Anatolii BRUSHKOV (BROUCHKOV), *et al.*
Bacillus cereus sp. F / Longevity

http://siberiantimes.com/science/casestudy/news/n0431-russian-scientist-admits-injecting-himself-with-35-million-year-old-eternal-life-bacteria/
29 September 2015

Russian scientist admits injecting himself with 3.5 million year old 'eternal life' bacteria

The result? He has avoided flu for two years, and works harder with renewed energy.

Russian scientists are making progress in the search for the elixir of youth by investigating a bacteria named Bacillus F which has remained alive in the permafrost for millions of years. Picture: Anatoli Brouchkov

The Siberian Times disclosed recently how Russian scientists are making progress in the search for the elixir of youth by investigating a bacteria named Bacillus F which has remained alive in the permafrost for millions of years.

Experiments are underway on mice and human blood cells but the man who made the 'sensational' discovery has now admitted he injected himself with the ancient bacteria found in the Sakha Republic, the largest region of Siberia. Anatoli Brouchkov, head of the Geocryology Department at Moscow State University, said he has become a guinea pig for the bacteria.

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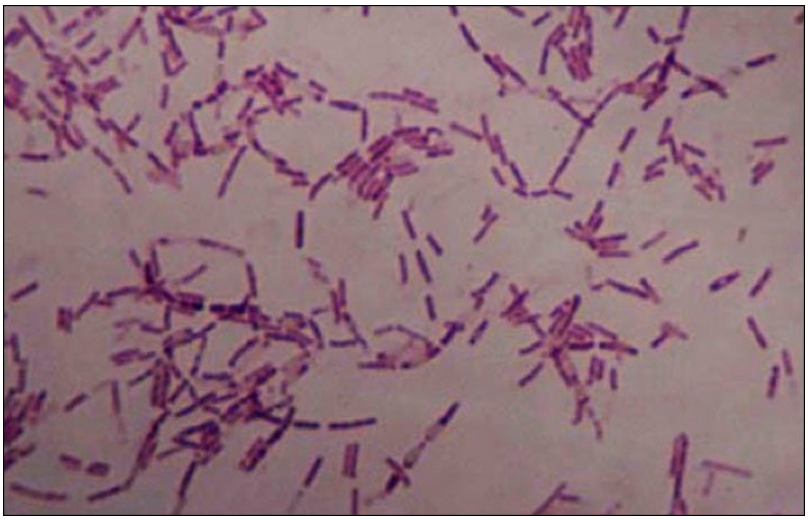
'I started to work longer, I've never had a flu for the last two years,' he said. 'After successful experiments on mice and fruit flies, I thought it would be interesting to try the inactivated bacterial culture,' he told The Siberian Times.

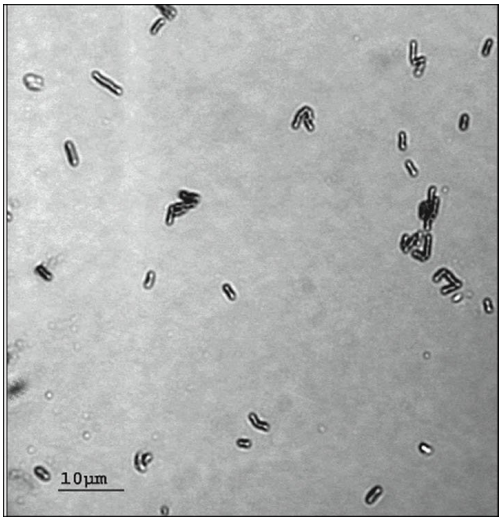
'Besides, the permafrost is thawing, and I guess these bacteria get into the environment, into the water, so the local population, the Yakut people, in fact, for a long time are getting these cells with water, and even seem to live longer than some other nations. So there was no danger for me. '

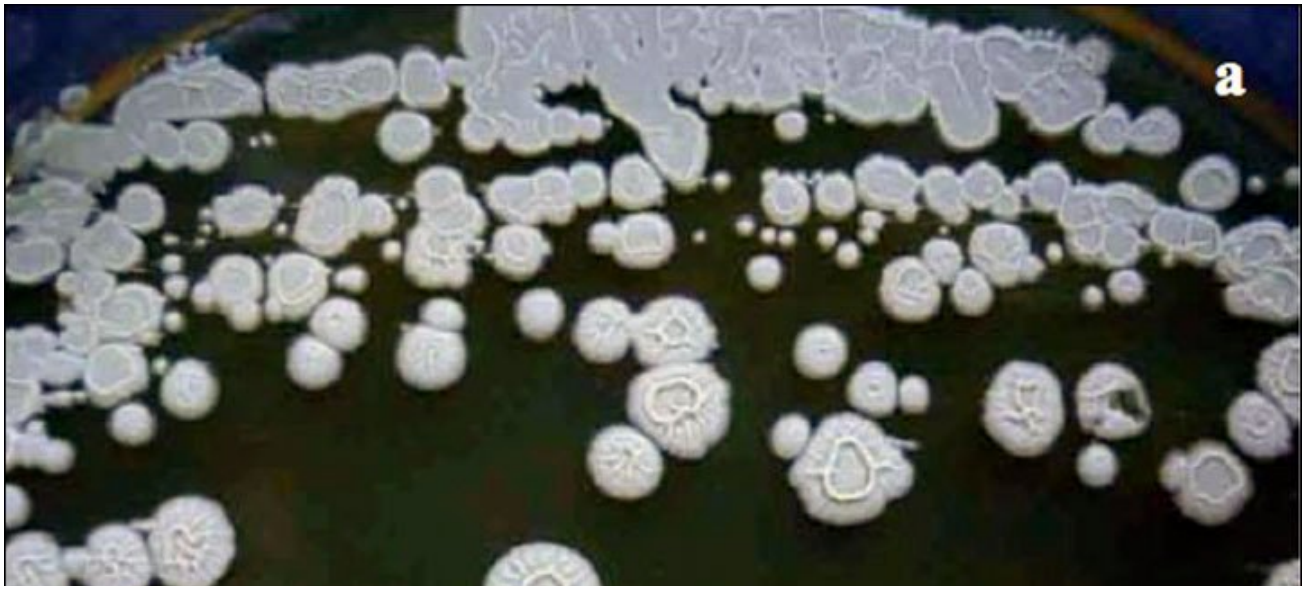
He stressed: 'It wasn't quite a scientific experiment, so I cannot professionally describe the effects. But it was quite clear for me that I did not catch flu for two years. Perhaps there were some side-effects, but there should be some special medical equipment to spot them. Of course, such experiments need to be conducted in clinic, with the special equipment and statistics. Then we could say clearly about all the effects.'

