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The sorption of bacteria of *Bacillus* genus on vermiculite

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Abstract: *The possibility of submerged sorption of probiotic cultures, such as Bacillus sp. 8 and Bacillus sp. 9 on vermiculite, as well as the survival ability of probiotic cells on the sorbent during prolonged storage were studied. Strains started to be sorbed on vermiculite in 2-3 hours. The sorption process lasted 7-8 hours. Cells that sorbed on vermiculite in ratio of 1: 1 showed the preservation of the cell titer at the control level for 6 months at room temperature, which showed their suitability for long-term storage.*

Keywords: *Bacillus subtilis, Bacillus licheniformis, sorption, vermiculite.*

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I. Introduction

One of the main tasks of agriculture is to provide the population with high-quality food products. The physiological state of agricultural animals and poultry and its productivity strongly depends on feeding it with high-quality and high-grade mixed fodder. Probiotics are commonly used to solve this problem since these microorganisms can increase productivity, improve the quality of meat and correct intestinal microflora [1]. Nowadays there are quite a lot of probiotic preparations in medicine and veterinary and they are presented both as monocultures and a combination of several cultures. *Bac. subtilis* is preferred of all the microbiological cultures. Preparations based on this species can positively change the bacterial background of the intestine, increase the resistance of the organism, preserve the livestock capita and the productivity of poultry and farm animals [2].

At the present stage of improving technological processes, the sorbed probiotics are intensively introduced into practice, in which the bacterial strains that form their basis are located on special substances namely sorbents, which facilitate the transportation of the active principle to the destination and enhance the biological activity of the preparations.

The use of this principle makes it possible to create complex preparations with high stability and efficiency [3]. While developing immobilized forms of biological preparations, the most important point is the choice of a sorbent carrier [4]. Used sorbents must meet medical requirements, i. e. not to be toxic, do not injure mucous membranes, evacuate well from the intestine, have good sorption properties, do not cause dysbiosis, have a convenient dosage form.

Mineral sorbents have proven themselves as fillers in the production of bio-feeds, premixes, meat-and-bone flour. The use of sorbents does not require special equipment. Wherein, production waste that pollutes the environment is reduced. Veterinary practice uses various local and imported sorbents of both natural and synthetic origin, such as zeolites, vermiculite, diatomaceous earth, marl, enterosorbite-B, mycophixes, etc.

At present time, a number of immobilized probiotics, such as "Bifidumbacterin - forte", "Probifor" and others have been developed with a long expiration date. It is known that the process of lyophilization of bacteria has a negative effect on the structure of their surface proteins, adhesion activity and also leads to the destruction of valuable bacterial metabolites. The liquid form of probiotics, on the contrary, promotes the realization of positive properties to the fullest extent. Their cells are in an active physiological state, so cultures retain all bacterial metabolites [3].

The aim of this work was the experimental confirmation of the possibility of immobilization of probiotic cultures, i.e. *Bacillus sp. 8* and *Bacillus sp. 9* on vermiculite for the purpose of creating a long-term storage preparation.

II. Materials And Methods

Bacillus sp. 8 and *Bacillus sp. 9*, which had antagonistic activity against a number of conditionally pathogenic bacteria of the gastrointestinal tract (GIT) of animals, grown in Nutrient broth were used as materials of study [5]. Vermiculite was used as a sorbent. Sorption of probiotic cultures was carried out in submerged conditions. The preservation of cells titer on sorbents was carried out by mixing the culture and sterile sorbent in a ratio of 1:1 at room temperature.

The immobilization process was led at temperature of 20°C and pH of 7.0-7.2. After preliminary sterilization of the sorbent (1 g of sorbent + 10 ml of sterile tap water) in the autoclave at 121°C for 30 minutes, 1 ml of culture suspension with a titer of 10^9 cells/ml was added. For a more complete contact of the sorbent with the cells, the vials with bacterial suspension and sorbent were periodically shaken on a shaker apparatus. The efficiency of bacterial sorption was calculated from the difference in microorganism cell concentrations in the cultural broth before and after the sorption process by the method of tenfold limiting dilutions, the number of cells was determined from the McCready table on Nutrient broth at 37°C after 24 hours of cultivation [6].

To obtain a "sorbent + probiotic" biocomposite for long-term storage, 10 g of sterile sorbent and 10 ml of one-day suspension of probiotic were thoroughly mixed and stored under sterile conditions at room temperature (20°C). Aliquots (500 mg each) were selected from the biocomposite in the dynamics of the experiment (immediately after mixing, after 1, 7, 15, 22, 30 days and 6 months), in which the cell titer was checked by successive tenfold dilutions up to 10^{-10} dilution on Nutrient broth. The titer of the culture cells on Nutrient broth without sorbent was considered as control sample. The number of cells in the biocosposite mixture was also calculated from the McCready table on Nutrient broth at 37°C by the method of limiting dilutions.

III. Results And Discussion

An activation analysis of vermiculite from Uzbekistan and abroad was conducted. The content of macro- and microelements is given in Table 1. The results of the analysis indicate a relatively high content of sodium, calcium, manganese, copper and zinc in the vermiculite obtained in Uzbekistan.

