ motor activity in the present study was assessed in terms of the total number of square crossings in the open field test. Overall motor activity in the open field (total number of turns to left and right) in mice of both groups decreased

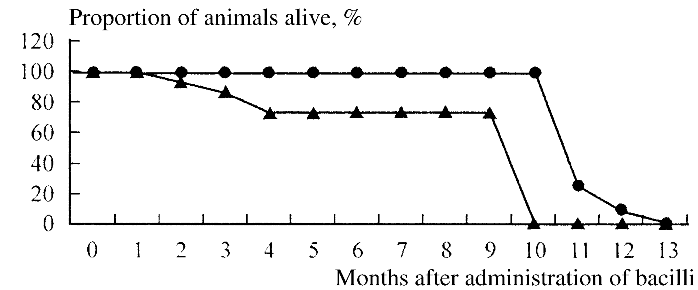

Fig. 1. Duration of life of laboratory animals after administration of strain 3M bacilli. ▲) Controls; ●) experimental. The ordinate shows the proportion of animals remaining alive, %. The numbers of animals at the beginning of the experiment were 30 in the control group and 24 in the experimental group.

TABLE 1. Characteristics of Motor Activity in Mice in the Open Field Test and Muscle Strength in the Load Lifting Test

Months of observation	Number of turns to the left		Number of turns to the right		Weight lifted, g	
	control	experimental	control	experimental	control	experimental
0	5.98 ± 0.41 n = 30	6.08 ± 0.42 n = 24	5.35 ± 0.46 n = 30	5.15 ± 0.45 n = 24	66.39 ± 5.51 n = 30	67.71 ± 5.59 n = 24
1	5.55 ± 0.22 n = 30	11.4 ± 1.25** n = 24	5.48 ± 0.15 n = 30	13.03 ± 1.29** n = 24	63.72 ± 5.67 n = 30	70.34 ± 4.98 n = 24
2	3.47 ± 0.15 n = 28	9.46 ± 0.61** n = 24	4.27 ± 0.21 n = 28	11.13 ± 0.49** n = 24	58.86 ± 5.61 n = 28	66.54 ± 6.62 n = 24
3	3.32 ± 0.12 n = 26	5.48 ± 0.53** n = 24	3.49 ± 0.16 n = 26	6.92 ± 0.54** n = 24	59.82 ± 4.89 n = 26	73.34 ± 5.95* n = 24
4	3.24 ± 0.17 n = 22	5.21 ± 0.44** n = 24	4.29 ± 0.21 n = 22	4.85 ± 0.55* n = 24	103.38 ± 4.81 n = 22	120.30 ± 5.15* n = 24
5	3.33 ± 0.25 n = 22	5.38 ± 0.30* n = 24	3.51 ± 0.10 n = 22	4.25 ± 0.41* n = 24	103.48 ± 5.37 n = 22	139.29 ± 6.05** n = 24
6	2.39 ± 0.15 n = 22	4.87 ± 0.10** n = 24	2.15 ± 0.20 n = 22	3.05 ± 0.21* n = 24	103.98 ± 5.80 n = 22	160.27 ± 6.7** n = 24
7	1.59 ± 0.19 n = 22	4.15 ± 0.30** n = 24	1.53 ± 0.01 n = 22	2.15 ± 0.17** n = 24	88.75 ± 9.01 n = 22	160.04 ± 4.48** n = 24
8	1.55 ± 0.02 n = 22	4.39 ± 0.20** n = 24	1.32 ± 0.21 n = 22	1.83 ± 0.12 n = 24	75.25 ± 5.96 n = 22	135.06 ± 6.4** n = 24
9	1.53 ± 0.14 n = 22	4.5 ± 0.39** n = 24	1.16 ± 0.10 n = 22	2.2 ± 0.15** n = 24	61.15 ± 6.41 n = 22	160.94 ± 4.06** n = 24
10	—	1.66 ± 0.11 n = 24	—	0 n = 24	—	88.07 ± 5.38** n = 24
11	—	—	—	—	—	—
12	—	—	—	—	—	—

Note (here and Tables 2–5). Significant differences between experiment and control groups: *$p < 0.05$; **$p < 0.01$.

gradually and in a stable manner, which was a consistent reaction and provided evidence of "assimilation" and "remembering" by the animals of the new living space. However, animals of the experimental group were significantly more active throughout the observation period, and motor activity in these animals during the first two months after administration of bacilli was more than two times greater than that in control animals. For example, one month after administration of bacilli, animals of the experimental group crossed 24.4 ± 0.9 squares during the 5 min of the test, compared with only 11.0 ± 0.5 in animals of the control group ($p < 0.01$).