In any case, it was too early to market the bacteria despite an undoubted demand for a potion offering eternal life. 'It still needs the experiments. We have to work out how this bacteria prevents ageing. I think
that is the way this science should develop. What is keeping that mechanism alive? And how can we use it for our own benefits?'

Bacillus cereus sp. F







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http://www.youtube.com/watch?v=lv0_Cu0FcPA

Russian scientist injects himself with 3,5-mn-yr-old 'immortality' bacteria to 'extend life'

Eternal youth... mankind's impossible dream through the ages... A Russian scientist thinks that - just maybe - the secret of eternal life may have actually been found. Deep in the permafrost of Siberia, a living bacteria was discovered that is old - very, very old.

Anatoly Brouchkov says that there is something about its DNA and genes that gives this cell extreme longevity and protects it from damage.

Anatoly Brouchkov

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http://siberiantimes.com/science/casestudy/features/f0159-russian-scientists-make-progress-on-secret-of-eternal-life/
19 September 2015

Russian scientists make progress on secret of eternal life

Living bacteria found in permafrost - '3.5 million years old' - is 'scientific sensation' which allows 'mice grannies' to reproduce.

Scientists have decoded the DNA of a bacteria found thriving in ancient permafrost, and are now seeking to understand the genes which provide its extraordinary longevity.

Work is also underway to study a so far unexplained positive impact on living organisms, notably human blood cells, mice, fruit flies, and crops. Professor Sergey Petrov, chief researcher of Tyumen Scientific Centre, said: 'In all these experiments, Bacillus F stimulated the growth and also strengthened the immune system. The experiments on human erythrocytes and leucocytes were also very optimistic. '

The bacteria were originally found on Mamontova Gora - Mammoth Mountain - in Siberia's Sakha

Republic, also known as Yakutia, in 2009 by Dr Anatoli Brouchkov, head of the Geocryology Department, Moscow State University. Similar bacteria were discovered by Siberian scientist Vladimir Repin in the brain of an extinct woolly mammoth preserved by permafrost.

'We did a lot of experiments on mice and fruit flies and we saw the sustainable impact of our bacteria on their longevity and fertility,' said Dr Brouchkov. 'But we do not know yet exactly how it works. In fact, we do not know exactly how aspirin works, for example, but it does. The same is true here: we cannot understand the mechanism, but we see the impact.'

Describing the discoveries as a 'scientific sensation' and an 'elixir of life', Yakutsk epidemiologist Dr Viktor Chernyavsky said: 'The bacteria gives out biologically active substances throughout its life, which activates the immune status of experimental animals.' As a result, 'mice grannies not only began to dance, but also produced offspring'.

If the same substance were to be given to people, it could cause a significant improvement in their health, leading to the discovery of an 'elixir of life', said Dr Chernyavsky.

A number of claims are now being made for the potential of three different strains of bacteria found in the permafrost, among them the rejuvenating of the life of living beings. Another is the potential development of organisms capable of destroying petroleum molecules, turning them into water, with the potential one day to create a new system for cleaning up oil spills. A third strain of ancient bacteria is capable of eliminating cellulose molecules.

Dr Brouchkov told The Siberian Times: 'We have completed the deciphering of Bacillus DNA and, more importantly, we have completely restored a sequence of genes in it. This work was ongoing for several years and it finished at the end of last year. Now we face the most complicated task - the attempts to find out which genes are providing the longevity of bacteria, and which proteins are protecting the DNA structure from damages.

'We want to understand the mechanisms of the protection of genome, the functioning of the genes. The key question is what provides the vitality of this bacteria, but it is as complicated as which human genes are responsible for cancer and how to cure it. The scale and complicity of the question are nearly the same.' This involves technically difficult research, he said.

He revealed that the bacteria has survived for millions of years deep in the Siberian ice. 'To state the exact age of bacteria, we need to date the permafrost rocks and this is not so easy,' he said. 'There are no exact methods to date the permafrost, but we have solid reason to believe that it is rather old.

'Eastern Siberia is not a warm place even now and 3.5 million years ago it was also rather cold. It already had nearly the same temperature mode as it has now. That is we believe that this permafrost was formed 3.5 million years ago. And we believe that the bacteria could not penetrate to the oldest layer from the earlier ones through the permafrost. This bacteria was isolated from the outer world in ice, so we are quite sure that this bacteria was kept in the permafrost for such a long time. Yet we are still working to prove this.'