Table 1. Activation analysis of vermiculite from Uzbekistan and abroad

Element	Content, mg/kg		Element	Content, mg/kg	
	Uzbekistan	Abroad		Uzbekistan	Abroad
Na, %	**0.84**	0.66	Sr	100	130
K, %	1.1	4.5	Cs	2.5	8.5
Ca, %	**10.0**	3.0	Ba, %	**0.24**	0.15
Sc	**53**	7.0	La	2.0	5.4
Cr	420	730	Ce	6.8	13
Mn	**680**	320	Nd	< 10	17
Fe, %	**6.0**	5.0	Sm	**1.6**	1.3
Co	68	74	Eu	**0.32**	0.22
Ni	220	280	Dy	**1.3**	< 1.0
Cu	**110**	10	Yb	**1.4**	< 1.0
Zn	**81**	45	Ta	< 0.2	0.32
As	**2.0**	< 0.2	Th	< 1.0	1.4
Rb	74	400	U	**0.50**	< 0.1

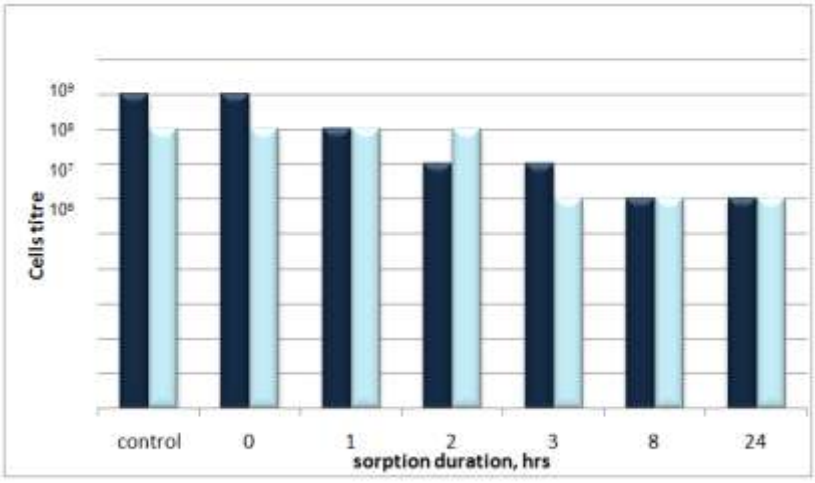

fig. 1. The sorptionof cells of *Bacillus sp. 8* and *Bacillus sp. 9* on vermiculite.

As can be seen from the data presented in Fig. 1, the sorption of the tested cultures on vermiculite occurs within 7-8 hours, beginning with a 2-3 hours contact of cultures with sorbents. In 8 hours the change in the concentration of cells on the sorbent reaches a plateau, which is measured by the dynamic balance between sorption and detachment of cells, i.e. depends on the action of the desorption forces.

In addition, to determine the possibility of immobilization of probiotic cells on vermiculite in submerged conditions, it was necessary to determine the ability of survival of probiotic cells on the sorbent during long-term storage. After the culture was mixed with the sorbent the titer of the cell was counted according to the McCready table every week up to 2 months, and then 1 time per month during the half-year. The results of the study are shown in Table 2.

The data presented in Table 2 demonstrate that probiotic cultures retain their primordial cell titer with mixing at a ratio of 1: 1 throughout the duration of the experiment (6 months).

Table 2. Cells titerof probiotic-sorbent 1:1 composite during the experiment

Sample	Cells titer/ml				Incubation duration
	control	initial	1st dilution	2nd dilution	
Bacillus sp. 8	$2.5.10^9$	$2.5.10^8$	$2.5.10^8$	$2.5.10^7$	0
	$2.5.10^9$	$5.0.10^8$	$2.5.10^8$	$2.5.10^7$	7days
	$5.0.10^9$	$2.5.10^8$	$2.5.10^7$	$2.5.10^6$	15days
	$2.5.10^9$	$2.5.10^7$	$5.0.10^7$	$5.0.10^5$	22days
	$2.5.10^9$	$2.5.10^7$	$2.5.10^7$	$2.5.10^6$	29days
	$2.5.10^9$	$2.5.10^8$	$2.5.10^8$	$2.5.10^5$	2 months
	$2.5.10^9$	$2.5.10^8$	$2.5.10^6$	$2.5.10^5$	3 months
	$2.5.10^9$	$5.0.10^8$	$2.5.10^6$	$5.0.10^5$	4 months
	$2.5.10^9$	$2.5.10^8$	$2.5.10^7$	$2.5.10^6$	5 months
	$2.5.10^9$	$2.5.10^8$	$2.5.10^7$	$2.5.10^6$	6 months
Bacillus sp. 9	$2.5.10^8$	$2.5.10^8$	$2.5.10^8$	$2.5.10^8$	0
	$2.5.10^8$	$2.5.10^8$	$2.5.10^8$	$2.5.10^7$	7days
	$2.5.10^8$	$2.5.10^7$	$2.5.10^7$	$2.5.10^6$	15days
	$2.5.10^8$	$2.5.10^8$	$2.5.10^7$	$2.5.10^6$	22days
	$2.5.10^8$	$5.0.10^7$	$2.5.10^6$	$5.0.10^6$	29days
	$5.0.10^8$	$2.5.10^8$	$5.0.10^8$	$2.5.10^5$	2 months
	$2.5.10^8$	$2.5.10^8$	$5.0.10^6$	$2.5.10^6$	3 months
	$2.5.10^8$	$2.5.10^8$	$5.0.10^7$	$5.0.10^6$	4 months
	$2.5.10^8$	$2.5.10^8$	$5.0.10^7$	$5.0.10^6$	5 months
	$2.5.10^8$	$2.5.10^8$	$5.0.10^7$	$5.0.10^6$	6 months

As can be seen from the data presented in Table 2, probiotic cultures retain their primordial cell titer throughout the entire experimental period while mixing at a ratio of 1:1 both with vermiculite.

IV. Conclusion

Thus, the sorption process of spore bacteria species on vermiculite in submerged conditions started from 2-3 hours and lasted for 7-8 hours. The vermiculite-sorbed probiotic cultures of *Bacillus* genus in a ratio of 1:1, intended for long-term storage, showed the preservation of the cell titer at the control level for 6 months at room temperature.

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