Muscle strength in mice was evaluated in terms of the ability to "lift" a load, clinging by reflex to a metal mesh from which loads were uniformly attached by nylon filaments (Table 1). In animals of the experimental group, muscle strength became greater than that in controls two months after administration of bacilli. Control animals could lift loads of more than 100 g for a period of three months (from month 4 to month 6), while animals from the experimental

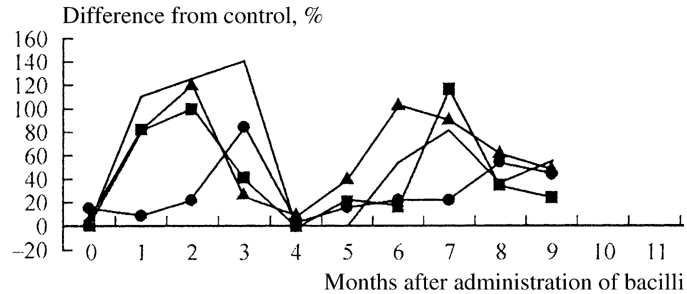

Fig. 2. Differences in the behavioral reactions of animals of the experimental group from controls in the open field test, %. ——) Number of excursions into the central squares; ■) number of vertical rearings; ▲) number of grooming acts; ●) number of defecations.

TABLE 2. Excursions to the Central Squares of the Open Field Test Polygon

Months of observation	Number of excursions to center		Months of observation	Number of excursions to center	
	control	experimental		control	experimental
0	1.54 ± 0.23 $n = 30$	1.49 ± 0.23 $n = 24$	6	0 $n = 22$	1.08 ± 0.15 $n = 24$
1	2.13 ± 0.01 $n = 30$	4.48 ± 0.46** $n = 24$	7	0.29 ± 0.02 $n = 22$	0.53 ± 0.03** $n = 24$
2	2.0 ± 0.16 $n = 28$	4.51 ± 0.09** $n = 24$	8	0 $n = 22$	0.36 ± 0.01 $n = 24$
3	2.0 ± 0.19 $n = 26$	4.82 ± 0.42** $n = 24$	9	0 $n = 22$	0.55 ± 0.04 $n = 24$
4	2.15 ± 0.15 $n = 22$	2.13 ± 0.08 $n = 24$	10	—	—
5	2.29 ± 0.21 $n = 22$	2.27 ± 0.21 $n = 24$	11	—	—

TABLE 3. Activity in Terms of Vertical Rearings

Months of observation	Number of vertical rearings		Months of observation	Number of vertical rearings	
	control	experimental		control	experimental
0	1.91 ± 0.12 $n = 30$	1.93 ± 0.13 $n = 24$	6	1.31 ± 0.15 $n = 22$	1.54 ± 0.13 $n = 24$
1	3.26 ± 0.26 $n = 30$	5.96 ± 0.37** $n = 24$	7	0.64 ± 0.03 $n = 22$	1.38 ± 0.13** $n = 24$
2	3.0 ± 0.24 $n = 28$	6.0 ± 0.28** $n = 24$	8	1.12 ± 0.10 $n = 22$	1.51 ± 0.14 $n = 24$
3	2.74 ± 0.21 $n = 26$	3.88 ± 0.28** $n = 24$	9	1.13 ± 0.11 $n = 22$	1.4 ± 0.13 $n = 24$
4	1.63 ± 0.08 $n = 22$	1.63 ± 0.09 $n = 24$	10	—	—
5	1.31 ± 0.17 $n = 22$	1.58 ± 0.10 $n = 24$	11	—	—

group lifted loads of more than 100 g for six months (from month 4 to month 9). Summing the weight lifted over the observation period showed that experimental animals lifted 60.5% more weight than control animals.

Figure 2 shows measures characterizing the animals' psychoemotional behavior in the open field. For convenience, results are presented as percentage differences from the value in the control group, which was taken as 0 (Table 2).

TABLE 4. Activity in Terms of Grooming

Months of observation	Number of grooming acts		Months of observation	Number of grooming acts	
	control	experimental		control	experimental
0	1.05 ± 0.09 n = 30	1.13 ± 0.03 n = 24	6	0.53 ± 0.03 n = 22	1.08 ± 0.07** n = 24
1	1.1 ± 0.10 n = 30	2.0 ± 0.16** n = 24	7	1.11 ± 0.12 n = 22	2.13 ± 0.10** n = 24
2	0.60 ± 0.04 n = 28	1.33 ± 0.15** n = 24	8	1.12 ± 0.09 n = 22	1.83 ± 0.15** n = 24
3	0.95 ± 0.09 n = 26	1.22 ± 0.09 n = 24	9	0 n = 22	0.5 ± 0.36 n = 24
4	0.90 ± 0.06 n = 22	1.01 ± 0.11 n = 24	10	—	—
5	0.78 ± 0.02 n = 22	1.10 ± 0.09* n = 24	11	—	—

TABLE 5. Activity in Terms of Defecations

Months of observation	Number of boluses		Months of observation	Number of boluses	
	control	experimental		control	experimental
0	0.96 ± 0.04 n = 30	1.1 ± 0.20 n = 24	6	0.95 ± 0.04 n = 22	1.16 ± 0.10 n = 24
1	1.6 ± 0.17 n = 30	1.75 ± 0.24 n = 24	7	1.33 ± 0.11 n = 22	1.62 ± 0.09 n = 24
2	1.53 ± 0.13 n = 28	1.86 ± 0.16 n = 24	8	1.50 ± 0.14 n = 22	2.33 ± 0.18**
3	0.83 ± 0.07 n = 26	1.53 ± 0.10** n = 24	9	0.83 ± 0.09 n = 22	1.20 ± 0.11* n = 24
4	0.86 ± 0.05 n = 22	0.90 ± 0.07 n = 24	10	—	—
5	0.92 ± 0.05 n = 22	1.07 ± 0.10 n = 24	11	—	—

Measures such as excursions into the central squares of the open field polygon (overcoming of fear) and vertical rearings (observations of space beyond the open field) were used to characterize the animals' orientational-investigative behavior. In animals of the experimental group, these values changed in a wave-like manner and were significantly greater than control levels from the first to the third month and beyond the sixth month after administration of bacilli (Tables 3 and 4).

