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DIET IN THE NORTH

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MICROBIOTA AND SANITATION OF UNDERGROUND GLACIERS DURING FOOD STORAGE

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ABSTRACT

Carrying out the relevant works on sanitation has difficulties due to the lack of effective means and scientifically-based regimes. The aim of this work is to study the microbiota and to find effective methods and regimes for the sanitation of glaciers in permafrost conditions for food storage. The work was carried out in the Laboratory for the Development of Microbial Preparations of the FSBSI the Yakut Scientific Research Institute of Agriculture, as well as in the underground glaciers of Yakutsk and the regions of the Republic of Sakha (Yakutia) in the period 2007 - 2015. The material for studying the quantitative and qualitative composition of the microbiota circulating in glaciers was air samples and scrapings from surfaces, air samples and scrapings from the glacier surfaces were collected in February, April, June, August, and November. At the same time, the external and internal air temperatures were taken into account. Air samples were taken by sedimentation method. The number of microorganisms in the air, on the surfaces was determined according to the generally accepted methods of sanitary-microbiological examination of environmental objects.

The microbiota of the underground glacier for food storage is mainly represented by soil spore-forming aerobic bacteria of the genus Bacillus, as well as toxic and mold fungi of the genera Aspergillus, Mucor and pathogens of yersiniosis, which can be dangerous in the contamination of food.

We were the first sanitation for the of the glaciers, contaminated with intestinal, coccal and spore infections in Yakutia developed effective modes of disinfection (up to -21.0±0.8°C) using electrochemically activated anolyte containing 0.1 mg/ml of active chlorine and peracetic acid in a concentration of 0.5% (ADV) at the rate of 300 ml/m2 and exposure time 5 hours, and 1% aqueous solutions of the PAA, at the rate of 300-400 ml/m2, exposure 18 hours.

Keywords: underground ice-houses, food storage, microbial contamination, sanitization.

Introduction. The whole territory of the Republic of Sakha (Yakutia) is occupied by permafrost, in which an enormous supply of cold is accumulated, which directly relates the climate to the sharp continentally and low temperatures. In the republic, for a long time and until now, storage of food raw materials and food products in glaciers is widespread. The use of natural cold in the processing and storage of food products in the conditions of Yakutia contributes to severe winter, which lasts in some of its regions up to 8-9 months and the presence of permafrost [1]. Inadequate design requirements and improper operation of underground refrigerators lead to premature deterioration of stored food and a decrease in quality [2, 6, 9]. After the ice is laid in the early spring (February-March), the glacier is loaded with products. During operation, the glacier is only opened as needed on certain days and hours. After the release of the products, at the end of November, the glacier is completely opened. Before and after operation, the glacier is mechanically cleaned.

Sanitary treatment of glaciers in most cases reduces only to mechanical cleaning of internal compartments and tambours, which leads to their high contamination by microorganisms in the process of exploitation. In the available literature there are no reports on methods and regimes for sanitation of glaciers in permafrost conditions.

In the opinion of several authors E.N. Bolotsky et al. [4], the trend of development of disinfection technology in recent decades throughout the world is not to create new disinfectants, but to search and activate already known means, in the development of regimes providing a high bactericidal effect with a minimum concentration of active substances and a weak toxic effect [4]. A technochemical activation technology has been developed in our country that allows synthesizing cheap biocidal solutions (anolytes) of the universal spectrum of action at the site of application. After use they spontaneously decompose without the formation of toxic compounds and do not require neutralization and subsequent washing. Therefore, a promising direction in the search for available sanitizing preparations is the use of biocides, which are created on the basis of unipolar electrochemical activation (ECA) of aqueous solutions of chlorides [5, 8, 13].

In the available literature it can be found an information on the use of an anodic fraction (anolyte) of an electrochemically activated (ECA) solution of 1% common salt as a disinfectant in medicine, processing industry, agriculture, fodder production, veterinary medicine [3, 14].

The minus temperature in the glacier complicates the sanitation of the ice surfaces. According to the research of N.P. Tarabukina, for the disinfection of wooden surfaces, seeded Sal. abortus equi BN-12, Str. equi N-34, Bac. subtilis TNP-3, at low temperatures up to -12°C there is an effective application of 1-3% (by active substances) solutions of peracetic acid (PAA) [15-17].

The aim of this work is to study the microbiota and to find effective methods and regimes for the sanitation of glaciers in permafrost conditions for food storage.