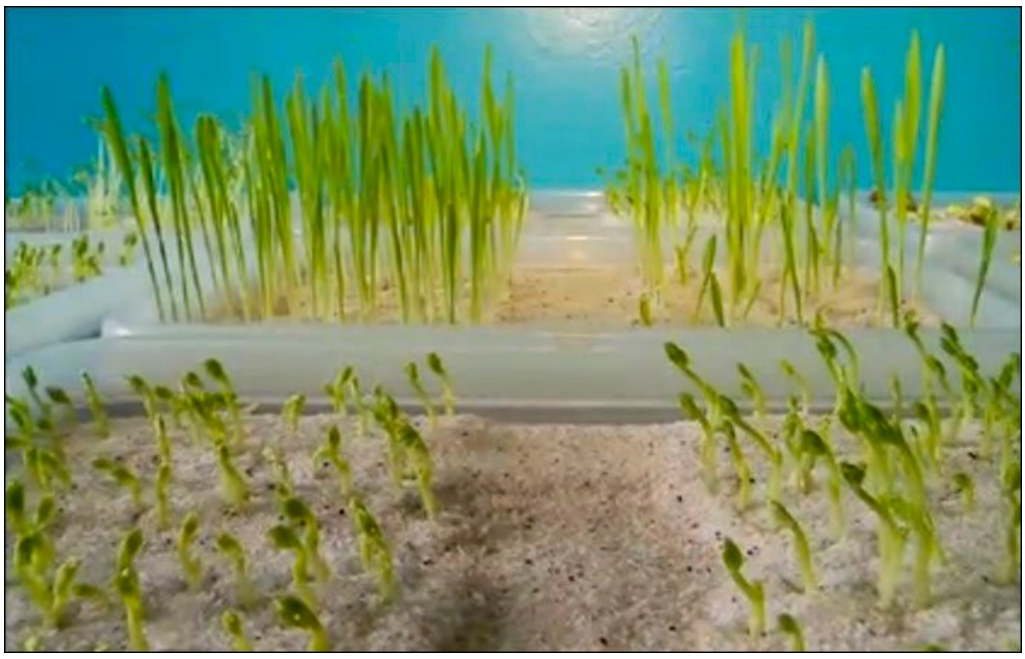
Bacteria also preserved in other extreme conditions, he said. 'Some of them were found in amber, some even in rock salt. More than this, in rock salt a bacteria aged half a billion years was found.'

He claimed: 'I would say, there exist (in the world) immortal bacteria, immortal beings. They cannot die, to be more precise, they can protect themselves. Our cells are unable to protect themselves from damage. These bacteria cells are able to protect themselves. It would be great to find the mechanisms of protection from ageing, from damage and to use them to fight with our ageing. It's is the main riddle of mankind and I believe we must work to solve it.

'Now we have a key, ancient bacteria, which scientists have found in an extreme and ancient environment.' He admitted that some people need convincing of the significance of the discoveries. 'Of course the discussions are ongoing. There are a lot of sceptics who do not believe that bacteria are really old.

'But the main thing is: we finally saw the light at the end of a long and hopeless tunnel. It is a great deed. I would be happy if people are interested in our research. It is much better than to follow the dollar rate or price of oil. Our researches globally has just started. The first (scientific) articles appeared about ten years ago, so it is at the first stage of the research.

'I believe that we all need to start studying these immortal beings, but at the moment not so many of us are doing this. We are arguing, discussing, instead of (doing sufficient) research. We need to use the fact that such bacteria were found in our permafrost. We have such opportunities for study. I believe that this bacteria could be very useful.'



In the laboratory we got very good results. The bacteria not only stimulates growth, but increases frost resistance. The seeds sprouted at a temperature 5C.' Pictures: SurgutInform TV

One place where active research is underway is in Tymen, western Siberia, under Prof Petrov. 'We conduct various studies of the impact of the bacteria on the living organisms,' he said. 'We made experiments with copepods, mice, crops and human blood cells. In all these experiments Bacillus F stimulated the growth and also strengthened the immune system. The experiments on human erythrocytes and leucocytes were also very optimistic.

'Now we are focused on experiments with the crops. The bacteria stimulates the growth of crops, increases productivity. This year we completed the laboratory studies and went to the field trials. We will look at the results. Before sowing seeds we put them into a solution containing a culture of the bacterium. We have harvested but the results are not completely processed yet.' The results of these trials will be announced later.

'In the laboratory we got very good results. The bacteria not only stimulates growth, but increases frost resistance. The seeds sprouted at a temperature 5C. It is very important in our Siberian harsh conditions, when a sudden frost can come in May and even June.'

He revealed: 'We can say that the bacteria enhances photosynthesis. It is also very important for our northern areas with short photo period. The plants have time to fully mature.

'We conduct the biochemical research to understand the mechanism, how exactly this bacteria influences on the plants, which exact stages of the metabolism it affects. At the moment we cannot say for sure. That is, we see the effects, but cannot fully explain this yet.

'At the moment the use of plant growth promoting bacteria is a very promising direction. It is clear that bacteria are much more effective than chemicals. Our Bacillus F has a great potential, as it managed to survive in permafrost. I believe its potential is much higher that other growth promoting bacteria. Besides it can help to withstand frost, which is so important for our conditions.

'Now we have applied for a grant to conduct further research, especially on human blood cells, and we hope that we will get it, because the research is extremely promising.'

Close to the site where the ancient bacteria was found lay the remains of woolly mammoths and rhinos. 'We found our bacteria in deeper, more ancient layers of permafrost, significantly lower that the layers where mammoths were found,' said Dr Brouchkov.