Measures of grooming activity (self-care) and defecation (metabolusesc processes and feelings of fear) reflect the level of emotional comfort and resistance to stress in animals. Grooming activity in mice of the experimental group was greater than that in controls, while defecation activity was to variable extents greater than that in controls throughout the observation period (Table 5).

A very important individual and prognostic characteristic of individuals, determining the animals' overall structure of behavior, is the preference for the direction of movement. A preference for turns to the left is known to provide evidence for predominant activation of the right hemisphere and turns to the right activation of the left. The ratio of the numbers of turns to one side and the other shows (Table 1) that during the first three months after exposure to strain 3M microorganisms, animals of the control group showed no preference for movement direction, though after month 5 there were more frequent turns to the left.

DISCUSSION

The results obtained here show that single parenteral doses of *Bacillus* strain 3M (5000 cells) given to "elderly" mice did not lead to radical increases in their lifetimes. Nonetheless, the increases in their maximal (by 7.7%), mean (by 9.2%), and minimal (by 41.9%) lifetimes compared with contemporaries from a control group show that bacilli from permafrost promote increases in longevity to the upper limit for the species, particularly in the weakest individuals. The relative increase in the animals' lifetime was accompanied by marked increases in the quality of life.

The uniform change in the activity of metabolusesc process in animals of the experimental group as compared with controls on the background of natural aging processes for forced "starvation" was interesting. Thus, at 10 months of living in natural conditions in the animal house and 18-h artificial "starvation", animals of the experimental group lost weight 2.2 times more slowly in both situations than mice of the control group. Recovery after "exit" from starvation was also quicker (by a factor of 1.5) than in contemporaries of the control group. These data suggest that after administration of this strain of permafrost bacilli, metabolusesc processes in "aging" mice transfer to a more economic regime of energy consumption and dissipation than seen in their contemporaries from the control group.

Activity of cellular immunity is known to some extent to reflect biological age. Our immunological studies showed that the level of reactivity of in vivo delayed-type hypersensitivity in animals of the experimental group with a chronological age of 25 months was double that in contemporaries of the control group and essentially equal to that in mice aged 6–8 months. We believe that the significant increase in cellular immunity cannot provide unambiguous evidence of a decrease in the animals' biological age, as the possibility of a hyperergic reaction is not excluded. Objective interpretation of this result requires further studies.

The increases in the muscle strength and motor activity of animals of the experimental group compared with the control group provide evidence of improvements in their physical status. When the animals found themselves in a new context (the open and well illuminated open field polygon), increases in motor activity provided evidence that their orientational-investigative behavior was stimulated, to a significantly greater extent in the experimental group. Comparison of measures of total motor activity and muscle strength in animals of the experimental group showed that these parameters changed in opposite directions – motor activity increased at the beginning of the experiment, while muscle activity increased after five months. Grooming activity was also tightly correlated with motor activity: in a calm context, the animal spent more time caring for itself than moving in space. Comparison of these parameters showed that in the open field mice showed dominance of motor (investigative) activity during the first three months, while grooming activity (indicating the state of comfort) was dominant after five months. These data indicate that strain 3M bacilli promote the retention of physical activity in "elderly" mice, with persistence of orientational-investigative behavior and the ability to self-care at a higher level than seen in their contemporaries from the control group, i.e., mice showed a real increase in survival ability.

Our data on the gradual shift in the balance towards a preference for turns to the left, which is evidence for activation of the right hemisphere, suggests that the positive changes in the physiological characteristics of the animals in the experimental group may be based on the regulatory actions of the CNS. This view is to a significant extent consistent with increases in the activity of cellular immunity, for which the right hemisphere is known to be largely responsible [1].

Thus, these studies showed that one strain of microorganisms isolated from permafrost can have systemic actions in mice, increasing the adaptive potential of the major physiological systems. Administration of strain 3M bacilli to "elderly" mice had the effect that the remainder of the animals' lives was lived at a higher quality than their contemporaries, which appears to have prolonged their lives to the upper limit of the species.

The present study is exploratory and does not allow identification of the mechanisms or mediators of the effects described. Permafrost bacilli may act as probiotic bacteria, though it remains possible that they synthesize particular substances with immunomodulatory or ageing-protective properties. Such effects have previously been observed with the synthetic thymus peptide Vilon, which increased endurance and physical activity in mice, decreased body temperature, and increased lifetimes [12].

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