Materials and methods. The work was carried out in the Laboratory for the Development of Microbial Preparations of the FSBSI the Yakut Scientific Research Institute of Agriculture, as well as in the underground glaciers of Yakutsk and the regions of the Republic of Sakha (Yakutia) in the period 2007 - 2015.

The material for studying the quantitative and qualitative composition of the microbiota circulating in glaciers was air samples and scrapings from surfaces, air samples and scrapings from the glacier surfaces were collected in February, April, June, August, and November. At the same time, the external and internal air temperatures were taken into account.

Air samples were taken by sedimentation method, the surfaces were examined using scrapes taken from ice walls with a 10x10 cm stencil in sterile Petri dishes. The number of microorganisms in the air, on the surfaces was determined according to the generally accepted methods of sanitary-microbiological examination of environmental objects. The generic and species identification of the isolated cultures of microorganisms was carried out according to the "Berjee bacteria determinant" (1997), and "The determinant of zoopathogenic microorganisms" (1995). Staining of smears was prepared according to Gram. The results of microbiological cultures were taken into account after 18 and 24 hours for bacteria, and microscopic fungi after 5 days. Elective media prepared according to GOST were used: meat-peptone agar (MPA) to determine the amount of MAFAnM - mesophilic aerobic and facultative-anaerobic microorganisms; MPA - to isolate spore-forming aerobic bacteria (after heating the main dilution at 80°C for 15 minutes); Endo - for the isolation and differentiation of enterobacteria; MBTB - medium with bromotymol blue to isolate Yersinia; Czapek - for the isolation of microscopic fungi.

As test cultures there were used the strains of bacteria Salmonella abortus equi BN-12, Streptococcus equi N-34, Bacillus subtilis TNP-3, which have been certified by the All-Union State Scientific and Control Institute of Veterinary Preparations (Moscow).

Preparations of solutions, determination of the active substance, disinfection quality control were carried out according to the "Rules for disinfection and disinvasion of objects of state veterinary supervision" (2002). To obtain an electrochemically activated neutral anolyte, an apparatus for the electrochemical synthesis of activated disinfecting solutions of AQUAEHA (mod. 40) of STEL type was used.

Results of the research. The total microbial contamination on the surface of the walls of glaciers is from $2.8x10^2$ to $60.0x10^3$ CFU/m^3. The total microbial contamination of the glacier air ranges from $1.4x10^2$ to $23.6x10^3$ CFU/m^3. The air temperature of the glacier during the study period remained stable and averaged 13.4 ± 2.1°C, regardless of the outside air temperature.

From the microbiota of the underground glacier spore-forming aerobic bacteria of the genus Bacillus were isolated in an amount from $1.1x10^2$ to $43.3x10^3$ CFU/cm^2, related to the soil saprophyte microflora. In addition, the causative agents of yersiniosis (from $1.5x10^2$ to $23.6x10^3$ CFU/cm^2) have been identified, related to sapronoses, which are classified as species: Yersinia pseudotuberculosis and Yersinia enterocolitic. Yersinia refers to psychotrophic bacteria with a wide range of adaptive and pathogenic properties. Researchers G.P. Somov, V.U. Litvin [12], E.M. Lenchenko [7] revealed the laws of the existence and circulation of Yersinia in the communities of the environment and believe that soil and aquatic ecosystems are obligatory components of natural foci of sapronoses. In this regard, the identification of the causative agents of yersiniosis in the microbiota of glaciers in the conditions of the cryolithozone confirms the authors' opinion and widens the range of their distribution.

Yersinia are steady to cold, well stand the temperature from -15 up to -20 °C, and in these conditions can exist for a long time. Psychrophilic properties of Yersinia pseudotuberculosis contribute to the emergence and development of the epidemic process, because the cold not only allows these bacteria to multiply and accumulate in environmental objects, but also is the trigger factor of the genetic and biochemical mechanisms that ensure the regulation of their virulence [11, 18]. Therefore, the pathogens of yersiniosis isolated from the surfaces of glaciers present the danger of food contamination

Results of industrial experiments on the disinfection of ice surfaces, (glacier temperature -21.0±0.8°C)