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http://www.jove.com/visualize?author=Anatoli%20V%20Brouchkov

Draft Genome Sequence of Bacillus cereus Strain F, Isolated from Ancient Permafrost.

Evgeniy V Brenner, Anatoli V Brouchkov, Alexander M Kurilshikov, Gennady I Griva, Elena Kashuba, Vladimir I Kashuba, O Melefors, Vladimir E Repin, Vladimir P Melnikov, Valentin V Vlassov.

Bacillus cereus strain F was isolated and cultured from a sample of permafrost, aged presumably about 3 million years, on the Mammoth Mountain (62°56N, 133°59E). These genome data provide the basis to investigate Bacillus cereus F, identified as a long-term survivor of the extremely cold and close environment.

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http://www.jove.com/visualize/abstract/19244426/glaciibacter-superstes-gen-nov-sp-nov-novel-member-family
Int. J. Syst. Evol. Microbiol.

Glaciibacter superstes gen. nov., sp. nov., a novel member of the family Microbacteriaceae isolated from a permafrost ice wedge.

Taiki Katayama, Tomoko Kato, Michiko Tanaka, Thomas A Douglas, Anatoli Brouchkov, Masami Fukuda, Fusao Tomita, Kozo Asano.

PATENTS

http://www.google.com/patents/WO2012060729A1?cl=en
[Machine Translation]

WO2012060729
BACTERIA STRAIN BACILLIUS SP. - RELIC MICROORGANISMS ISOLATED FROM PERMAFROST ROCKS AND HAVING IMMUNO-MODULATING AND GERO-PROTECTIVE ACTIVITY

The invention relates to biotechnology, in particular to strains of microorganisms, and can be used for stimulating immune system activity and increasing animal and human life expectancy. The relic microorganism - a bacteria strain Bacillus sp. VKPM (Russian National Collection of Industrial Microorganisms) ?-10130 - is isolated from permafrost rocks and has immuno-stimulating and gero-protective activity.

TECHNICAL FIELD

The invention relates to biotechnology, in particular, to strains of microorganisms and may be used to stimulate the immune system and increase the life span of animals and humans.

BACKGROUND

At a time when the fundamental mechanisms of aging are still an issue of concern, the study of cells able to survive for thousands of years may be of interest to Gerontology. Evidence of the viability of microorganisms in frozen ground appeared in the nineteenth century. SS Abizov in 1979 found in the ice at the Antarctic station Vostok bacteria, fungi, diatoms and other microorganisms (Abizov SS, Bobin NE, Kudryashov BB, 1979. Microbiological studies glacier in Central Antarctica. Proceedings of the Academy of Sciences of the USSR, Series Biology, 6, pp. 828-836). Without denying the likelihood of microorganisms in the permafrost, we note that their growth is probably difficult. Even in vitro aging culture, as is known, stop growing. Crystallization of water and stopping External metabolism reduces the ability to grow. Therefore, we can assume that the bacteria in the permafrost are fossils relic organisms. Their age is confirmed by geological conditions of the location (Baranov Yu, Elijah IA, Nikitin VP, Pniewy GN, Fradkin AF, NY Shvareva Miocene Mammoth Mountain. Trudy GIN SB RAS USSR. Science. Moscow. 1976. 284 s), radiocarbon dating (Katayama T., Tanaka M., Moriizumi J., Nakamura T., Brouchkov A., Douglas TA, Fukuda M., Tomita R, Asano K . Phylogenetic Analysis of Bacteria Preserved in a Permafrost Ice Wedge for 25,000 Years. Appl. Environ. Microbiol., Apr. 2007: 2360-2363), the study of the optical isomers of amino acids (Brinton KLF, Tsapin AI, Gilichinsky D., McDonald GD. Aspartic Acid Racemization and Age-Depth Relationships for Organic Carbon in Siberian Permafrost. Astrobiology, Volume 2, Number 1, 2002, p.77-82), indirectly, biodiversity encountered species (Friedmann EI. 1994. Permafrost as microbial habitat. In Viable Microorganisms in Permafrost. Russian Academy of Sciences: Pushchino, Russia; 21-26).

The nature of long-term viability of microorganisms in ancient permafrost does not have a full explanation. Ability relic organisms remain viable for a long time suggests the existence of a mechanism that prevents the accumulation of damage. We consider it possible to use this mechanism to treat disease and prolong life in animals and humans.

The present invention relates to bacteria of the genus Bacillus, found in ancient permafrost Yakutia.

Known strain of bacteria Bacillus subtilis 1719, highlighted in vivo and exhibits a wide range of antagonistic activity, a low adhesion activity and immunomodulatory activity (patent RU 2298032 C2, published on 27.10.2007). Known bacterial strain Bacillus macroides Excel 00 isolated from the mycelium of medicinal mushroom Agaricus Blazei Murill, a developing immune activity and the ability to prevent aging and disease (application JP 2008-005702 A, published on 17.01.2008).

SUMMARY OF THE INVENTION

Being patented strain Bacillus sp., In contrast to the known, isolated from permafrost age is many thousands of years, and is characterized by exceptional viability and ability to survive for long time at low temperatures. In experiments with its culture, an increase in muscle strength, increased physical and mental activity, immunomodulation and anti-aging.

The inventive microorganism - a strain of Bacillus sp., Isolated from permafrost and possess immunomodulatory activity and geroprotective.

Strain Bacillus sp. zadeponirovan in the Russian National Collection of Industrial Microorganisms (VKPM) FGUSH osNIIGenetika 30.01.2009, registration number VKPM: B-10130.

1. MATERIALS AND METHODS isolated microorganisms

For microorganisms from permafrost samples were collected from natural outcrops. They are located on the left bank of the Aldan, 325 kilometers upstream from its confluence with the Lena, at Mammoth Mountain. Samples were selected 0.9-1 m deep layer of seasonal thawing. Exposure of the river destroyed (more than a meter a year), so that the deposits from which samples were taken, were obviously in a state of permafrost. Thus there is an annual spring flushing collapses, preventing blockages and mixing breeds. The latter are fine-grained sands and siltstones; their age corresponds to the Middle Miocene (Baranov Yu, Elijah IA, Nikitin VP, Pniewy GN, Fradkin AF, NY Shvareva Miocene Mammoth Mountain. Trudy GIN SB RAS . Science. Moscow. 1976. 284 s). Cooling began here in the late Pliocene, about 3 - 3.5 million years ago. January temperature at that time was measured from -12 to -32 ° C, and in July of +12 to + 16 ° C. Deposits apparently not thawed Pleistocene of the cold climate Yakutia. Thus, the age of permafrost on Mammoth mountain can probably reach 3.5 million years. In addition, samples were taken from the wedges of ice ice complex in Yakutia and Alaska: Fox in the tunnel and in the gold mine near Fairbanks, and the walls of the underground Permafrost Institute. Melnikov in Yakutsk.

11. Sampling

Samples were collected using sterilized with alcohol and burnt in the flame of metal tools (drills, forceps, scalpels). In order to conduct surface sterilized samples sample weighing about 50 g was placed in a beaker with 96% ethanol for one minute and then the flame at about 2 seconds, and finally in a sterile tube. Selected rocks stored at -5 ° C, which was close to the natural conditions. Transportation of samples was carried out in cold boxes with refrigerants in a frozen state.

12. Growth on artificial media

Samples of various dilutions were added aseptically in petri dishes containing medium YPD, MRS and NA (see user manual Manual of environmental microbiology. 1997. Ed. Hurst CJ FSM Press. Washington DC. 894 p.). Samples were added in liquid meat peptone in anaerobic and aerobic conditions.

13. Preparation and Sequencing of DNA

Ribosomal DNA extracted microbial culture by using Fast DNA kit for soil (BIO 101 Inc., Vista, CA), where the method is based on the destruction of the cells with glass beads. Nucleic acids were precipitated from the solution using a solution consisting of 0.1 parts of 3 M sodium acetate (pH 5.2) and 2.5 parts of ethanol, incubated on ice, and then centrifuged for 30 minutes at a speed of about 12,000. / Min. The precipitated nucleic acid is then dissolved in distilled water (free of RN-DN-az and AZ), and stored at -20 ° C. Fragments of 16S rRNA were amplified Poland pursued with bacterial primers (27F; 5'-AGAGTTTGATCCTGGCTCAG-3 ', 1492R;

5 '-TG ACTG ACTGAGG YTACCTTGTTACGACTT-3'). PCR was performed in a volume of 20-µ1 using GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) as follows: 4 min at 94 ° C, followed by 30 cycles of 1 min at 94 ° C, 1 min at 50 ° C, and 1.5 min at 72 ° C, then 7 min at 72 ° C. PCR amplicons were subjected to electrophoresis and purification using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA). Purified amplicons were cloned using pCR2.1 vector,

culture of E. coli, as well as TA cloning kit (Invitrogen) according to manufacturer's recommendations. From the daily culture of plasmid DNA containing the 16S rDNA, obtained by Mini prep spin kit (Quiagen, Crawley, UK). Purified plasmid DNA was sequenced on ABI PRISM 3100 Genetic Analyzer using Big Dye Terminator cycle-sequencing kit (Applied Biosystems). Amplitsitsirovannye 27F-1492R products were sequenced in both directions with primers 27F, 357F (5'- CTACGGGAGGCAGCAG -3 '), 520R (5'-ACCGCGGGGTGCTGGC- 3'), 920F (5 '- AAACTC AAAGGAATTGACGG-3'), 1080R (5'- CCCAACATCTCACGAC-3 ') and 1492R as described Mori et al. (1997). The length of the sequence was 1488 bp. The resulting sequence was compared with others using BLAST (Altschul et al., 1997). The phylogenetic tree was constructed by the method of Saitou and Nei (1987), using the CLUSTAL W software package (Thompson et al., 1994). The nucleotide sequence of 16S rRNA has been deposited in the DDBJ / EMBL / GeneBank under the number AB 178889, identification number 20040510203204.24251.

2. RESULTS OF ALLOCATION, studying the growth and identification of microorganisms

In Miocene deposits frozen on Mammoth mountain it was found cultivated bacteria capable of aerobic and anaerobic growth in the medium YPD, MRS and NA; optimum growth temperature is set at + 37 ° C. The microorganism is psychrotolerant since capable of metabolic activity at -5 ° C. Bacillus is a relatively large ((1- 1.5) x (3-6) microns) wand, which is connected to the culture circuit, and is capable of forming spores circular shape. She slabopodvizhna, gram-positive, non-pathogenic. The microorganism belongs to the genus Bacillus is a new kind. The greatest similarity of species isolated bacilli marked with B. cereus and B. macroides., Homology with the 16S rRNA which is 99 and 97%, respectively.