Disinfectants	Concentration of active substance (in %)	Consumption	Growth of microorganisms														
			Exposition (in hours)														
			1,5			3			5			18			24		
			Sal. abortus equi BN-12	Str. Equi N-34	Bac. subtilis TNP-3	Sal. abortus equi BN-12	Str. Equi N-34	Bac. subtilis TNP-3	Sal. abortus equi BN-12	Str. Equi N-34	Bac. subtilis TNP-3	Sal. abortus equi BN-12	Str. Equi N-34	Bac. subtilis TNP-3	Sal. abortus equi BN-12	Str. Equi N-34	Bac. subtilis TNP-3
PAA	1	300 ml/m²	-	-	+-	-	-	+-	-	-	+-	-	-	-	-	-	-
PAA	1	400 ml/m²	-	-	+-	-	-	+-	-	-	+-	-	-	-	-	-	-
PAA	1	500 ml/m²	-	-	+-	-	-	+-	-	-	-	-	-	-	-	-	-
Anolyte neutral with an active chlorine content of 0.5 mg/ml		200 ml/m²	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anolyte neutral with an active chlorine content of 0.5 mg/ml + PAA	0,5	300 ml/m²	-	-	+-	-	-	+-	-	-	+-	-	-	+-	-	-	+-
Anolyte neutral with an active chlorine content of 0.1 mg/ml + PAA	0,5	300 ml/m²	-	-	+-	-	-	+-	-	-	-	-	-	-	-	-	-
Tap water	Control	300 ml/m²	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: PAA - Peracetic acid, ADV-active ingredient, (+) - growth of test cultures; (+ -) - single growth of test cultures; (-) - no growth of test cultures.

during storage.

Also, the results of our studies indicate the presence of toxigenic and mold species of the genera Aspergillus and Mucor in the microbiota of glaciers at temperatures from -14 to -22°C, although, according to the published data, the criterion for the temperature existence of microscopic fungi is up to -9°C [10].

The results obtained allow us to conclude that it is necessary to find effective measures for the rehabilitation of underground glaciers used for food storage.

For the first time for the sanitation of glaciers have been tested an electrochemically activated neutral anolyte with a content of 0.5 mg/ml and 0.1 mg/ml of active chlorine, with the addition of 0.5% peracetic acid (PAA), as well as 1% peracetic acid solutions (PAA), at a flow rate of 200-500 ml/m², an exposure of 1.5; 3; 5; 18 and 24 hours. As a control, ice surfaces contaminated with test cultures are treated with tap water, at a rate of 200-500 ml/m². Solutions are applied in the form of small-drop spraying with a non-propellant balloon at a glacier temperature of -21.0±0.8°C. The results are shown in Table.

As the data in Table show, 1% solutions of PAA, at a flow rate of 300-400 ml/m², an exposure of 18 hours, reliably disinfect the ice surfaces contaminated with intestinal, coccal, spore infections. When the flow rate increases to 500 ml/m² an exposure of a harmful effect on Sal. abortus equi BN-12, Str. equi N-34, Bac. subtilis TNP-3 is reduced to 5 hours. It should be noted that the use of a large number of solutions is not desirable for the sanitation of glaciers.

According to the results of the research, solutions of anolyte neutral with an active chlorine content of 0.5 mg/ml, with the addition of 0.5% PAA, the ice surfaces contaminated with Sal.abortus equi BN-12, Str. equi N-34, Bac.subtilis TNP-3 are not completely disinfected, and the use of dilute solutions of anolyte neutral with an active chlorine content of 0.1 mg/ml with the addition of 0.5% PAA, at a flow rate of 300 ml/m², starting with a 5-hour exposure completely destroy these microorganisms.

Conclusion. Thus, the study showed that in the underground glaciers used for food storage (with an additional installation for maintaining the cold), at a temperature of -13.4±2.1°C, the total microbial contamination on surfaces is up to 60.0×10³ CFU/cm² and in air - up to 23.6x10³ CFU/m³. From the microbiota of the underground glacier in winter (February-April), the yersiniosis pathogens Yersinia pseudotuberculosis, Yersinia enterocolitica and toxigenic fungi of the genus Aspergillus (fumigatus, niger, mucor sp.) had been isolated.

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SCIENTIFIC REVIEWS AND LECTURES

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GENETIC POLYMORPHISMS OF THE HEMOSTASIS SYSTEM

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ABSTRACT
Despite advances in treatment of chronic viral hepatitis, search of predictors of poor outcome is still needed. One of them is the hemostasis system. The decoding of the human genome has made it possible to determine genetic markers that lead to blood coagulation disorders. It is widely known that more than 10 single nucleotide polymorphisms (SNP) are responsible for some form of coagulation disorders. The genome-wide