Survival and growth of germs at low temperatures observed previously; It is known, for example, Bacillus anthracis that easily tolerates freezing (Luyet BJ, Gehenio PM 1940. Life and death at low temperatures. Biodynamica: Normany, Missouri; 99 p., a well Baross JA, Morita RY Life at Low Temperatures: Ecological Aspects. In Microbial Life in Extreme Environments, Kushner DJ (ed.). Academic Press: London 1978; 9 - 71). However, the optimum growth temperature is high enough bacilli found. Despite the fact that it was able to grow on an artificial medium, and below zero, visible colonies on frozen samples is not observed. How active is her life in the permafrost, it is not clear; This applies to the microorganisms isolated from the ice in Central Yakutia and Alaska (Katayama T., Tanaka M., Moriizumi J., Nakamura T., Brouchkov A., Douglas TA, Fukuda M., Tomita R, Asano K. Phylogenetic Analysis of Bacteria Preserved in a Permafrost Ice Wedge for 25,000 Years. Appl. Environ. Microbiol., Apr. 2007: 2360-2363).

Strain Bacillus sp. VKPM B-10130 has the following characteristics.

Cultural and morphological characteristics of the strain: Morphological: straight rods with rounded ends, slabopodvizhnye 1-1, 2 x 3-10 mm, 1-2, chains and 7. It forms endospores oval, located centrally and terminal, not exceeding the size of the cells vegetetivnyh . Gram-positive.

Cell culture: The optimal conditions (temperature + 30 to 37 ° C, aerobic conditions), and yet is capable of growing at temperatures between + 5 ° C and 43 ° C, and spread over a wide pH range.

In dense agar nutrient media polymorphic form, opaque, shiny, slightly rough, soft colony slightly yellowish, with a wavy edge.

The strain is undemanding to growth factors, grows well on common nutrient media (SPA, MPA, MPB).

For the cultivation of Bacillus sp. VKPM B-10130 is used medium of the following composition, g / l Peptone 10, yeast extract 5; Distilled water the rest. Cultivation was carried out at 37 ° C and intensive aeration until the deceleration phase of growth.

The culture was maintained on solid medium reseeding, stored in glycerol solution at -70 ° C or freeze-dried state.

Physiological symptoms.

Has no hemolytic activity, does not inhibit growth of the type strains of Bacillus cereus, Escherichia coli, Candida albicans, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumonia, katalazopolozhitelen growing NaCI at a concentration of up to 5% but not 10%, resistant to rifampicin, oxacillin, polymyxin, ceftazidime, cefepime; chustvitelen streptomycin, benzylpenicillin, kanamycin, gentamicin, amikotsinu, erythromycin, meropenem, ampicillin, tetracycline, levomycetin at 37 ° C and at 10 ° C, even the minimum concentration of antibiotics inhibits bacterial growth.

Biochemical signs of strain are shown in the table. 1.

Table 1.
Performance Characteristics

Gram stain +
Pigmentation -
Form E
N Location
Disputes:
Fan
- Sporangium
Mobility +
Growth under anaerobic conditions +
Kata ase activity +
Oxidase activity cl +
Voges-Proskauer test -
Use of the citrate -
Reduction of nitrate + gas
Casein -
Hydrolysis gelatins +
Starch -
The formation of glucose - acid from mannitol -
Arabinose -
Xylose -
Lactose -
Mannose +
Sorbitol -
+ 2 ° C -
Growth at
+ 8 ° C +
temperature:
+ 43 ° C +
6,5% NaCl + SL
10% NaCl -
15% NaCl - pH 4 - pH 5 + CO
Reaction to
pH 5.5 +
Wednesday
pH 8.5 +
pH = 9 +
pH + 10
pH 10.5 +
pH + 11 SL
Ammonia -
Education in
Indole - BCH:
H 2 S +
Fixing N 2 +
E - elliptical dispute; C - central location disputes - - negative reaction; + - Positive reaction; + Cl -

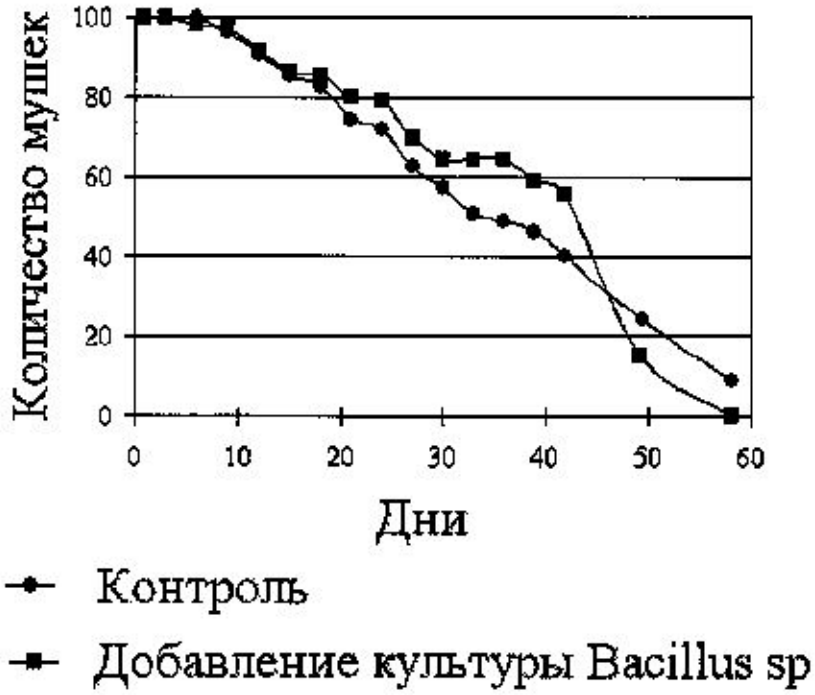
weakly positive reaction. 3. TESTING CULTURE Bacillus sp. AT higher organisms

LIST OF FIGURES

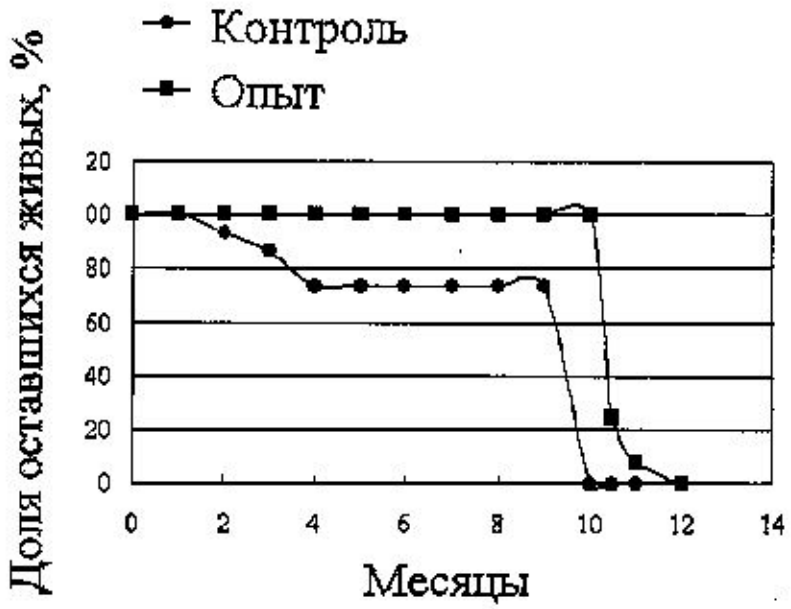
FIG. 1 shows the influence of culture Bacillus sp. lifespan Drosophila melanogaster

FIG. 2 - the influence of culture Bacillus sp. when administered parenterally 5000 cells lifespan of laboratory mice 17 months of age.

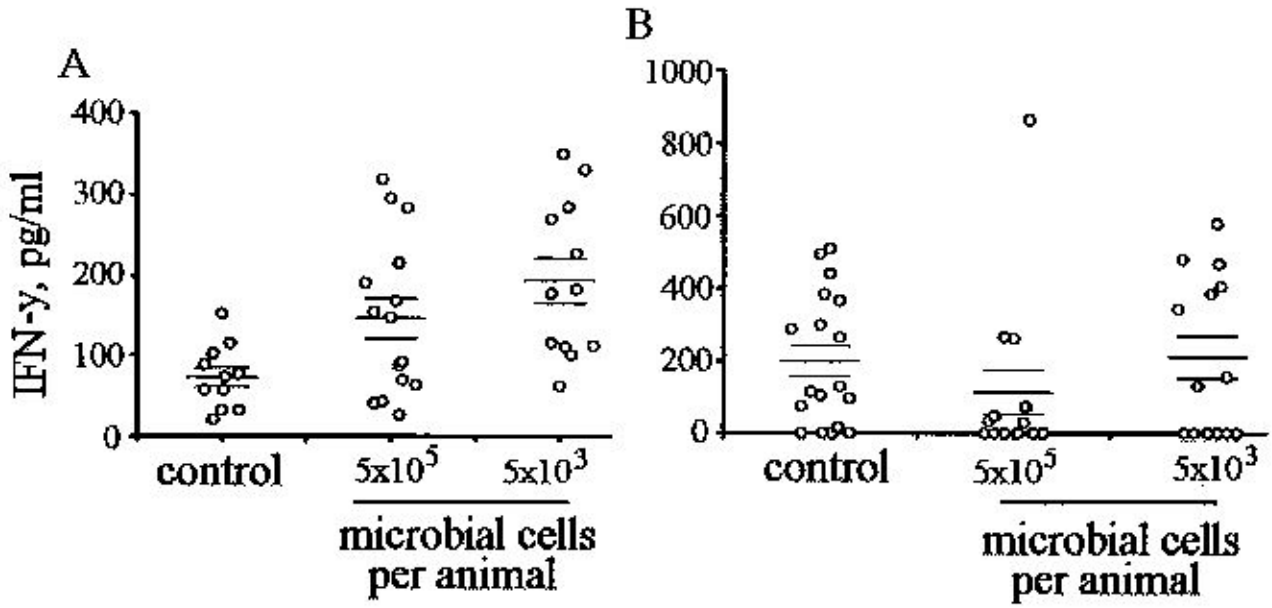
FIG. 3 - the levels of IFN-? after 6 days (A) and TNF-a after 90 min (B) in the serum of mice after intraperitoneal injection of Bacillus sp.



Фиг. 1



Фиг. 2



Фиг. 3

REALIZATION EXAMPLES OF THE INVENTION

3.1. Testing flies Drosophila melanogaster

For the experiment were selected Drosophila melanogaster individuals of the same age (1 day). They were placed in tubes containing culture medium (5-7 ml) in 5 pairs. The sample size for each variant was 100 flies. The selection of individuals for the experiment was carried out by esterification, the victims and survivors of flies counted every third day. The experiment was carried out overnight culture Bacillus sp. MZ grown on meat-broth. In test tube culture was added in the amount of 20 l. The experiment included a control variant in which the flies were kept on a medium supplemented with yeast; and experienced embodiment, when the first 5 days of flies were kept on a medium with the addition of yeast, then one day in an environment with bacillus (alternating entire observation period). To determine the fertility of selected Virginia flies the day of departure. Males and females were placed separately and held 5 days when they reach maximum fertility. Then the tubes were placed in pairs with removable lids. The bottom lids poured agar-agar with the addition of sugar confectionery. Accounting fertility conducted daily for 6 days. We consider the number of eggs laid, and a day determined by the number undeveloped eggs.

When adding nutrient medium to the surface 75 of the strain Bacillus sp. in saline solution (1 billion. cells / ml), there was a reduction of fertility in relation to control 5 times. The average fertility of the females was in control of 58,1 ± 8,61 pcs., In a test version this value is 10,2 ± 3,44 units. When adding the bacterial culture after incubation flies for yeast, despite destruction of flies in the control experiment and after 50 days, it was observed excess proportion surviving flies from 24 to 42 th day of the experiment compared to the control (FIG. 1).

3.2.Testirovanie on laboratory mice

Preparation of the bacterial culture test was conducted in a similar manner on fruit flies; used daily culture Bacillus sp., but it was carried out before the introduction of freezing and thawing. Experiments were performed on CBA mice Fl / V1ask-6, an average of 15 animals in each group. In the first series of

experiments investigated the effect of doses culture parameters of the immune system of young animals (aged 3-4 months). The two control groups of animals were used, one of which was intact, while the second group was treated with saline. Bacterial culture Bacillus sp. intraperitoneally administered to the animals once 5000; 50000; 500,000; 5 000 000 and 50 million microbial bodies (bw) per animal. In a second series of experiments evaluated the effect of the bacterial culture on the physiological and behavioral responses of "older" mice (17 months), the culture was administered once intraperitoneally in the dose of 5000 bw The control group was represented by animals of the same age. Euthanasia of animals held by cervical dislocation. Evaluated according to standard procedures morphophysiological thymus and spleen activity on the index body (body weight ratio to body weight,%), nonspecific activity factors immunoresistance in the level of phagocytic (FI%) and metabolic (NBT-test,%) of splenic macrophage activity, cellular immunity DTH reaction in vivo, the activity of humoral immunity by the number of antibody-forming cells (AFC) in one million nucleated cells in the spleen, muscle strength test animals in cargo lifting, behavioral reactions in the test "Open Field" and lifespan.

It was found that Bacillus sp. at a dose of 5000 microbial bodies increases the index of thymus and spleen. Culture bacilli in the low dose (5,000 MT) promote and, in moderate doses (500,000 and 5,000,000 MT) - inhibit the phagocytic activity of splenic macrophages. Culture bacilli Bacillus sp. almost all doses increases the activity of the humoral immunity, and a dose of 5000 bw increases the functional activity and cellular and humoral immunity.

In this regard, for investigation on the influence of culture longevity dose was chosen in 5000 bw The minimum lifespan of mice in the control group was 589 days, and the maximum - 833 days. The minimum lifespan of mice from the experimental group was 836 days, and the maximum - 897 (FIG. 2). Body weight in the treated group was higher than in control animals after 2 months after administration of the culture. Muscle strength increased in the experimental animals (60%) relative to their peers in the control group. On increasing the ability of the animals to the orientation in space and research activity indicating more frequent visits to their domestic sectors open field, increasing the number of vertical posts and visiting mink. Apparently, the bacterial culture at parenteral administration stimulates the immune system and improves the emotional stability of laboratory animals. Increased life span of mice testifies to the possible presence in the culture of Bacillus sp. geroprotectors.

In addition, the strain was investigated as immunomodulators biocorrectors capable of launching an innate immune response in animal models. Activation of immunity was investigated in the experiments of metabolic change in macrophage activity, levels of interferon-gamma (IFN-?), tumor necrosis factor (TNF-a) and nonspecific cytotoxic effects of T-lymphocyte cells under the action of Bacillus sp., Introduced into mice C57B1 / 6 . It is found that intraperitoneal injection of the strain in the dose range 5 000 - 5 000 000 m. M. To animals leads to a dose-dependent activation of metabolism intraperitoneal macrophages, assessed by the level of reactive intermediates of aerobic metabolism (ROIs) by more than 2 times. A single intraperitoneal injection at a dose of 5000 MT lead to increased levels of IFN-? by 2.6 times, and also contribute to a significant reduction in TNF-a (FIG. 3). The cytotoxic effect of T-lymphocytes obtained from the spleen of animals after injection Bacillus sp., Was studied in vitro against mouse lymphosarcoma cells RLS. It is found that reliable activation of unspecific T lymphocyte clones occurs at a dose of 5 OOO OOO bw, wherein the cytotoxic effect of T-cell lymphocytes against lymphosarcoma was 1.8 times higher than their action without activating the strain. Thus, increased metabolism of macrophages, increasing interferon-gamma, decreased tumor necrosis factor and activation of cytotoxic T-lymphocytes acts under the action of Bacillus sp. indicates activation of innate immunity in laboratory animals and pronounced immunomodulatory properties of the strain Bacillus sp.

UA103574
USE OF BACILLUS SP. F STRAIN OF CONNATE BACTERIUMS AS ANTY-INFLAMMATORY AGENT

The invention relates to a strain of primordial bacteria Bacillus sp. F, isolated from permafrost, with strong anti-inflammatory properties. The strain is capable to be cultured under standard conditions, has anti-inflammatory properties can be used in clinical and experimental medicine as an anti-inflammatory agent.

UA102047
USE OF STRAIN OF RELIC BACTERIA BACILLUS sp. F AS HEPATOPROTECTOR AND ANTIOXIDANT

The invention relates to the use of the strain of relic bacteria Bacillus sp. F as hepatoprotector and antioxidant.

UA96709
STRAIN OF EPIBIOTIC BACTERIA BACILLUS SP. F WITH IMMUNOMODULATING ACTIVITY AND HEROPROTECTIVE ABILITY

The invention relates to a strain of epibiotic bacteria Bacillus sp. F with highly expressed heroprotective properties and immune resistance.
